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

Commencing in 1979 with Volume XXXIII of the Proceedings of the North Dakota Academy of Science, a new format appeared. The Proceedings changed to an 8½ x 11 format, it is produced from camera-ready copy, and it is issued in a single part prior to the annual meeting (i.e. in mid-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 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 of the Proceedings contains the 29 papers presented in the five symposia at the 1984 annual meeting of the Academy. The papers are presented in the same sequence as presented at the meeting, and are numbered as they appeared in the meeting program.

The second section of this volume of the Proceedings contains the 51 communications presented in the Professional section of the 1984 annual meeting of the Academy. 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 numbered in the sequence in which they appear in the meeting program.

The third section of this volume contains the 22 collegiate communications representing 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 subject to review prior to publication herein. The Denison Awards Committee judged the oral presentation and the communication in arriving at their decision for the first and second place awards in both the graduate and undergraduate competition. The collegiate communications are numbered in the sequence in which they appear in the meeting program.

Readers may locate papers by presentation number within the major sections of these Proceedings or by referring to the author index in this volume for a page reference.

A. William Johnson Editor

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NORTH DAKOTA ACADEMY OF SCIENCE

I. Rules for Preparation of Proceedings Communication

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.

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.

Authors are encouraged to utilize the full space available in order to provide sufficient information to fully describe the research reported.

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, 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.

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.

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.

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) Brit. Med. J. 3, 474-475. (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 *The Pharmacological Basis of Therapeutics*, 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.

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.

Abbreviations: Only standard abbreviations should be used, and should be written out the first time used with the abbreviation following in parentheses.

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.

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.

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

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.

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.

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. Timed rehearsals with slides are highly recommended. There is usually time for a maximum of 6 or 7 slides for a presen-

tation of this kind.

SYMPOSIUM

on

PLANT TISSUE CULTURE AS A RESEARCH TOOL-BASIC AND APPLIED

Presiding: David G. Davis

Metabolism and Radiation Laboratory - ARS, USDA

Fargo, ND

1. Plant Tissue Culture as a Research Tool - Basic and Applied

David G. Davis*

Metabolism and Radiation Laboratory - ARS, USDA

Fargo, ND

2. The Use of Cell Cultures in Leafy Spurge Physiology Studies

Donald S. Galitz*

Botany/Biology Department, NDSU

Fargo, ND

3. Extracellular Phosphohydrolases of Plant Cell Cultures

Arland E. Oleson*

Biochemistry Department, NDSU

Fargo, ND

4. Organogenesis and Secondary Products in Plant Cell Cultures: Their

Possible Relationships in Plant Development

David G. Davis*

Metabolism and Radiation Laboratory - ARS, USDA

Fargo, ND

5. The Potato Protoplast System

Gary A. Secor*

Department of Plant Pathology, NDSU

Fargo, ND

6. Plant Variants From Wheat Cell Cultures

Edward L. Deckard*

Agronomy Department, NDSU

Fargo, ND

7. Regeneration of Maize From Tissue Cultures

W. F. Sheridan*

Biology Department, UND

Grand Forks, ND

 * This number reflects the sequence of this presentation in the meeting program.

Plant Tissue Culture as a Research Tool - Basic and Applied

Introduction

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The terms biotechnology and genetic engineering are very much in vogue at present. Both terms are being used in the scientific community and the lay press to describe areas of research and technology that are a synthesis of several scientific disciplines working together to produce new and unusual products or organisms that were expensive or impossible to aquire using previous techniques. Two of the best known examples are interferon, which was hailed by many as a miracle cure for cancer, and the production of human insulin through genetic engineering techniques.

In agriculture there have been tremendous strides in biotechnology and genetic engineering in recent years, and great interest in the potential has been created by some significant advancements. The transfer of genes from one plant to another, the fusion of tomato and potato protoplasts to produce a new hybrid, and the transfer of herbicide resistance from one plant to another are three examples of the important areas of plant research being explored. Plant tissue culture techniques have been used for key roles in these new advancements. The impact of dramatically new forms of plant life that may arise from genetic engineering are not as likely to be as visible as are new animal forms, nor are they as likely to be applied directly to humans. Therefore, concerns have not been expressed about plant genetic engineering research as much as in animal research.

The symposium speakers are individuals who have been working with plant tissue cultures for several years and are well qualified to discuss their individual topics. There are other scientists in the state of North Dakota and nearby areas of Minnesota who might have been included in this symposium, but the few chosen represent an overall summary of the work in North Dakota.

There may also be others working or thinking of working in plant tissue culture, of whom I am not familiar. If so, this symposium may serve a purpose by drawing us all together so we may exchange thoughts and ideas as to how best advance our knowledge and the use of the techniques.

Table 1. Plant Tissue Culture - Areas of Research and Basic Techniques

Culture of plant cells, tissues and organs
Callus, cell suspensions and isolated roots
- initiation and maintenance

- Differentiation
- specialized cells, organs, plants Protoplasts
 - isolation, fusion, hybridization

Aseptic techniques
Nutrition; media
Somatic embryogenesis
Anther and pollen cultures
Secondary product formation
Biotransformation of
foreign chemicals

The Use of Cell Cultures in Leafy Spurge Physiology Studies

2

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Leafy spurge (<u>Euphorbia esula L.</u>) is a perennial weed which infests millions of acres of uncultivated lands across the northern tier of the United States and across Canada. Its economic impact has become staggering because of the costs of control surpass the economic returns of these lands of marginal productivity.

Vegetative differences have been observed in field grown leafy spurge. Some of these are due to environmental conditions, for this plant is found to grow in a diversity of environments. However, some of these differences are genetic. The different plant forms have been termed "Biotypes" and variations in their physiology and chemistry may be responsible for the variable control which is obtained with present control programs.

Tissue culture techniques have been employed to acquire additional evidence supporting the concept of genetic differences between biotypes. From each of the nineteen type specimens in our biotype collection, we have generated callus tissue and from the callus tissue we have obtained cell suspension cultures. These cell suspension cultures have been characterized in terms of growth rates, several biochemical parameters and relative sensitivity to different concentrations of dicamba (3,6-dichloro-o-anisic acid).

Conditions will be described for the development of callus from young spurge stem tissue and procurement of viable cell suspension cultures from the callus tissue. Comparative growth rates of untreated cultures show biotypic variation. Similarly, differences in sensitivity of cultures of leafy spurge biotypes to dicamba provide biochemical evidence for a genetic basis for leafy spurge biotypes.

Cell suspension cultures provide a mechanism for studying the cellular basis for the response of a plant species to herbicide treatment. The effects of dicamba on parameters such as protein content, nitrate assimilating capacity, DNA, RNA and total acid soluble nucleotide content of cell suspension cultures of spurge biotype will be reported.

EXTRACELLULAR PHOSPHOHYDROLASES OF PLANT CELL CULTURES

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Release of hydrolytic enzymes into the medium during growth of cell suspension cultures is a common occurrence in many plant species. Some of these enzymes are phosphohydrolases that catalyze the degradation of polynucleotides added exogenously to cultured cell systems (1), and they may interfere with transformation or transfection of plant cells or protoplasts with biologically active nucleic acids. The amount of nucleolytic activity present in the medium varies from one species to another. Culture filtrates of tobacco (Filner cell line (2)) have been found to contain 50- to 100fold greater DNase and RNase activity than those of wheat, soybean, or <u>Haplopappus</u>, and studies in this laboratory over the past few years have been directed at characterizing the properties and regulation of nucleases and nucleotidases from the tobacco cultures. The phosphohydrolases present in this system include acid phosphatase (APase), acid phosphodiesterase (PDase), ribonuclease I (RNase), and nuclease I (3). The latter enzyme is active on DNA, RNA, and 3'-nucleotides at pH 5-6 (4). It is subject to a moderate level of inhibition by inorganic orthophosphate (Pi), and is strongly inhibited by pyrophosphate, ATP, and certain other nucleotides (5). This enzyme has been purified by chromatographic and affinity adsorption methods (6). Extracellular nuclease I is a metalloglyco-protein which exists as two isozymic forms of identical size $(35,000 \text{ M}_{\text{P}})$ but different charge (pI)values of 5.2 and 5.6) (7). Recent studies indicate that the two isozymes are of similar amino acid, sugar and metal ion composition and have identical catalytic properties, but they can be distinguished immunochemically. The amount of extracellular nuclease I and its relative substrate specificity is related to the concentration of nutrient Zn^{2+} initially present in the culture medium (8).

The release of both nuclease I and PDase by tobacco cells cultured in B5 medium has been found to occur continuously from early log to stationary phase of growth. In contrast, APase and RNase were not detected in the medium until after nutrient Pi exhaustion in mid-log cultures. Massive synthesis and release of these two enzymes occurred in late-log phase. Addition of 20 µM cycloheximide to late-log tobacco cultures abolished protein synthesis and prevented further synthesis and release of all phosphohydrolases, while treatment with 1 mM Pi caused cessation of synthesis and secretion only of APase and RNase. Synthesis of RNase is thus subject to Pi regulation, like the previously studied (9) APase system.

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Organogenesis and Secondary Products in Plant Cell Cultures: Their possible relationships in plant development.

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Certain compounds (secondary metabolites or secondary products) are produced by plants and are unique to a few species or individual cultivars (Table 1). Many are economically and/or ecologically important. Some products are considered as end products. Many move into vacuoles of plants, and were previously thought to be entrapped there. However, some of these compounds have been shown to be recycled back into the plant's normal metabolic cycle.

Plants are an important commercial source of many chemicals and medicinal compounds (1). Many pharmaceuticals, flavorings and perfumeries have not been duplicated economically by chemical synthesis or bacterial fermentation. Also, certain chemical reactions can occur in plant cell cultures that are difficult or expensive to duplicate in other systems. Certain compounds used for treating cardiac patients have been selectively altered so that useless compounds are converted into useful materials. Staba (2) lists several patents for compounds for which cell cultures have been the means of production. Many laboratories and pharmaceutical companies are spending considerable sums of money to develop plant tissue cultures that may produce these compounds, or to produce regenerated plants that are high yielders of these chemicals. Although in theory plant cell cultures offer an ideal system for producing secondary products of plant origin, practical limitations are such that plant cell suspension cultures have not been used extensively. Low production, slow growth rates and the deposition of many of these metabolites into vacuoles all create difficulties that are hard to overcome.

Useful areas of plant tissue culture are in genetics and agronomy, to produce new cultivars that will increase production of secondary products or high yielding crops. Organogenesis and regeneration of a new plant from cell cultures frequently are the biggest roadblocks to the use of plant tissue cultures. A usual situation is to produce a callus and/or a cell suspension, to expose the culture to growth regulators or selection pressures (chemical or physical), produce shoots and roots, grow the plant aseptically and then in a greenhouse and field for evaluation. This approach is usually somewhat empirical.

Organogenesis, regeneration and secondary product formation will be discussed for <u>Euphorbia</u> <u>esula</u> (<u>Euphorbiacea</u>), a noxious weed commonly called leafy spurge. These research areas are linked because there may be a cause and effect relationship between these processes in many plants (3).

Very real and significant differences between cultures of different plant origin cause major problems. The success of experiments frequently depends on selection of a particular cultivar or biotype. Regeneration of leafy spurge plants from cell suspension cultures of one biotype has been accomplished in this laboratory. Anthocyanin production in this same biotype will be discussed as an example of secondary product formation in cell cultures. Five other biotypes have given almost no indication of the capacity for organogenesis, regeneration or anthocyanin production. Until the reasons for this are known plant tissue culture will remain somewhat of a hybrid between art and science.

Table 1. Secondary Products Produced by Plants

Alkaloids Antibiotics	Volatile Oils Resins Tanins	Sterols Cardiac glycosides	Saponins Latex

References:

4

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- (2) E.J. Staba. 1977. Tissue Culture and Pharmacy. <u>In</u>: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture (ed. J. Reinert and Y.P.S. Bajaj). Springer-Verlag, Berlin. 694-702.
- (3) M.M. Yeoman, K. Lindsey, M.B. Miedzybrodzka and W.R. McLauchlan. 1982. Accumulation of Secondary Products as a Facet of Differentiation in Plant Cell and Tissue Cultures. <u>In: British Society for Cell Biology Symposium (eds. M.M. Yeoman and D.E.S. Truman)</u>. Cambridge Univ. Press, Cambridge, England 4: 65-83.

The Potato Protoplast System

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Cell and protoplast culture systems are new tools that can be used to produce variability from which improved plants can be selected. The potato (Solanum tuberosum) has been especially amenable to this manipulation. Complete regeneration of plants from protoplasts (cells without cell walls) has become fairly routine. Extensive laboratory, greenhouse and field testing has been done with potato plants regenerated from protoplasts (protoclones). A high degree of variability has been observed in these protoclones, including yield, sugar levels, maturity, tuber type and color, vine architecture and disease resistance. Sixty five clones of the variety 'Russet Burbank' were analyzed extensively for many characteristics. The minimum variability occurred in one character, the maximum in 17 characters, with an average variability of four characters. Compared to the control, 70% of the characters were judged to be the same, 20% were inferior and 10% superior. The protoclones appeared to be stable for at least four years. Environment affects the performance of some protoclones. One of the better performing protoclones will be entered into regional field trials in 1984. The varieties 'Crystal' and 'Norgold Russet' have been regenerated and will be evaluated as to whether they are improved clones. Variability of potato protoclones has been verified by workers in Great Britian. Screening systems are being developed to select stress resistant clones in culture. Variability has been documented in other plants from cell and protoplast culture, including sugar cane, tobacco, lettuce, alfalfa, corn, celery and others. Future application includes the techniques of protoplast fusion and DNA insertion.

PLANT VARIANTS FROM WHEAT CELL CULTURES

6

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Cell culture generates genetic variability that is expressed in regenerated plants. Since plant breeding is dependent upon genetic variability, cell culture has the potential of contributing to the plant breeding process. Any contribution is dependent on that crop lending itself to cell culture techniques, and techniques for the cereal crops have developed slowly. However, many of the cereal crops have now been cultured, and plants have been regenerated from the cultured cells.

Most tissues of the grass plant have been cultured, but we have found for wheat that immature embryos (about 14 days after anthesis) are the most predictable tissue for forming regenerable cultures. Immature embryos were placed on a solid agar medium containing the Murashige and Skoog inorganic components and 150 mg L-asparagine, 0.5 mg Thiamine, 20 g sucrose, and 7.0 g agar per liter. For callus initiation, 5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) was added to the medium. After callus initiation, callus growth was promoted when the 2,4-D concentration was reduced to 3 µM and the callus was subcultured every month. The most responsive genotypes exhibited growth rates of about 1.5 g fresh weight/month for at least 8 months. Other genotypes exhibited a decreasing growth rate with time.

Regeneration of plants is a two-step process. When the 2,4-D concentration was reduced to 0.5 µM, shoot formation occurred. Removal of 2,4-D resulted in root formation. We have regenerated 10 to 200 plants from about 60 hard red spring wheat (Triticum aestivum L.) genotypes. Most regenerated plants appeared altered in some way. Several plants were of no practical use because they lacked chlorophyll or did not produce seed. However, the frequency of change was not so high through the culture process that most plants possessed deleterious alterations. Many plants exhibited apparent differences in plant height, plant color, enzyme level, and leaf size. We have not made appropriate genetic analyses to characterize any alteration, but the majority of alterations were transmitted meiotically.

Other researchers working with other crops have reported genetic variability induced by cell culture. The types of variability include morphological, agronomic, yield, and disease resistance. Single gene mutations, changes in chromosome number and changes in chromosome structure have been suggested as responsible for causing the genetic variability. The mechanism(s) causing the genetic change(s) is not known.

Plant breeders improve a specific crop only by altering the physiology or biochemistry of that crop, and yet we know little of the specific physiological-biochemical processes that limit crop production. Not only does our poor understanding of these important processes limit crop improvement by standard breeding methods, it also limits the assistance of cell culture techniques. Therefore, cell culture techniques may contribute new useful genetic variability either serendipitously or in specific problem areas, but a more wide-spread directed approach awaits a more complete understanding of the present genetic limitations.

REGENERATION OF MAIZE FROM TISSUE CULTURES

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Maize is the most important crop plant in the United States. It is also especially well suited for selection as the plant species for the study of the molecular biology of plants. A major block persists to the full utilization of maize in molecular studies, as well as for the application of the results of such studies to the improvement of this major crop-the difficulty in regenerating maize plants from in vitro culture systems. Yet, the capacity to regenerate plants is an essential bridge for linking molecular biology approaches on the one hand with crop improvement by conventional plant breeding procedures on the other.

Successful regeneration of plants from maize tissue cultures dates from the original report of Green and Phillips (1975). They reported that a regenerative culture could be obtained by culturing immature embryos on a modified Murashige and Skoog medium (MS). The scutellar tissue proliferated to produce a callus culture containing semi-organized structures that would regenerate plants upon subsequent subculture.

The most significant new development in maize tissue culture was the isolation of callus lines that are friable, grow rapidly, and regenerate plants by somatic embryogenesis (Green 1982, Green and Rhodes 1982, Green 1983, Green, Armstrong and Anderson 1983). These lines have arisen spontaneously in callus derived from the scutellar tissues of immature embryos of the inbred A188. They produce large numbers of somatic embryos which mimic the developmental pathway of zygotic embryos. Callus of this type has appeared in cultures growing on both the MS and N6 media containing 0.5 to 1.0 mg/l of 2,4-D.

The formation of callus of this type directly from embryos has occasionally been observed, but its frequency is dramatically increased when the free amino acid proline is included in the N6 medium used for the initial culturing of immature zygotic embryos; but proline addition to the MS medium did not result in such an increase (Green, Armstrong and Anderson 1983). Initially proline levels of 0, 3, 6, and 9 mM were tested but, since the 9mM level yielded the highest frequency (14.5% of the cultured zygotic embryos produced embryoids), higher levels were tested. When concentrations of 0, 6, 12, 25, 50, and 100mM proline were included in the medium, the optimum level was found to be between 25 and 50mM, with the former level resulting in 63% embryoid formation and 27% of the cultured embryos yielding friable callus lines while the 50mM level resulted in 65% embryoid formation and 20% of the cultured embryos yielding friable callus lines (Green, Armstrong, and Anderson 1983).

Although Green and his co-workers have observed that the higher levels of proline are more effective for induction of the friable embryogenic callus, they have noted that the long-term maintenance of this type of callus can be obtained with a 6mM level and that its long-term maintenance at the 25mM level is questionable. They also noted that whereas their early experiments were conducted with media containing 0.5 mg/l 2,4-D, they sought to determine a 2,4-D level that would "prevent the advanced development of the embryoids in favor of proliferation of embryogenic callus" and found that not only did 1.0 mg/l produce the desired result, but that it also allowed for the maintenance of these lines by subculturing for more than a year (possibly longer) "without apparent loss in friability or embryogenic potential." (Green, Armstrong and Anderson 1983).

The friable embryogenic callus was observed to form well-dispersed suspension cultures when 2 g of friable callus was placed in 100 ml of liquid N6 medium containing 1.5 mg/l 2,4-D, 6mM proline and 0.1 M (.026 mg/l) abscisic acid (ABA). The resulting suspensions were subcultured weekly by a 10 fold dilution with fresh medium of the same composition. The inclusion of the ABA "was found to be very effective in maintaining culture homogeneity and stability." The cells in suspension remained mostly in clumps of 25 to 500 cells. When these cells were plated on solid N6 medium containing 1.5 mg/l 2,4-D and 6mM proline, they grew into a callus that was embryogenic and had the same properties as the original friable callus (Green, Armstrong and Anderson 1983).

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SYMPOSIUM

on

NON-PARAMETRIC APPLICATIONS TO RESEARCH

Presiding: David W. Smith

Mathematical Science Division, NDSU

Fargo, ND

43.* The Wilcoxon Rank Sum Test
William D. Slanger*
Animal Science Department, NDSU
Fargo, ND

44. A New Distribution for the Analysis of Correlated Bernoulli Data Jon R. Peterson* Statistics Department, NDSU Fargo, ND

45. A Non-parametric Procedure for Ranking Laboratories
K. G. Janardan*
North Dakota State University
Fargo, ND

46. Non-parametric Statistics and Some Alternatives
Douglas H. Johnson*
U.S. Fish and Wildlife Service
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 $^{^{\}star}$ This number reflects the sequence of this presentation in the meeting program.

THE WILCOXON RANK SUM TEST

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Many statisticians consider the Wilcoxon Rank Sum Test (1) (WRS) the best nonparametric test for equivalent locations of two populations. The WRS test statistic is independent of the form of the parent sampled populations, but the theory (2) assumes that the distributional forms of the two populations are equal. This means the two sampled populations are assumed to have equal variances. It has been shown that nonparametric tests are inferior to tests that form a statistic that is approximately distributed as a Student's t and require synthesis of degrees of freedom (3). The form of the two populations does not have to be symmetric in order to use the WRS test. The Mann-Whitney (4) test statistic has a linear relationship to the WRS test statistic. The two tests are therefore equivalent and accounts for the procedure sometimes being described as the Mann-Whitney-Wilcoxon test.

The test is easy to conduct, either by hand for small sample sizes or with PROC NPARIWAY of SAS (Statistical Analysis System) software package. An example using Veterinary Science data will be given during the presentation. The n_1 observations from the first population and the n_2 observations from the second population are combined and jointly ranked. The test statistic T^2 (5) is the sum of the ranks for sample 1. A T that is either extremely small or large is evidence for rejection of the null hypothesis of equal location. The midrank method is easily applied to handle the problem of ties (5). A moderate number of tied observations seems to have little effect on the probability distribution (6). The critical value for various values of probability of type I error, n_1 and n_2 can be found in several sources (7,8,9,10). The test statistic T starts to approximately follow the normal distribution when both n_1 and n_2 are greater than 10. The test statistic can then be:

$$Z = (T - \mu_T)/\sigma_T$$
, where $\mu_T = n_1(n_1 + n_2 + 1)/2$ and $\sigma_T^2 = n_1 n_2(n_1 + n_2 + 1)/12$.

There is a correction to σ_T^2 if there are ties (5). The T statistic is symmetric about its mean. If tables are not readily available and a quick test is wanted, the normal approximation is probably accurate enough for most practical purposes when both n_1 and n_2 are greater than 5.

The WRS test can be efficient. The asymptotic relative efficiency of T relative to Student's t is never less than .864 (6).

The Kruskal-Wallis test (5) is an extension of the WRS test for more than two populations. Wilcoxon's Signed Rank (1,5) test is for analyzing paired data and is not the same as the WRS test.

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A NEW DISTRIBUTION FOR THE ANALYSIS OF CORRELATED BERNOULLI DATA

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A typical problem encountered in research is the following example from the Journal of Speech and Hearing, p. 532-562, September 1978. The experiment conducted by Frances J. Freeman, dealt with stimulating different laryngeal muscles during fluent, and stuttered utterances, and recording either a positive or a negative response. Since there are two muscle types and two sets of responses, there is a question of whether or not these responses are correlated. This paper is an attempt to model the distribution if a correlation exists.

Laryngeal Muscles

Subject	PCA (x ₁)	INT (x ₂)
1	1	0
2	0	1
3	0	1
4	1	1
5	1	0
Total	3	3

1 = positive response
0 = no response

An observation consists of either a 0 or 1 on each of the two variables. The p.d.f. can be set up in the following manner where x_1 is marginally binomial $b(1,p_1)$, and x_2 is marginally binomial $b(1,p_2)$.

The following p.d.f. has been derived to model this population

$$f(x_1x_2) = (1-p_1-p_2+p_{12}) \qquad (p_1-p_2) \qquad x_1(1-x_2) \qquad x_2(1-x_1) \qquad x_1x_2$$

$$f(x_1x_2) = (1-p_1-p_2+p_{12}) \qquad (p_1-p_2) \qquad (p_2-p_{12}) \qquad p_{12}$$

$$f(x_1x_2) = (1-p_1-p_2+p_{12}) \qquad (p_1-p_2) \qquad (p_2-p_{12}) \qquad p_{12}$$

Subject to: $0 \le p_{12} \le 1$ $p_{12} < p_{1} < 1$ $p_{12} < p_{2} < 1$ $p_{1} + p_{2} \le 1 + p_{12}$

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For a random sample of size n the likelihood function can be obtained in the usual manner. Sufficient statistics are obtained by utilizing the likelihood function and the factorization theorem. The completeness property can be shown by reducing the bernoulli distribution to a power series form, which is known to have a completeness property.

Estimates for the parameters are obtained by the method of maximum likelihood estimation and are given by $\hat{p}_1 = \sum x_1/n \qquad \hat{p}_2 = \sum x_2/n \qquad \hat{p}_{12} = \sum x_1x_2/n \ .$

The following tests of hypothesis were investigated: H_0 : $p_{12} = p_1 p_2$ H_0 : $p_1 = p_2$ H_A : $p_{12} \neq p_1 p_2$ H_A : $p_1 \neq p_2$

Likelihood ratio tests were obtained of the form -21N λ . This test is known to be a chi-square distribution with 2 degrees of freedom.

Other applications to this type of problem can be found in the Journal of Veterinary Medicine, December 1981, in a Paper on Pursuing a Practical Solution to Bovine Pinkeye, by George E. Staples and David W. Smith. The paper dealt with whether the presence of IBK along with other bacteria were correlated in some way thus causing this pinkeye condition.

A NONPARAMETRIC PROCEDURE FOR RANKING LABORATORIES

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- 1. To assure the quality of the analytical work done, quality control programs are instituted by some state environmental laboratories. A quality control program can serve many functions. It can provide the information on the relative performance of labs, measure the precision and/or accuracy of a procedure, and improve the validity of analytical data, among others. The first phase of such a program requires ranking of the labs in terms of precision and accuracy. For this purpose identical, unmarked, spiked and split samples are sent to the labs. True spike values are not revealed until after the results are received. Since each lab analyzes and reports only one value per spike (e.g. inorganic metal), the distribution of the concentrations can not be determined. Thus, no parametric procedure can be used.
- 2. Since the data set of concentrations constitutes a multivariate sample from each lab, a statistical technique for representing the sample in a small number of dimensions, ideally in a single dimension, is desired. Thus, a procedure which maps several observations from a single laboratory into the small closed interval 0 to 1, so that the existing differences in accuracy and precision for each element are still preserved, is developed into a single index. This index combines optimally all the parameters (metal concentrations) in such a way that it provides a single valued function of the standardized distance of each observation from the known spiked value. The index values of the 'm' labs are then ranked to determine the hierarchical rating.
- 3. Assume that the data consists of ℓ mutually independent n (m+ 1 spike) variate random variables $(X_{i1}, X_{i2}, \ldots, X_{in})$, for i = 1,2,..., ℓ . The random variable X_{ij} stands for the value of the analysis for the i-th metal performed by the j-th lab (i = 1,2,..., ℓ , j=2,3,...,n). The first observation X_{i1} (for i = 1,2,..., ℓ) stands for the spike value. Let R_{ij} = $R(X_{ij})$ be the rank of the observation X_{ij} , from 1 to n, within row (metal) i. That is, for metal i the observations $X_{i1}, X_{i2}, \ldots, X_{in}$, including the spike value, are compared with each other and the rank 1 is assigned to the smallest observed value, the rank 2 to the second smallest and so on to the rank n which is assigned to the largest observation within each row. Ranks are assigned in this way to all observations within each row. If the observations within a row (metal) are tied, then tied observations are given the average rank of the observations assuming no ties.

Let R_j denote the sum of the ranks in the j-th column (lab). That is

$$R_j = \sum_{i=1}^{n} R_{ij}$$
, for $j = 2, 3, ..., n$.

The variance of the data ranks within each row is computed using the formula

$$V(R_i) = \sigma^2 (R_i) = [n^3 - n - \sum_k (t^3 - t_k)] / 12n$$
, for $i = 1, 2, ..., l$,

where n is the number of observations plus the spiked value for the i-th metal, t_k is the number of observations in a tie of the k-th type. k may be 0 or 1, or 2, ..., or n.

Let the standardized variate Z be defined as

$$Z_{ij} = [R_{ij} - R_{is}] / \sigma(R_i)$$
, for $i = 1, 2, ..., l$ $j = 2, 3, ..., n$

where $R_{i\,j}$ is the rank of the i-th value in the j-th lab, $R_{i\,s}$ is the rank of the spike value for the i-th metal, and $\sigma(R_i)$ is the standard deviation of the data ranks for the i-th metal.

Compute

$$P_j = \sum_{i=1}^2 P_j$$
 , for $j=2,3,\ldots,n$. Then the index C_j for the j-th lab is calculated as

$$C_j = [D_j/(R_j+D_j)]^{1/2}$$
.

This index is a random variable which can take any value from 0 to 1. Smaller the value of C_j higher is the precision and accuracy of the analysis of the samples by the Lab j. Thus, the value of C_j can be looked upon as a quantitative measure, in a single dimension of over all precision and accuracy. The values of the index for several labs can then be ranked to determine the hierarchical rating of the laboratories in terms of their precision and accuracy.

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Nonparametric procedures have been variously defined. Under the strictest definition, they include only methods that do not refer to any parameter involved in the distribution of the data. The broadest usage of the term encompasses any method that relaxes assumptions required by the corresponding parametric (usually normal) procedure. Most nonparametric methods are somewhat less efficient than their parametric counterparts when the data meet the more stringent assumptions of the latter, but are less misleading when assumptions fail.

Nonparametric procedures are not always the most appropriate, however, even for data that fail to meet the assumptions of parametric methods, because (1) the assumptions of nonparametric techniques are not as weak as is often thought, and (2) nonparametric methods are not as well suited for estimation and model-building as they are for hypothesis testing. In many applications cost is a concern and an investigator must know the magnitude, as well as the statistical significance, of a treatment effect so the treatment can be evaluated in terms of its cost. In this context, the parameter--which nonparametric methods attempt to evade--is a vital concern.

The primary purpose of this paper is to discuss some alternatives to nonparametric statistics. The first is simply to employ the usual methods based on the normal distribution. Many of them are, for large samples, robust with respect to nonnormality, by virtue of the central limit theorem. A second alternative is to transform the data in such a way that the critical assumptions are more nearly met and then proceed with the parametric analysis. Transformations can be invoked for any of several purposes, and can be chosen either from theoretical considerations or after examining the data themselves. The third and final alternative is to employ robust methods. These encompass a variety of techniques designed to reduce the influence of outlying observations, thus making them "robust" with respect to errors, aberrant data points, or misspecifications of the underlying distribution. They achieve their goal usually by transforming the data or by giving certain data points less weight.

For many applications, particularly those involving ordinal data, the most appropriate method is a nonparametric one. This situation does not always hold, however, and parametric procedures may enhance the value of an analysis. Transformations and robust methods are two ways of facilitating a parametric analysis.

SYMPOSIUM

on

THE HUDSON BAY ARCHIVES AS A SOURCE OF GEOGRAPHICAL DATA

Presiding: Douglas C. Munski

Geography Department, UND

Grand Forks, ND

57. * Archives and Geography: Interpreting Past Landscapes Through Historical Records

D. C. Munski*

Geography Department, UND

Grand Forks, ND

58. Changes in the General Circulation of the Atmosphere in the 1760's at Churchill and York Factory, Nanitoba

T. F. Ball*

Geography Department, University of Winnipeg

Winnipeg, Manitoba

59. Assessing Andrew Graham's Observations on Hudson's Bay, 1767-1791 as a Form of Evidence in Historical Geography

D. C. Munski, A. Stone, and R. Keys*

Geography Department, UND

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60. Hydrologic Applications of Archival Data

W. F. Rannie*

Geography Department, University of Winnipeg

Winnipeg, Manitoba

61. Examining Materials Produced by the Champlain Society for the Hudson's Bay Record Society as an Example of Studying Evidence Itself in Historical Geography

D. C. Munski and A. Asbeck* Geography Department, UND

Grand Forks, ND

62. Hudson's Bay Record Society Publications as Springboards for Analysis of Macro-Historical Geography of Post-1870 Settlement of Manitoba

D. C. Munski and B. Kontz* Geography Department, UND

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* This number reflects the sequence of this presentation in the meeting program. ARCHIVES AND GEOGRAPHY: INTERPRETING PAST LANDSCAPES THROUGH HISTORICAL RECORDS

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Although there is much controversy concerning what constitutes modern historical geography (5), one can assume that Sauer's admonition that initial reconstruction of past landscapes involves the use of archives is a methodology that remains at the heart of this sub-discipline of human geography (6). While mastery of the written records of the past is used for a variety of purposes within current trends of historical geography study, Harley contends that a tradition is lacking within historical geography for editing archival materials for research and teaching activities (4). This reflects what Guelke has pointed out concerning the need for historical geographers to become more versed in the concepts and methodologies of social science (2). Thus, Harley's point that historical evidence should be a focus of theoretical investigations by historical geographers provides a springboard for examining historical records in their own right as a source of data for interpreting past landscapes (4).

When examining the nature of archival material, the historical geographer would be well-advised to assess those items in the following contexts: 1) form of the evidence; 2) type of control; and 3) availability of use. When considering the form of the evidence, one should refer to the general categories of archives: 1) manuscripts; 2) cartographic and related records; 3) still pictures and slides; 4) motion pictures; 5) sound records; and 6) machine-readable records (1). Under the category of manuscripts there are such items as correspondence, diaries, and related written material. Surveyor's notes, architectural drawings, and contemporaneous maps would be classified as being cartographic-oriented forms of evidence. Air photos and LANDSAT imagery would be considered as still photographs while motion pictures and videotapes would be categorized together in a separate fashion. Oral histories having inhabitants describe environments as remembered would come under the topic of sound records. Machine-readable records are of increasing importance; computerized census data is a major example, but it must be pointed out that many items that have been considered manuscripts, e.g., ships' logs, railway timetables, parish records, and various business and government documents are on microfilm/microfiche.

Type of control over records underlies type of control over archives. Three forms of control exist: 1) personal; 2) corporate; and 3) governmental. Government agencies generate materials at three different levels with jurisdiction being local, state, or federal in general. Thus, records which are produced become archives if retained and processed. Yet, once these items are forwarded to a repository, there is an alteration of the nature of that control, especially in terms of availability.

Availability of archival material generally is made through a formal repository. Often times personal, corporate, and governmental records will be found in the same repository. This reflects the nature of the disposition of archival material by the donator. In the United States there are the following repositories: 1) the National Archives and presidential libraries; 2) state and local public archives; 3) business and labor archives; 4) college and university; and church archives. Depending upon the nature of the historical geographer's topic, it will be necessary for the researcher to consult a variety of materials from different sources in numerous repositories.

Whenever any data is used from archival sources, historical geographers must be cautious. How accurate are the materials and do they reflect overall patterns is a point made by Hall (3). Oftentimes, data is missing or has been presented in the first place to present a particular position with deliberate distortions included by the contemporaneous author. Thus, historical geographers must view archival material in terms of what Harley has described as contact, code, and context (4). With these reservations in mind, archives serve historical geographers as the foundations upon which to reconstruct past geographies, validate historic preservation activity, and generally interpret past land-scapes.

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CHANGES IN THE GENERAL CIRCULATION OF THE ATMOSPHERE IN THE 1760'S AT CHURCHILL AND YORK FACTORY, MANITOBA

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Unlike Europe or Asia there are relatively few historical sources of climatic information in North America. The Hudson's Bay Company Archives provides one of the few long term comprehensive sources of historical climatic material which includes weather diaries, daily journals, and instrumental records that are some of the earliest in North America (1).

A content analysis methodology was used to categorize the range and type of climatic variables that occur in the records. A coding system was developed that provided quantitative data for analysis of long term variations in the climate. The records for Churchill and York Factory, Manitoba, were coded and analysis of the frequency of occurrence and the first seasonal occurrence of climatic events provide a picture of a very variable climate from 1714 AD to 1852.

A very significant change in the frequency of rain events, the percentage frequency of winds and the number of days with thunder indicate that a significant change in the general circulation occurred in the 1760's. The pattern of changes are those that would be expected with a shift in the mean summer position of the Arctic Front. They are also the changes that would mark the changes related to the end of the Little Ice Age.

The frequency of rainfall events, that is the total number of occurrences of rain of all types, show that Churchill and York Factory both have similar patterns prior to 1760. After that year there was a dramatic increase in rainfall events at York Factory while Churchill showed no change. Prior to 1760, during the Little Ice Age, both locations were north of the mean summer position of the Arctic Front and experienced tundra type conditions. After 1760 the Arctic Front moved to a more northerly position between Churchill and York Factory so that the latter was in a boreal forest climate (2).

An increase of rainfall is one change that would be expected with a transition to a boreal regime but it is not, on its own, evidence of a change in circulation. Support for the hypothesis is found in an increase of southerly winds, an increase in the number of heavy rainfall events and an increase in the number of days on which thunder and lightning was observed. The latter are climatic events associated with an increase of southerly flow and the related increase of instability in the atmosphere. It would appear that this reflects the warning accompanying the end of the Little Ice Age resulting in a movement of the Arctic Front. In the 100 years after 1760 the climatic pattern showed a great deal of variability, particularly in the period from 1800 to 1840, but York Factory did not return to the tundra climate it had shared with Churchill prior to 1760.

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ASSESSING ANDREW GRAHAM'S OBSERVATIONS ON HUDSON'S BAY, 1767-1791 AS A FORM OF EVIDENCE IN HISTORICAL GEOGRAPHY

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One of the most controversial sources of contemporaneous data for studying the historical geography of Hudson Bay during the late eighteenth century is the materials produced by Andrew Graham. Because of its overall value in providing general information and personal opinion from one of Hudson's Bay Company's prominent Bay-side officers, it is appropriate to follow Harley's suggestion on examining evidence in its own right as an object of theoretical studies by historical geographers (1). Such a project is possible in large measure because of the availability of Andrew Graham's Observations on Hudson's Bay, 1767-1791 as issue 27 in the series of printings of archival material from the Hudson's Bay Company archives by the Hudson's Bay Record Society (3).

Because Andrew Graham's Observations on Hudson's Bay, 1767-1791 has been printed in an edited form, use of this Hudson's Bay Record Society publication to conduct a genetic study of evidence, or what Harley cites as the study of processes by which evidence is created itself (1), becomes a less than satisfactory exercise in scholarship. Instead, it is more appropriate to use this modified data base as a starting point to conduct either a static study of evidence (retrieving data on key time periods for critiquing contemporaneous landscape interpretation) or a dynamic study of evidence (examining data with respect to the impact which the original document had upon its initial audience). This paper emphasizes a static study of Andrew Graham's Observations on Hudson's Bay, 1767-1791 with its focus upon Section IX, "Life and Trade in the Bay"; particular attention has been given to Graham's commentary and descriptions of the posts and life at the posts. Jakle's approach to studying images of the Ohio Valley is used as the conceptual framework for this analysis (2).

In order to assess Andrew Graham's Observations on Hudson's Bay, 1767-1791, it first is necessary to consider that:

"Although his (Graham's) own service was limited to the Bay-side posts of Churchill, York and Severn, Graham was an avid collector of information from officers at the more southerly posts, from the (Hudson's Bay) Company's inland traders, and not least from the Indians; and this volume reflects the breadth of his interests (3)."

Furthermore, one must remember that:

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"Some of Graham's entries are repeated from volume to volume of the 'Observations,' but others differ in length and content according to the date of writing. A short paragraph in one volume may swell to several pages in a later; new information appears and disappears; revisions are made, and then apparently unmade; some of the volumes are dated, others not (3)."

Fortunately, "editorial policy in Section IX has been to print Graham's own comments, regardless of which volume of the 'Observations' they appear in (3)," a boon for the researcher attempting to interpret what was to be found at each post in terms of physical and cultural elements as well as the lifestyle at each post. Graham's descriptions of the posts at Prince of Wales (Churchill), York, Albany, Severn, Henley House, Moose Factory, and East-Main emphasize the transportation accessibility, fortifications, and population of each site. Life at the posts, as described by Graham, centered upon maintaining trade, keeping warm and reasonably well-fed, and daily adventures.

Using Jakle's approach to studying the images presented by Graham reveals that the typology of images has the following hierarchy: general images of Hudson Bay, images of individual posts as defensive sites, images of individual posts as commercial nodes, and images of individual posts as frontier residences. Such a typology is useful in beginning to assess the nature of the evidence presented in Andrew Graham's Observations on Hudson's Bay, 1767-1791.

- Harley, J.B. (1982) In Period and Place: Research Methods in Historical Geography (Baker, Alan R.H.B. and Billings, M., eds.), pp. 261-273. Cambridge, New York
- 2. Jakle, J. (1977) <u>Images of the Ohio Valley</u>, pp. 3-20. Oxford, New York
- 3. Williams, G., ed. (1969) Andrew Graham's Observations on Hudson's Bay, 1767-1791, pp. ix-xi, 242-256, 292-315. Hudson's Bay Record Society, London

HYDROLOGIC APPLICATIONS OF ARCHIVAL DATA

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For much of western North America, analysis of long-term streamflow fluctuations is frustrated by short data bases and historical sources are increasingly being used to determine conditions in the pre-instrumental period. The Red River valley is particularly well-suited to such an analysis because it lies astride a significant bioclimatic transition zone where climatic changes are liable to be strongly expressed and because it has a long history of settlement and abundant archival materials. Several types of archival information may be recognized; while the categories outlined below are not intended to be exhaustive or mutually exclusive, they illustrate the nature of observations which may be of use in hydrological reconstruction.

1. General observations which characterize a particular season.

"The travelling was tedious from the heavy rains . . . In many places we found several feet of water; every little hollow formed a pond and every rivulet appeared like a river. Our horses often sunk up to their knees in mud and at times had water up to their bellies . . . The water (of the Red River) was very high . . . They attempted to go there but found the country almost entirely overflowed." (Alexander Henry travelling north from Pembina on July 7, 1806.)

As these observations indicate conditions which seem abnormal, they become more interesting, although care is necessary to avoid placing undue emphasis upon them.

- 2. <u>Observations of discrete, irregular events</u> of importance. Most commonly, such observations yield only binary data (either they occurred or did not occur), but occasionally sufficient detail is included to permit estimates of magnitude or rank order.
 - ". . . the S. Branch or proper Red River was so overflowed that Mr. Henry's House in the Pabina was under water for 28 days the bed of the River generally 5 feet deep was increased to 55 & the Country on both sides deluged forming a Lake thro' its whole Course of about 8 miles wide . . . " (W. Auld to A. Wedderburner, Oct. 5, 1811.)

Observations of stages in the great floods of 1861, 1852, and particularly 1826 enabled discharge to be estimated, thereby improving the design information on which damage reduction measures in Winnipeg were based. Other floods in 1776, 1798, 1809, 1815 and 1850 have been noted by several writers although the commonly-cited 1809 event probably refers to a flood in 1811, apparently of great magnitude as the quotation above indicates. Closer examination of archival sources reveals that overbank flow at Pembina also occurred in 1801, 1806, 1825, 1827, 1828, 1849 and 1851. Noteworthy are the June-July floods in 1806, 1825, 1850 and 1851 since modern data at Emerson suggest a Return Period for summer bankfull flow in excess of 60 years. The reporting of observations in this category is influenced by what Gerard and Karpuk (1) called the 'perception level'--that is the threshold above or below which an observer is likely to comment on a phenomenon. For example, a frost is likely to be mentioned only if it has an effect on vegetation or crops.

"On the 8th of June, 1836, a severe frost killed most of the barley and cut down the wheat, and on the 19th of August of the same season, a very heavy frost so injured the wheat that it was not even fit for seed." (Peter Garriock, Red River Settlement.)

- 3. Recorded data. Instrumental records are rare and generally of short-term. Nevertheless, even a short record may yield significant information. Gunn measured an astonishing 28.6 inches of precipitation in July and August, 1855, at Fort Garry, an amount which could be dismissed except that extreme depths were also recorded at Lac Qui Parle, Minnesota.
 - 4. Phenological observations which indicate the timing of recurring natural events.

 "The ice being now sufficiently strong people cross the (Red) river without danger." (J. Smithhurst, Red River Settlement, Oct. 29, 1843.)

 servations of this sort have great potential in providing a proxy data base which may

Observations of this sort have great potential in providing a proxy data base which may be related to climatic variables. For example, breakup and freezeup dates of the Red River at Winnipeg assembled by the writer from archival sources (2) indicate a median 19th Century freezeup date 12 days earlier and breakup date 10 days later than in the 20th Century, implying average spring and fall temperatures about 2.5 °C cooler. Early freezeup, late breakup, and cool spring temperatures have been widelycited as major factors contributing to flooding.

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EXAMINING MATERIALS PRODUCED BY THE CHAMPLAIN SOCIETY FOR THE HUDSON'S BAY RECORD SOCIETY AS AN EXAMPLE OF STUDYING EVIDENCE ITSELF IN HISTORICAL GEOGRAPHY

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Sauer, in his seminal work, "Foreward to Historical Geography," emphasized that investigation of past cultures should focus upon the use of primary sources; he listed land surveys, parish records, diaries, letters, and tax records as among the types of materials that historical geographers should be using while conducting research at appropriate archives (4). Access to such repositories, however, may be difficult for many researchers engaged in field work beyond their immediate environs. Thus, publication of archival materials are of great value for aiding historical geographers in focusing research projects on specific topics in various regions. Of particular importance for historical geographers studying past landscapes in the hinterland of the Hudson's Bay Company are items published jointly by the Champlain Society and by the Hudson's Bay Record Society.

Since its establishment in 1670 through to the mid-nineteenth century, the Hudson's Bay Company has had a significant impact upon the historical geography of much of North America. Operating over a wide territory and in time periods in which record management was not a high priority, the primary sources for information on the regions served by the Hudson's Bay Company as well as documents relating to the firm itself were scattered. In 1920 the archives of the Hudson's Bay Company were consolidated in London as part of the 250th anniversary of the firm (1). Yet, prior to 1924, archival materials were merely in storage and unavailable to all but invited researchers; the problem of accessibility was compounded by moving the repository in its entirety from its site in London to different facilities in London in 1924 and again in 1927 (2). However, interest in making documents from the archives of the Hudson's Bay Company existed among the executives of this firm. As Gower pointed out,

"In the spring of 1928 the Board decided to publish some of the most interesting journals and other documents relative to the period of Sir George Simpson, and for the next three years the services of the archives staff were concentrated on the collection and preparation of material to that end. This entailed a more detailed examination and arrangement of the archives, especially those relating to the period of 1821-1860, than had previously been undertaken (2)."

Consequently, the repository was moved to better facilities in 1932.

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Accessibility to materials from the archives of the Hudson's Bay Company improved considerably during the Great Depression, a condition that was promoted by the Champlain Society. As early as 1913 the Champlain Society, a rare books publication consortium, had suggested that materials from the Hudson's Bay Company archives be published for public use, but it was not until the 1930's that this could be accomplished (5). Thus, Journal of Occurances in the Athabasca Department by George Simpson, 1820 and 1821, and Report, was published for the Hudson's Bay Record Society by the Champlain Society in 1938 as the first of 12 volumes of primary sources from the Hudson's Bay Company archives to be disseminated in this cooperative fashion. The last jointly sponsored work was the volume, James Isham's Observations on Hudson's Bay, 1743, and Notes and Observations on A Book Entitled A Voyage to Hudson's Bay in the Dobb's Galley, 1749, published in 1949. These 12 volumes, when examined as sources of evidence in themselves using Harley's approach in historical geography (3), reveal a diversity of usefulness for pursuing themes of historical geography as suggested by Sauer in biogeography, cultural geography, and political geography (4). Thus, historical geographers should recognize the limitations of such materials but continue to use them and begin to analyze them as evidence in themselves for gaining greater insights into historical geography understandings as cited by Harley (3).

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- 2. Gower, R.H.G.L. (1933) The Beaver, 264-265., 40-42.
- 3. Harley, J.B. (1982) In <u>Period and Place:</u> Research Methods in Historical Geography (Baker, Alan R.H. and Billinge, M., eds.), pp. 261-273. Cambridge, New York
- 4. Leighly, J., ed. (1969) <u>Land and Life: A Selection from the Writings of Carl Ortwin Sauer</u>, Pp. 351-378. University of California, Berkely
- 5. Wallace, W.S. (1934) The Beaver, 264-265., 40-42.

HUDSON'S BAY RECORD SOCIETY PUBLICATIONS AS SPRINGBOARDS FOR ANALYSIS
OF MACRO-HISTORICAL GEOGRAPHY OF POST-1870 SETTLEMENT OF MANITOBA

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Moodie and Lehr have demonstrated that the archives of the Hudson's Bay Company provide a key data base in the creation of macro-historical geography (4). Yet, access by many researchers to the repository of those documents in Winnipeg may be limited for a variety of reasons. Funding for travel to archives as a part of field work by students as well as by faculty members is not a high priority item in many academic institutional budgets. Thus, printed archival items on Hudson's Bay Company's role in settling Manitoba can be found in publications from the Hudson's Bay Record Society. While many of the volumes in that series provide selected data on pre-1870 settlement in what is today Manitoba, two volumes are focused upon post-1870 settlement. Both volumes are collections of letters of Charles John Brydges, the second Hudson's Bay Company Land Commissioner (1,2).

Charles John Brydges was one of the most influential figures in developing the settlement pattern of Manitoba during 1879-1889 in the sense that he discharged his duties in the sale of Hudson's Bay Company reserves with a greater vision than a simple land speculator. First, it must be remembered that in the Dominion Lands Act of 1872

"In each township, the (Hudson's Bay) Company was to receive section eight, section twenty-six in each township with a designation number divisible by five, and the southern half and northwest quarter of section twenty-six in all other townships. The government, in other words, established a policy by which free homestead land alternated with land expected to provide a revenue for the railway and profits to the Hudson's Bay Company (2)."

Yet, as Bowsfield points out,

"To the irritation of some of the (Hudson's Bay) Company's officers, particularly Chief Commissioner James A. Grahame, he (Brydges) he involved himself in the renovation of the Company's general trade and transport systems both of which, he believed, had to be modernized to satisfy the demands of homesteaders and pioneers of the new towns on the Company townsites and along the route of the transcontinental railway (1)."

Thus, Brydges' correspondence is of particular value to historical geographers interested in reconstructing past landscapes in Manitoba during 1879-1889. His letters provide insights into behaviors of a leader of a business firm that had an important part in organizing and shaping the geographical personality of a particular region of Canada. Access to such documents can provide data for what Johnston has cited as the behavioral approach in historical geography (3). Such a conclusion can be drawn because

"...Brydges saw earlier than the (Hudson's Bay Company Executive) Board, the emergence of a western community with which the Company must not only come to terms but even to assume responsibility and leadership. His forcefulness was innate; his confidence was founded on years of practical management and participation in civic affairs in Canada. His boldness between 1879 and 1882 came of the knowledge of his secret mandate to displace Smith and Grahame. Armed with this sense of the Board's confidence, he seldom hesitated to set his mark upon every aspect of the Company's operation and of the environment in which it could flourish. He operated from instinct and mandate as if he were Chief Commissioner, not Land Commissioner—and as if it were his task to create a new image of the Hudson's Bay Company as a citizen of the Canadian Northwest (1)."

Therefore, Brydges' correspondence provides the base for studying land transactions as part of macrohistorical geography for the post-1870 settlement of Manitoba.

- 1. Bowsfield, H. (ed.) (1977) The Letters of Charles John Brydges, 1879-1882, pp. ix-x, 1xxiv. Hudson's Bay Record Society, Winnipeg
- Bowsfield, H. (ed.) (1981) The Letters of Charles John Brydges, 1883-1889, pp. xi-lxii. Hudson's Bay Record Society, Winnipeg
- Johnston, R.J. (1983) Geography and Geographers, 2nd edition, pp. 140-144. Edward Arnold, New York
- 4. Moodie, D.W. and Lehr, J. (1981) Can. Geog. 25, 267-271.

SYMPOSIUN

on

MATHEMATICS-SCIENCE EDUCATION IN NORTH DAKOTA HIGH SCHOOLS: Where Are We And Where Are We Going?

Presiding: L. Elliot Shubert

Biology Department, UND

Grand Forks, ND

80. * Hathematics and Science Education in North Dakota: A Status Report

on Curriculum and Teachers

Vito Perrone*

Center for Teaching and Learning, UND

Grand Forks, ND

81. Mathematics and Science Education in North Dakota: Student Achievement

Ron Torgeson*

ND Department of Public Instruction

Bismarck, ND

82. Nathematics and Science Education in North Dakota: An Administrator's

Perspective

Richard Hill*

Center for Teaching and Learning, UND

Grand Forks, ND

Panel Discussion With Panelists

Clair Bergene, Principal

Kindred High School, Kindred

Mike Burton, Teacher

Fargo High School, Fargo

Steve Fogarty, Teacher Jim Hill Junior High School, Minot

Dan Hoff, Teacher

Velva High School, Velva

Kathy Kadrmas, Member

District Board of Education, Kindred

David Looysen, Teacher

Jim Hill Junior High School, Minot

Niomi Phillips, Past-President

Board of Education, Grand Forks

Glen Schimke, Superintendent

Wahpeton School District, Wahpeton

This number reflects the sequence of this presentation in the meeting program.

MATHEMATICS AND SCIENCE EDUCATION IN NORTH DAKOTA: A STATUS REPORT ON CURRICULUM AND TEACHERS

Vito Perrone*
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Over the course of the past year, there have been numerous national reports deploring the state of mathematics and science education. To a large degree, the reports suggest increased math-science requirements in the schools, closer monitoring of academic achievement, and better teacher preparation. Public interest in mathematics and science education has, as a result of the reports, enlarged. This is to be valued. But before too much public policy activity occurs or too many final judgments are made, especially here in North Dakota, we ought to examine closely the current status of mathematics and science education in the schools. My focus in the Symposium relates to curriculum and teachers.

In regard to curriculum, almost <u>all</u> secondary schools in North Dakota make available introductory coursework in Physical Science (essentially a ninth grade course in earth science), Biology, and Chemistry. Further, an introductory course in Physics is offered in approximately seventy percent of the secondary schools. In contrast, only twenty-five percent of the secondary schools—for the most part, the State's largest schools—offer any <u>advanced</u> coursework in the Biological or Physical sciences. In the area of mathematics, almost <u>all</u> secondary schools offer the Algebra I, Geometry, Algebra II sequence; however, only forty-five percent offer any advanced mathematics (Trigonometry, pre-Calculus or Calculus). In general, the opportunities for students are considerably greater in the large high schools (those with over 400 students) than in the small high schools. This, of course, is a longstanding issue in North Dakota, and not exclusively in the math-science fields.

To outline the existence of courses is only one way of describing math-science in North Dakota, or, for that matter, anywhere else. One might also consider enrollment patterns, laboratory facilities, and teacher preparation. In regard to enrollment, approximately twenty-three percent of all students (1983) are enrolled in or have completed Algebra II and sixteen percent of all seniors (1983) are enrolled in an advanced mathematics course. Virtually all ninth graders and tenth graders in North Dakota (1983) are enrolled in Physical Science and Biology respectively. In contrast to the universality of these courses, only twenty-one percent of the juniors and seniors (1983) are enrolled in Chemistry and nine percent (1983) in Physics. In addition, seven percent are enrolled in an advanced science course, generally in the biological science field. While we might wish for more, these figures are similar to those reported for the nation as a whole (National Assessment, 1983). In regard to instructional processes, North Dakota teachers are, like their counterparts across the country, tied heavily to textbooks and an information-giving orientation. Inquiry as an instructional mode is not commonplace. Laboratory facilities are mixed, in general being better equipped in the larger schools. In regard to teacher preparation, fewer teachers in North Dakota hold a graduate degree than is the case nationally. In the math-science area, principally because of the size of North Dakota's high schools and the need for teachers capable of teaching in two or more fields, many teachers, especially in the smaller schools, have composite majors (science) rather than subject majors (Physics or Chemistry). This represents a limitation.

There is a need for ongoing programs to support the State's math and science teachers, to encourage their continued growth in pedagogy as well as academic background. Support mechanisms appear, however, to be limited.

We are in a position, especially with the heightening of public interest, to give greater attention to the areas of mathematics and science in the schools. Yet, we ought not to be too quick with our remedies, and whatever is done should be thoughtfully conceived, viewed as long-term, and must draw upon teachers and administrators in the public schools. It would also help if math and science faculty in the State's higher education institutions were more deeply involved in the life of the schools.

MATHEMATICS AND SCIENCE EDUCATION IN NORTH DAKOTA: STUDENT ACHIEVEMENT

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Over the course of the past year, there have been numerous national reports deploring the state of mathematics and science education. To a large degree, the reports suggest increased math-science requirements in the schools, closer monitoring of academic achievement, and better teacher preparation. Public interest in mathematics and science education has, as a result of the reports, enlarged. This is to be valued. But before too much public policy activity occurs or too many final judgments are made, especially here in North Dakota, we ought to examine closely the current status of mathematics and science education in the schools. My focus in the Symposium will relate to the achievement of North Dakota students on a variety of standardized measures.

North Dakota elementary and secondary students have participated in a variety of standardized testing programs in mathematics and science over the past decade. While standardized tests--whether norm or criterion referenced--have serious limitations, they can be useful in status studies and curriculum reviews.

In general, North Dakota students in the elementary and secondary schools score on all standardized tests well above national norms. In grades 3, 5, and 7, on the Iowa Test of Basic Skills, North Dakota students have had increasing scores over the past decade. In mathematics, the scores have risen from the sixtieth percentile to the seventy-sixth percentile. On the Test of Academic Proficiency, given over the past three years to 9th and 11th graders, scores in math have increased from the seventy-ninth percentile to the eighty-third percentile among 9th graders and from the seventy-first percentile to the eighty-first percentile among 11th graders. Similar performance directions in mathematics exist for students participating in the SRA (Science Research Associates) testing program at grades 9 and 11. On this measure, 9th and 11th graders have moved from the sixtieth percentile in 1980 to the seventieth percentile in 1983. While mathematic's scores on the foregoing norm referenced tests have increased substantially, math scores for North Dakota students taking the ACT have declined over the past decade. In 1973, the average math score was 19.1 (slightly above the national average); in 1983, the average math score was 15.8 (slightly below the national average). ACT scores in science have remained, in contrast, stable. In 1973, the average science score was 21.5; in 1983, the average score was 21.0. These science scores are above the national average for each of the years. In regard to the ACT, it should be noted that a much higher percentage of North Dakota juniors and seniors take the ACT than is true nationally. In addition, it is significant to note that the decline in scores among females is considerably greater than the decline among males.

In addition to these measures, the State Department sampled in 1978 and 1981 a population of North Dakota students in grades 4, 8, and 11 on a criterion referenced test which made use of items from the National Assessment program. Science and math scores at all three levels were above the national average.

A review of test data does not suggest a crisis in mathematics and science. But the scores ought not to cause complacency. The tests don't measure validly everything that is important to continued success in mathematics and science; for example, critical thinking, logical reasoning, creativity, etc. To engage in the kinds of discussion about achievement that have the potential to move us beyond where we are, we need to give more attention to purposes and to assessment activities that are consonent with those purposes.

MATHEMATICS AND SCIENCE EDUCATION IN NORTH DAKOTA: AN ADMINISTRATOR'S PERSPECTIVE

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Over the course of the past year, there have been numerous national reports deploring the state of mathematics and science education. To a large degree, the reports suggest increased math-science requirements in the schools, closer monitoring of academic achievement, and better teacher preparation. Public interest in mathematics and science education has, as a result of the reports, enlarged. This is to be valued. But before too much public policy activity occurs or too many final judgments are made, especially here in North Dakota, we ought to examine closely the current status of mathematics and science education in the schools. I will concentrate my attention in this Symposium on what the schools can and ought to do in the face of current concerns about mathematics and science education. My response grows out of my long experience as a school administrator and as a person actively involved in preservice and inservice programs relating to school administrators.

At the outset, it is critical to acknowledge the school administrator's need to view education broadly, to provide for all students the fullest educational opportunities possible. Within this particular outlook, math and science are important but they are no more important than English, foreign language, social studies, health, and the arts.

In North Dakota, given the fiscal constraints and the geographic isolation and rural nature of many of our school communities, it is a struggle to offer a well balanced, high quality academic and extracurricular program. Some schools, because of size, location, taxing capacity, and community support have organized exemplary programs in many fields including mathematics and science. A larger number of schools, however, have been able to offer only a minimal basic program, essentially thirty or fewer units. Our challenge is to seek ways of assisting these schools to offer a broader and higher quality program. This might mean more cooperative, cross-district activities as well as increased involvement of the State's higher education institutions. It might also demand more school consolidations. But school consolidation, often seen as a simple solution, is not likely to occur, nor should it, on any large scale.

A question that has been debated in North Dakota for the whole of the century revolves around teacher preparation. Should the colleges and universities insist on traditional subject matter majors exclusively or should they provide less traditional composite majors? Given the typical size of our schools, a person with a major in Physics—though acknowledged as well prepared—is not likely to be as attractive as a person with a composite major in science, able to teach courses in General Science, Physical Science, Biology, and Chemistry. This needs to be understood. Because we have in North Dakota a number of teachers who teach in several areas, the needs for staff development are particularly high. Unfortunately the opportunities don't match well the needs.

Further, it needs to be acknowledged that science is a high cost activity, especially if carried out well with a strong laboratory base. While some progress was made in the late sixties and early seventies in updating science laboratories, in large measure as a result of federal support, there has been an erosion in quality in the past decade, particularly as the electronics and optics fields have become more central to some areas of science. To bring science laboratories and the related equipment to an advanced stage, consonant with the best thought that exists in the area, would take far more resources than can be generated from current state and local tax resources. School administrators must address these needs as sensitively as possible but they face other fiscal pressures as well.

There are no simple roads to high quality math and science education in North Dakota. While we have come far in our efforts, we clearly cannot rest where we are.

SYMPOSIUM

on

RECENT DEVELOPMENTS IN ENGINEERING ELECTROMAGNETICS

Presiding: David A. Rogers

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND

83. Engineering Electromagnetics in 1984

David A. Rogers*

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND

Reduction of Intermodulation Distortion in Optical Fiber Communication 84.

Systems

Donald A. Smith*, Gary L. Larson, and Guocong Chen

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND

Design of Medium Power Nicrostrip GaAs Fet Amplifier Using High 85. Dielectric Networks

Behrooz Resvani and Banmali Rawat*

Department of Electrical Engineering, UND

Grand Forks, ND

Analytical Methods for Dispersion Minimization in Single-Mode Fibers 86. David A. Rogers* and Guocong Chen

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND and Wuhan Research Institute of Posts and

Telecommunications, Hubei, China

Survey of Recent Results for the Singularity Problem in the 87.

Electromagnetic Integral Equation

Douglas B. Miron*

Department of Electrical Engineering, SDSU

Brookings, SD

88. Modal Investigation of Striplines Via a Spectral-Domain Variational Nethod

Bing H. Liu*

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND

89. Measuring the Receiving Pattern of an Electrically Short Antenna Using

Natural Electromagnetic Fields as a Source

James C. Rogers* and Charles T. Young

Electrical Engineering Department and Geology and Geological

Engineering Departments, Michigan Technological University

Houghton, MI

90. Potential Calculations for Floating Conductors with Axial Symmetry

R. M. Nelson* and R. G. Ölsen

Electrical and Electronics Engineering, NDSU

Fargo, ND and Department of Electrical Engineering

Washington State University

Pullman, WA

91. Harmonic Returns from Zero-Bias Detector Diodes

D. J. Krause*

Department of Electrical and Electronics Engineering, NDSU

Fargo, ND

This number reflects the sequence of this presentation in the meeting program.

ENGINEERING ELECTROMAGNETICS IN 1984

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Since the time of the writing of George Orwell's <u>1984</u> in 1948, electromagnetics has been a steady contributor to the growth and development of electrical engineering both as a discipline in colleges and as a source of ideas and devices for business and technology. Throughout those years most electrical engineering students have taken at least one course in electromagnetic field theory and many have studied related courses in transmission lines, microwave devices, propagation, and antennas.

These courses, for the most part, have been a permanent fixture in the curricula of our institutions. Research, however, has been a more volatile area, with funding being very heavily dependent on the condition of the national economy. Most areas of electromagnetics, however, have maintained a reasonable growth. Radio science continues to be a productive area although the strongest interest probably was in the 1960's. The federal government continues to maintain good efforts in radio astronomy and for the nuclear electromagnetic pulse problem. Microwave engineering and fiber optics is heavily financed by private industry with hundreds or perhaps thousands of companies making an investment in these areas. The basic electromagnetics research of the future may be in the area of device interactions, as the development of microelectronics approaches near-atomic size limits.

Even though many engineers doing electromagnetics have managed to experience decades of forward progress, some have witnessed the gradual smothering of their discipline and concerns by developments in other areas. This may be seen in the growth of solid-state electronics, digital electronics, and computer engineering over the last 25 years. Many radio scientists have entered the area of solid-state electronics since the mathematical tools they are familiar with are useful in this area. A very fine microwave engineering professor of 20 years ago at a major west coast university subsequently became a leader in computer science. Many, seeing the growth potential outside of electromagnetics, have made similar moves. Nationally, few undergraduates engage in serious study of electromagnetics beyond what is contained in required courses. The majority of electrical engineering students seems to gravitate toward areas that are at least tangentially related to computer engineering.

This has been recognized nationally in articles in the last few years in the newsletters of the various IEEE societies in the areas of antennas and propagation, microwaves, and education. The IEEE Microwave Theory and Techniques Society has discussed supporting academic efforts in electromagnetics, but they have not been able to establish an appropriate mechanism. Some schools recently have made a renewed commitment to electromagnetics education. A notable national example is the program at the University of Massachusetts. The universities represented by the presentors of the papers in this symposium have expressed their support of this discipline by the employment of individuals committed to quality programs in electromagnetics.

Dr. James C. Rogers is making important contributions in the VLF area and in basic research at Michigan Technological University. In addition to his research, Dr. Douglas B. Miron has introduced a senior elective in microwaves at South Dakota State University. Dr. Banmali Rawat has made significant improvements in both the graduate and undergraduate offerings at the University of North Dakota. The remaining presentors are from North Dakota State University. Four of them (Nelson, Smith, Liu and Rogers) actively teach engineering electromagnetics courses. Two of them (Liu and Rogers) are specialists in electromagnetics. Nelson is a doctoral student in electromagnetics who also teachs in computer engineering. Smith is a solid-state electronics specialist whose research bridges both areas. Krause is a communications theorist whose research interests involve electromagnetics applications.

The symposium presentors demonstrate the capability this region has for contributions to electromagnetics and the expertise available within the region's universities for support of high-technology industries engaged in microwave and fiber optics device development or the study of electromagnetic effects in any electrical or electronic systems where the concern may range from device interactions due to electromagnetic fields to the effects of fields on human beings or other life forms.

Engineering electromagnetics in the upper midwest is alive and well in 1984. Its health is demonstrated by the communications that follow in this Symposium on Recent Developments in Engineering Electromagnetics.

REDUCTION OF INTERMODULATION DISTORTION IN OPTICAL FIBER COMMUNICATION SYSTEMS

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The light emitted from a light-emitting diode (LED) depends on the current through the LED. The nonlinearities in this relationship cause intermodulation distortion in multichannel communication systems. By reducing intermodulation distortion an improved received signal-to-noise ratio may be obtained. Investigations have been made to characterize the nonlinear relationship in LED modulation characteristics. Methods of compensation of the nonlinearities have been implemented and evaluated.

The characteristics of LED's from several manufacturers were measured. Regression analysis, which minimizes mean square error, was used to mathematically describe the characteristics obtained. It was found that a sixth order polynomial adequately described the LED characteristic. From this mathematical description of the characteristic, intermodulation distortion due to the nonlinearity may be calculated. This calculated intermodulation distortion compares favorably with measured results. In good quality multichannel television, it is desired to keep intermodulation distortion at -45 dB or lower with respect to the modulation signal. Of the light-emitting diodes considered, this would limit modulation depths to around twenty percent.

One method of compensation of the nonlinearity of the LED investigated is the use of predistortion. A network which is complementary to the LED's nonlinearity can be used in series with the input. The exact complement of the LED characteristic may be very difficult to realize. To simplify the analysis and the compensating network, a piece-wise linear network using diodes and resistors was used. Breakpoints and slopes of the characteristic of the compensating network may be controlled by adjusting resistances. A simple compensating network is one which employs a single breakpoint in its characteristic. For a single breakpoint compensating network, the location of the breakpoint is best predicted at the same location as the dominant breakpoint of the LED characteristic, that is, where the change in slope of the characteristic is greatest in magnitude. This is represented by the maxima of the second derivative or zeroes of the third derivative of the LED characteristic. If a fourth order best fit curve is used to describe the characteristic, only one breakpoint is predicted. Experimental measurements using compensation networks with a single breakpoint showed a reduction in intermodulation distortion of 6 dB to 15 dB for most LED's. This is sufficient compensation to keep intermodulation levels to -45 dB at 50% modulation. This allows a significant increase of signal-to-noise ratio over the 20% modulation limit without compensation. If the single breakpoint approach does not adequately increase the linearity of the system, a two or more breakpoint network can be implemented.

A second method of compensation in order to reduce intermodulation distortion which was investigated is the use of a quasi-feedforward technique. This approach uses two matched LED's in the transmitter. A modulating signal is supplied to the first LED. The optical signal emitted from the first LED is detected and amplified. This output, which contains both the signal and distortion, is subtracted from a reference signal yielding an error control signal. The error control signal is combined with the original modulating signal giving the signal predistortion. The predistorted signal is used to modulate the second LED. If the two LED's have similar characteristics the distortion introduced by the first LED is cancelled by the distortion introduced by the second LED. Experimental measurements using this approach showed a reduction of 15 dB to 20 dB in intermodulation distortion.

The two approaches for the reduction of intermodulation distortion in optical fiber communication systems that were investigated have both shown encouraging results. In both cases the reduction of intermodulation distortion is sufficient to allow at least 50% modulation depths to be utilized while keeping intermodulation to -45 dB or lower.

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DESIGN OF MEDIUM POWER MICROSTRIP GaAS FET AMPLIFIER USING HIGH DIELECTRIC NETWORKS

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In the design of microstrip GaAs FET amplifier, the input and output matching networks are the most important and critical parts to design. The use of conventional microstrip matching techniques becomes ineffective as transmission line widths become large and result in dispersion effects. But the use of dielectric substrates with high dielectric constants, high Q and high temperature stability [1] for matching networks makes the microstrip configuration quite suitable.

For the design of microwave amplifier the most important parameters are bandwidth, noise figure and power handling capability of the device. A simple design technique of a microstrip GaAs FET amplifier has been developed with the use of high dielectric (a ceramic Ba2Ti9020, $\epsilon_{\rm r}$ =40) networks for inputoutput matching and biasing circuit. The design was carried out for a center frequency f₀=10GHz, with more than 8dB gain over the entire frequency band (8-12GHz) and approximately 3dB noise figure with 30dBm output power.

For the design of input and output matching networks the broad band theory has been used. At microwave frequencies, the reverse transmission scattering parameter S_{12} of GaAs FET is very small, therefore the unilateral mode (S_{12} =0) can be used. With the unilateral model the transistor input and output matching networks can be designed separately to match the input impedance of the transistor to its generator resistance and its output impedance to the load.

As shown in Fig. 1 the scattering matrix of a loss less (reactive) matching two-port network which is normalized to the passive load $Z_L(j\omega)$ on the right and to the resistor R_S on the left is given as,

given as,

$$S(j\omega) = \begin{bmatrix} S_{11}(j\omega) & S_{12}(j\omega) \\ S_{21}(j\omega) & S_{22}(j\omega) \end{bmatrix} -----(1)$$

The transducer power gain can be given as,

$$G(\omega^2) = |S_{21}(j\omega)|^2 = 1 - |S_{22}(j\omega)|^2 -----(2)$$

where $S_{22} = \frac{Z_2(s) - Z_L(s)}{Z_2(s) + Z_L(s)}$

Thus if $Z_L(s)$ and $G(\omega^2)$ are known, then $Z_2(s)$ can be determined. The response $G(\omega^2)$ can be selected to have a nonsloped (flat) response within the pass band or to have a sloped response with roll-up to compensate for the roll-off of the transistor. For determining the idealized situation for the formulas used in the design, the maximum unilateral gain $G_{u,max}$ is examined over the desired band using the relation [2],

$$G_{u,max} = |S_{21}|^2 \frac{1}{1-|S_{11}|^2} \cdot \frac{1}{1-|S_{22}|^2}$$
 -----(3)

 $G_{u,max}$ at 6GHz is found to be 14.85dB and at 12GHz as 7.04dB, resulting in a gain roll-off of about 5.81dB/octave which is close to 6dB/ octave. Now $G(\omega^2)$ can be selected to have a nonsloped response within the pass-band or to have a sloped response with roll-up of 6dB/ octave to compensate for the roll-off of the transistor. This is to be used for both input and output matching networks. The GaAs FET used for the design was HFET-1000 from Hewlett-Packard with stability over the entire band. Moreover the FET was in chip form suitable for a simplified unilateral model. The maximum unilateral gain of FET was above 8dB over the entire band. For biasing the GaAs FET the standard microstrip configuration with Series/Shunt $\lambda/4$ sections have been used.

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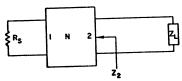


Fig. 1 Two-Port matching network.

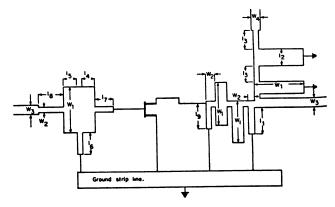


Fig. 2 Microstrip configuration of Ga As FET amplifier.

ANALYTICAL METHODS FOR DISPERSION MINIMIZATION IN SINGLE-MODE FIBERS

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Pires et al. (1) developed a method for exact calculation of the wavelength, $\hat{\lambda}$, for minimum dispersion in a single-mode fiber based on solution of the total dispersion equation. Following slightly different procedures, an approximate method for fiber design based on the wavelength of an available light source has been developed (2-3). In this work we completed the process by showing an exact solution for the design problem and an approximate method for finding $\hat{\lambda}$ based on an independent solution of the total dispersion equation. Comparisons have been made between the various cases showing the consistency of results and the accuracy of the methods. In each major case, a full circle of analysis was made. For example, the $\hat{\lambda}$ result of the analysis method was used in the design procedure to obtain \hat{a} , and this core radius was found to be virtually identical to the radius used in the analysis procedure.

The method developed for analysis of pulse dispersion in single-mode optical fibers with step-index profiles (1) is based on solutions of the exact characteristic equation. In this work, through an exact solution to the total dispersion equation for the core radius when the wavelength assigned was assumed to be that necessary for minimum total dispersion, we found the optimum value of the radius of the core. A computer program (FORTRAN IV, double precision) was implemented, the inputs of which were the wavelength and the coefficients of Sellmeier's three-term equation for the refractive indexes of the core and cladding.

There are two core radius solutions corresponding to a single wavelength. The normalized frequency is either V < V_p or V > V_p for each radius ($V_p \approx 1$). The computational program is designed so that the initial value of V can be set to either 0.6 or 1.0. For each given wavelength, these two values provide two different radius values. The full range of normalized frequencies is 0.6 < V < 2.5.

In solving the exact characteristic equation several subroutines in the International Mathematical and Statistical Library (IMSL) were used. Muller's method was used to solve the transcendental equations. Necessary derivatives were computed by the IMSL subroutine DRVTE. Finally, Muller's method was used again to solve the total dispersion equation to compute the required value of the core radius, â. The study made involved wavelengths in the 1.45 - 1.55 μm range yielding core radii in the 2.45 - 1.94 μm range.

In the approximate solution for the analysis problem the approximate formula for the parameter u, which was proposed by Miyagi and Nishida (2-4), is used in the total dispersion equation. For a given value of core radius a, one can find the desired laser source wavelength, $\hat{\lambda}$, for minimum total dispersion. Due to the approximate procedure used, the range of the normalized frequency is confined to V > 1.0. Core radii in the 1.3 - 2.0 μ m range yielded wavelengths in the 2.01 - 1.52 μ m range.

To verify the consistency of the results and the accuracy of the methods, in each case a full circle of analysis was made. For example, the $\hat{\lambda}$ result of the analysis method was used in the design procedure to obtain a, and this core radius was found to be virtually identical to the radius used in the analysis procedure. For the approximate methods, the programs are in single precision, and the relative difference after a full circle is on the order of 10^{-5} . For the exact method, the programs are in double precision giving a relative difference that is on the order of 10^{-11} .

Comprehensive analysis and design procedures are now available for a large class of single-mode, step-index optical fiber problems. System planners may select the method that best matches their available computing facilities and satisfies the computational precision requirements for the problem at hand.

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87 SURVEY OF RECENT RESULTS FOR THE SINGULARITY PROBLEM IN THE ELECTROMAGNETIC INTEGRAL EQUATION

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The integral for electric intensity is valid everywhere except in the source region. The second derivatives of g(R) force the integral to diverge as R goes to zero. Starting about twenty years ago, as described by Yaghjian[1] in 1980, it was found that, if a small region around the observation point is excluded from the integral, a convergent and correct result could be obtained for the volume case by treating the integral as a Principal Value problem, and finding a correction term, as in (1). $\overline{L(J(r))}$ is obtained by surface integration around the excluding region.

$$\overline{E}(\overline{r}) = \frac{1}{j\omega\epsilon_0} PV \int_{V} [(\overline{J}(\overline{r'}) \cdot \nabla)\nabla g(R) + \beta^2 \overline{J}(\overline{r'})g(R)] dV' + \overline{L}(\overline{J}(\overline{r}))$$
(1)

The correct result is obtained when the excluding region is shrunk to zero, keeping its shape constant. Shortly thereafter, Lee et.al. [2] published (2a), which yields a correct result for finite excluding volume. In 1983 Miron [3] published (2b) for surfaces. In these formulations, u, v refer to an orthogonal coordinate set. I is a typical scalar component of the integral. For (2a), N is the outward normal to the surface $_{\rm u,v}$ S(V) surrounding the excluding volume, V . For (2b), the conducting surface S is a coordinate surface of the system. n, tangent to S is the outward normal to the contour C(S_0) around the excluding region, S_. u and v refer either to the same coordinate or to two successive coordinates in a right-handed sequence.

$$I_{uv} = \int_{V-V_0} J(\overline{r}') \frac{\partial^2 g}{\partial u \partial v} dV' + \int_{V_0} (J(r') \frac{\partial^2 g}{\partial u \partial v} - J(\overline{r}) \frac{\partial^2 g}{\partial u \partial v}) dV' + \frac{-J(\overline{r})}{4\pi} \int_{S(V_0)} (\hat{V} \cdot \hat{R}) (\hat{U} \cdot \hat{n}) \frac{dS'}{R^2}$$
(2a)

$$I_{uv}(S) = \int_{S-S_o} K(\overline{r}') \frac{\partial^2 g}{\partial u \partial v} dS' + \int_{S_o} (K(\overline{r}') \frac{\partial^2 g}{\partial u \partial v} - K(F) \frac{\partial^2 g}{\partial u \partial v}) dS' + \frac{-K(\overline{r})}{4 \pi} \int_{C(S_o)} (\hat{v} \cdot \hat{R}) (\hat{u} \cdot \hat{n}) \frac{d1'}{R^2}$$
(2b)

Some study of specific cases leads to the conclusion that (2b) generalizes to (3). The line integrals of the cross-coupling terms go to zero.

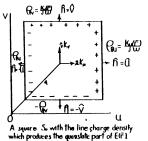
$$\begin{split} \overline{E}(\overline{r}) &= \frac{1}{\mathrm{j}\omega\varepsilon_{0}} \left\{ \int_{S-S_{0}} \left[(\overline{K}(F') \cdot \nabla) \nabla g + \beta^{2} \overline{K}(\overline{r}) g \right] dS' + \int_{S_{0}} \left[(\overline{K}(\overline{r}') \cdot \nabla) \nabla g - (\overline{K}(\overline{r}) \cdot \nabla) \nabla g - (\overline{K}(\overline{r}) \cdot \nabla) \nabla g \right] \right\} \\ &+ \beta^{2} \overline{K}(\overline{r}') g dS' - \frac{1}{4\pi} \int_{C(S_{0})} \left[\widehat{U}K_{U}(\overline{r}) (\widehat{U} \cdot \widehat{R}) (\widehat{U} \cdot \widehat{n}) + \widehat{V}K_{V}(\overline{r}) (\widehat{V} \cdot \widehat{R}) (\widehat{V} \cdot \widehat{n}) \right] \frac{d1}{\rho^{2}} \right\} \end{split}$$

$$(3)$$

Physical interpretation of specific cases leading to (1) was given in 1977 by K-M Chen [4]. He showed that the correction term is due to the electric field in V produced by the quasistatic surface charge density equivalent to J(r). This field is finite even when V goes to zero. The same reasoning applied to (3) shows that the contour integral is the electric field from the line charge density equivalent to K(r). The figure shows this for a square S_0 . The surface integral over S_0 is the contribution to E(r) from all the current except K(r).

An essential difference between the formulations in (1) and (3) is that, in numerical implementation, (1) requires that the excluding region be identified with the integration cell, whereas (3) and (2) allow the excluding region to be any size. This is particularly important for the surface case, because the first and last terms contain cancelling singularities which grow as S shrinks. To avoid this, one maintains S at some convenient size while the integration cell is made smaller for more accuracy. For closed surfaces, S_0 may be made equal to S, removing the first and third integrals from (3).

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In various applications, modal properties of guided modes of planar stripline structures can be characterized by means of dispersion curves, which relate the propagation coefficients of guided modes to the operating microwave frequencies as well as other physical and geometrical parameters. From the point of view of their derivation, these relationships are transcendental and quite complicated, often including the necessity of solving integral or integrodifferential equations numerically. However, from the viewpoint of their final results as represented by the dispersion curves, only very smooth curves are usually involved. This observation seems to lend the hint that simpler methods may be used as the zeroth-order approximation to the dispersion relations for the guided modes. In this report, a spectral-domain variational method for this purpose will be presented and illustrated with the aid of single and coupled strip lines buried in a two-layer medium as depicted in Fig. 1. The configurations correspond namely to the case of a covered microstrip line and the case of edge-coupled strip lines.

Starting from Maxwell's equations in the Fourier transform domain (or the "spectral domain"), exact hybrid-mode equations can be obtained:

$$j \omega (Z_{11}I_z + Z_{12}I_x) = F_z$$

 $j \omega (Z_{21}I_z + Z_{22}I_x) = F_x$ (1)

Here F_z , F_x are tangential electric field components at the interface containing the conductor strip(s) and I_z , I_x are surface current densities on the strip(s). Note that the Z_{ij} contain the information of the layered medium. Note also that they are real and symmetric (i.e. $Z_{12}^{=Z_{21}}$), so that variational principles, or their equivalents, can be formulated quite straightforwardly in the spectral domain.

To do this, the functionals M and N are first introduced:

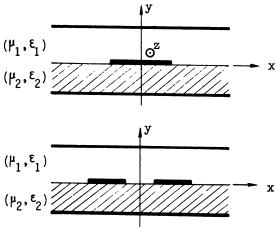


Fig. 1 Examples of planar striplines

$$M(I_{z}, I_{x}) = \int_{-\infty}^{\infty} (Z_{11} I_{z}^{*} I_{z} + Z_{12} I_{z}^{*} I_{x}) dp$$

$$N(I_{z}, I_{x}) = \int_{-\infty}^{\infty} (Z_{21} I_{x}^{*} I_{z} + Z_{22} I_{x}^{*} I_{x}) dp$$
, (2)

both of which have the stationary value of zero in case that I $_{z}$ and I $_{x}$ are true solutions to equations (1). Here $_{p}$ is the spectral domain variable. If the $_{z}$ unknown currents now assume a small variation $_{z}$ around the true solutions such as

$$I_{za} = I_z + si_z, \qquad I_{xa} = I_x + si_x, \qquad (3)$$

where s is real, i and i are some "suitable" functions in the spectral domain, and the subscript a denotes assumed x current functions. By substituting (3) into (2) and investigating the stationary condition $\partial M/\partial s = \partial N/\partial s = 0$ at s = 0, criterions for i , i to be suitable and, hence, those for I can be established. These include suitable asymptotic behavior at $|P| \rightarrow \infty$. In

$$\int_{2}^{\infty} Z_{12} (I_{z}^{*} i_{x} - I_{x}^{*} i_{z}) dp = 0$$

is obtained, which takes into account the layered structure of the medium in question. As application examples, the stripline configurations of Fig. 1 will be discussed. Further applications of the present method will also be pointed out.

MEASURING THE RECEIVING PATTERN OF AN ELECTRICALLY SHORT ANTENNA USING NATURAL ELECTROMAGNETIC FIELDS AS A SOURCE

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The radiation pattern of an electrically short antenna in an isotropic environment can be determined from elementary antenna theory. If the environment is anisotropic the pattern can differ significantly from the isotropic case; in particular, ELF communication antennas can have their pattern shifted several degrees from the isotropic case. It is expensive and time consuming to measure the far field pattern of an ELF antenna. The measurements reported here require only minutes of measurement time at the antenna terminals and avoid the need for extensive far field measurements.

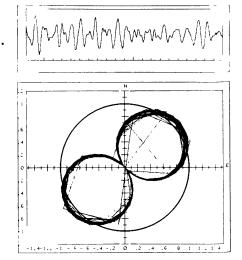
A magnetotellurics (MT) system, normally used for geophysical measurements, is capable of simultaneously measuring three orthogonal components of magnetic field and two horizontal orthogonal components of electric field at the surface of the earth (1). The bandwidth of the Michigan Tech MT system is $384~\mathrm{Hz}$ to $.8~\mathrm{milli}$ Hz and it has been used for deep earth studies of value for siting low frequency antenna systems (2)(3). Natural sources, lightning and magnetic storms, provide significant signals at these frequencies. By measuring two orthogonal components of magnetic field at the surface of the earth, one can deduce the apparent direction, frequency content and amplitude of electromagnetic waves traveling from these transient sources. This information, when coupled with a simultaneous measurement of voltage at the antenna terminals of interest, is sufficient to determine the antenna receiving pattern. Our measurement used two orthogonal horizontal induction coils to measure H and H (north and east directed magnetic fields). If the signal source is far away the traveling field can be treated approximately as incident plane waves and their direction of travel can be determined by simple trigonometry. By narrow band filtering the three signals, $\mathrm{H_x}$, $\mathrm{H_y}$ and the antenna voltage, it is possible to prepare a polar antenna pattern at a particular frequency. The pattern is constructed from the antenna voltage normalized by the total magnetic field amplitude, which is plotted at the direction determined by tan^{-1} ($\mathrm{H_x/H_y}$).

A test of the method has been conducted on a small loop antenna consisting of approximately 20,000 turns around a mumetal core which was placed with its magnetic axis oriented 30 degrees west of north. The sampling rate was set to three Hz, and 10,000 samples representing about 30 minutes of time were recorded. About 14 minutes of time series data are plotted at the top of the figure. They have been band-pass filtered with a center frequency of 0.03 Hz and a Q of 3. The pattern calculated is presented in the figure. Since not all incident natural signals are sufficiently strong to overcome background and system noise, we set a signal threshold above which the pattern plotting computer routine makes a plot. Thus the routine may start and stop several times as it displays the filtered time series. Lines such as the two that form a large x in the NE quadrant are artifacts of this process. Also, the pattern indicated has not been compensated for differences in the response of the H_x and H_y channels. However, these are of secondary importance and it is judged that the utility of the method is demonstrated.

Antenna measurements have been made at the US Navy's ELF antenna test facility. We have observed the terminal voltages on two approximately orthogonal grounded dipole antennas which are about 12 and 14 miles long respectively. A more sophisticated pattern analysis routine, based upon standard MT response algorithms, has been used to determine a preliminary pattern that is in good agreement with independent pattern measurements of the antennas. A pattern skew of several degrees from

the isotropic case is indicated. The work on this project has been supported by the US Navy Underwater Systems Center under Contract Number N00140-84-M-LG22. We acknowledge the valuable support of Edward Wolkoff for initiating and guiding the project.

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POTENTIAL CALCULATIONS FOR FLOATING CONDUCTORS WITH AXIAL SYMMETRY

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Engineers who design high voltage power systems need to know how the electric potential field distribution will be affected by the design of the system. Using this knowledge, the engineer can design a system which will minimize the energy loss through corona processes on the line. Knowing the potential field distribution will also help the engineer to quantify the exposure of operating personnel to high electric fields.

Many methods have been used to determine the electric potential distribution in high voltage systems. These methods include using resistive analog circuits, flux plotting, or making measurements on models in electrolytic tanks. In more recent years, computers have been used to obtain theoretical solutions to this problem by using various numerical techniques, such as the finite difference and finite element techniques, simulation techniques, Monte Carlo methods, and others. Criteria which are used to compare these methods include such items as computer time needed for solution, computer space needed, and the accuracy of the numerical solution. Another method which is used to solve Laplace's equation for the electric potential distribution is the integral equation technique. In this method, the electric potential is expressed as a surface integral over unknown surface charges. An integral equation is obtained by setting this integral equal to known values of potential on electric conductors. The integral equation is then solved for the unknown surface charge densities by approximating the integral as a sum over small surface elements with assumed uniform charge densities. This sum is then set equal to the known potential at the center of each surface element, yielding a set of linear algebraic equations which can be solved by standard matrix techniques. Once the charge densities are known, the potential and/or electric field at any point can be determined.

The integral equation technique described above can only be used to solve problems which have conductor-dielectric boundaries and on those boundaries the potentials need to be known. Olsen and Daffe (1) extended this method to include problems that have dielectric-dielectric boundaries by deriving a pair of coupled integral equations for the real and polarization charge at all interfaces. These integral equations can be solved numerically for rotationally symmetric electrostatic problems.

The theory and computer program developed by Olsen and Daffe is limited, however, to problems in which the potential is known on all conductor-dielectric boundaries. Often in electrostatic problems, conducting bodies are at a 'floating potential', i.e., the potential is an unknown constant, and the total charge on the conductor is known. This condition may happen for a cap and pin of a set of insulators near the center of an insulator string, where the potential is constant but unknown and the total charge (usually zero) is known. In our study, the work of Olsen and Daffe was extended to be able to solve rotationally symmetric electrostatic problems for which three types of interfaces are allowed: conductor-dielectric interfaces where the potentials are known, dielectric-dielectric interfaces, and conductor-dielectric interfaces where the potential is constant but unknown, and the total charge is known. In the third case, the total charge is assumed to be zero. Four coupled integral equations are derived using the fact that a unique solution to Laplace's equation in the region can be found if one of the following conditions holds on every part of the boundary: the potential is known, the normal derivative of the potential is known, or the potential is constant but unknown, with the total surface charge known (which is assumed zero). A set of linear algebraic equations can be obtained by requiring the appropriate boundary condition to be satisfied on each surface. These linear equations can then be solved for the unknowns, which are the surface charges and the constant but unknown conducting potentials. Using the values of the charges, the potential and/or electric field can be calculated at any point in the region.

The results of the work were checked by solving problems which also have a closed-form solution. One problem checked was a set of four concentric conducting spheres, separated by two different dielectric media, where two of the spheres had known potentials and two of the spheres had constant, but unknown potentials. For the geometry chosen the unknown potentials were calculated to be 152 volts and 105 volts, and the closed-form solution gave potentials of 153.66 and 105.86 volts on these spheres. Another problem solved with both the computer and with a closed-form solution was that of a disk capacitor with three conducting plates which were separated by two different dielectric media. The top and bottom plates were at a known potential, and the middle plate was at a constant, unknown potential. For this problem, for a set geometry and given values of plate voltages, the unknown plate voltage was calculated to be 59.8 volts, which compared favorably with the 60 volts obtained by a closed-form solution

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HARMONIC RETURNS FROM ZERO-BIAS DETECTOR DIODES

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Zero-bias Schottky detector diodes when used in conjunction with an appropriate antenna can be used as a tag to monitor insect movement. The tag is identified and the insect's position monitored by measuring the harmonic return from the diode tag. Tags of this form can be made small since the device is passive and does not require a power source other than what is received by the tag at the fundamental frequency.

An experimental radar system (1) was developed to locate and monitor the movement of screwworm flies. The system was based on the detection of the second harmonic generated by a diode tag attached to the fly. The diode used was custom fabricated by Microwave Associates of Burlington, MA. The diode has no external enclosure and is a chip 0.5 mm square with two gold leads 0.03 mm in diameter bonded to the anode and cathode. The gold leads serve as a simple dipole antenna. A critical parameter that was missing for a complete analysis of the radar system was the amount of power returned from the diode tag at the second harmonic frequency. A measure of performance for the diode tag which yields the harmonic return power is the radar cross section. The experimental radar system was used to measure the maximum radar cross section for the diode tags.

The radar cross section, σ , of the diode tag is determined from the radar equation:

 $\sigma = \frac{-16\Pi^2 P_r R^4}{P_t G_t A r} \quad \text{where:} \quad \begin{array}{c} P_t = \text{ transmitted power} \\ G_t = \text{ transmitting antenna gain} \\ R = \text{ range of tag} \\ P_r = \text{ received power and } Ar = \text{ area of receiving antenna.} \end{array}$

For conventional radar systems, σ is independent of the power density at the target since the target is only serving as a reflector. In the case of harmonic returns from the diode, the cross section can be expected to be a nonlinear function of the power density. The cross section was not measured as a function of the tag's angular rotation. Only the maximum cross section was measured.

The fundamental transmitting frequency was 5 Ghz. Measurements of the cross section were made at two ranges of 50 ft with a power density of 0.2 mw/cm² and 100 ft with a power density of 0.05 mw/cm². The cross section of 17 diodes were measured and are tabulated in Table 1.

TABLE 1 RADAR CROSS-SECTIONAL AREA OF DIODE TAGS

Lead Lengths	Area at 50 ft (10 ⁻⁶ cm ²)	Area at 6 2.
(cm)	50 ft (10-cm-)	at 100 ft (10-6cm ²)
	,	
.75, 1.5	45	112
1.5, 1.5	18	28
.75, 1.5	111	45
1.5, 1.5	70	18
.75, 1.5	70	28
.75, .75	1	
1.9, 1.9	2	*
1.9, 1.5	7	*
.75, 1.5	111	6
.75, 1.5	111	11
.7, 1.4	*	*
.75, 1.5	176	178
.75, 1.5	176	71
.75, 1.5	35	45
.75, 1.5	279	178
.75, 1.5	111	56
.75, 1.5	176	178
.75. 1.5	176	56
.75, 1.5	176	355
.75, 1.5	351	71
.75, 1.5	70	224
.75, 1.5	18	-
	1.5, 1.5 .75, 1.5 1.5, 1.5 .75, 1.5 .75, .75 1.9, 1.9 1.9, 1.5 .75, 1.5	.75, 1.5

*Return undetectable

With the simple dipole antenna configuration used on the tag it is critical that the lead lengths be 1/4 wavelength. There was not a significant difference between leaving both leads $1/4\lambda$ of the fundamental or have one $1/4\lambda$ of the fundamental and the other $1/8\lambda$ of the fundamental. If both leads are cut to $1/8\lambda$, the cross section becomes small as indicated with diode #6. Reducing the leads slightly from $1/8\lambda$ and $1/4\lambda$ had a dramatic effect in that the return from the diode could not be detected as indicated with diode #8.

The nonlinear behavior of the diode tags differs substantially from diode to diode. Originally it was thought that the frequency doubling mechanism was from the square-law characteristics of the diode (2). The data obtained from diodes 9-20 do not verify this. A complete model of the frequency doubling depends on the nonlinear junction capacitance. This still does not account for the erratic differences in the measured cross section.

The measurements of the radar cross section indicate that zero-bias detector diodes with a simple dipole antenna can be used as suitable tags.

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Previous ice-margin correlations in north central North Dakota have been made only on the basis of geomorphic characteristics because of the lack of datable organic material (1). In this study, surface and shallow subsurface tills were sampled behind each defined ice-marginal position to test whether tills from different advances could be differentiated by textural and lithologic parameters.

The two areas selected were southwestern Ward County and the Minot area. Ice-marginal positions are considered to decrease in age from southwest to northeast in this region (1). Samples were collected from surface exposures, power-auger borings, and coal exploration rotary-drill test holes. Lab analyses of the till samples included texture (sand, silt, and clay percentages) and lithology of the very-coarse-sand (1-2mm) fraction, the more useful technique for differentiating tills of different advances. The parameters determined included percentages of grains in igneous/metamorphic, carbonate, and shale groups normalized to 100 percent (Table I). Lignite percentage in the very coarse sand

fraction, a qualitative indicator of till lithology, was also determined.

Till units in Table I are listed from oldest (bottom) to youngest (top). The surface till exposed

Till units in Table I are listed from oldest (bottom) to youngest (top). The surface till exposed in front (southwest) of the Blue Mountain ice margin in extreme southwestern Ward County has a smooth, low relief, erosionally modified surface suggesting that it is pre-Late Wisconsinan in age. Clayton and Moran (1) correlate this till with the Snow School Formation, a till postulated to be Early Wisconsinan in age (phase C). Blue Mountain till, northeast of the Blue Mountain ice-marginal position, has a fresh surface morphology little modified by erosion and a thick oxidized zone at the surface (2). The oxidized zone is traceable in the subsurface throughout the Missouri Coteau beneath younger till units (2). For example, a buried oxidized zone containing till of Blue Mountain lithology was encountered at a depth of 46m in a coal exploration test hole (NDGS N-4107) located about 20km northeast of the ice margin. The Blue Mountain till is considered to represent the first Late Wisconsinan ice advance into the area. The thick oxidized zone is interpreted to be the result of a major ice retreat followed by a dry non-glacial period during which southwestern Ward County had a water table deeper than present. Based on the surface topography and the large areal extent of the buried oxidized zone, the Blue Mountain till is correlated with phase D of Clayton and Moran (1) instead of their interpretation as phase E.

Mortheast of the Blue Mountain till, Pettyjohn (2) mapped the Makoti, Ryder, and Martin drift units. The Ryder and Martin units correlate approximately with till behind the phase I and K ice-marginal positions, respectively, of Clayton and Moran (1). The relatively few samples collected for this study from these three till units suggest that the Makoti, Ryder, and Martin tills can be differentiated by very-coarse-sand lithology. Oxidized zones were not present at the tops of the Makoti and Ryder tills when encountered in the subsurface beneath younger tills. Contacts between till units are commonly marked by boulder concentrations.

In the Minot area, numerous till samples were analyzed behind (Unit A) and in front of (Unit B) the phase L ice margin of Clayton and Moran (1). Although the texture and lithology of Units A and B are similar, differences between the mean values of the very-coarse-sand lithologies (Table I) are significant at the 95 percent confidence level. Unit B is present behind margin L below the contact with Unit A, which is characterized by a sand bed or boulder concentration. Further work is needed to determine whether Unit B is the till deposited by the ice advance to the phase K (Martin) margin.

Table I. Very Coarse Sand Percentages

	n	1g/i	et.	Car	b.	Sh		Li	g.
Unit		x	SD	x	SD	x	SD	x	SD
Α	63	68.5	4.3	28.3	7.3	3.3	1.4	0	0
В	94	74.3	4.1	21.3	3.8	4.3	2.5	3.8	6.2
Martin	4	49.9	5.5	28.7	4.3	21.4	3.9	4.1	2.2
Ryder	6	34.6	3.6	30.1	4.1	35.5	5.4	0.5	1.2
Makoti	13	40.9	8.2	22.8	2.4	36.3	9.1	7.7	5.6
Blue Mtn.	22	33.4	5.6	24.1	4.1	42.6	7.4	0.3	1.1
Snow School	6	43.6	5.0	35.0	3.0	21.4	3.2	*	*

* - abundant lignite in sample

⁽¹⁾ Clayton, Lee and Moran S.R., (1982) Quat. Sci. Reviews 1, p. 55-82.

⁽²⁾ Pettyjohn, W.A., (1967) North Dakota Geol. Surv. Misc. Series 30, p. 123-130.

INTERRELATIONSHIPS OF MAJOR, MINOR, AND TRACE ELEMENTS WITH SULFIDES IN A NORTH DAKOTA LIGNITE

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Sulfides are among the most important inorganic phases in coals because they can contain many elements that are of economic and environmental interest. The most common sulfides found in coals include pyrite, marcasite and melnikovite-pyrite. Often small quantities of sphalerite, galena, and chalcopyrite are present. The major sulfide found in Beulah lignite is pyrite. Both epigenetic and syngenetic pyrite are present.

Studies (1,2) have shown that pyrite begins forming syngenetically as small framboids during the peat stage of coal formation. Sulfur bacteria have an important role in the formation of pyrite and marcasite. Pyrite in peat evidently can form only by bacterial activity given in ample supply of sulfur and iron. Sulfur originates from bacterial protein or sulphate ions carried in solution groundwater carries in Fe ions.

The epigenetic sulfides are deposited in cracks and fissures during the second stage of the coalification process. The mineralization is brought about by the action of iron bearing solutions within the more porous portion of the coal body such as fusain.

Samples collected within a stratigraphic sequence at the Beulah mine, North Dakota were analyzed by a variety of analytical techniques including neutron activation, x-ray fluorescence, x-ray diffraction, and scanning electron microscopy. Examination of these data revealed high concentrations of many major and trace elements at 1.5 meters from the base of the coal seam. The elements found to be concentrated at 1.5 meters include: Se, Cd, As, Fe, Zn, La, Mg, I, Al, Cl, and Cu. Of these elements the following have chalcophilic characteristics: Se, Cd, As, Fe, Zn, and Cu. Results of the scanning electron microscope/microprobe study revealed that pyrite was found in the form of framboids averaging 20 to 30 microns in diameter and massive bands within the woody structure of the coal (3). The major coal lithotypes present at 1.5 meters include by weight 22.7% fusain, 77.3% vitrain, and 0% attritus. This sample has an unusually high concentration of fusain which is a charcoal-like lithotype. Finkelman (4) has reported a correlation between coal mineral and macerals indicating that the macerals concentrated in fusain have significant concentration of sulfides.

Investigation of the interrelationships of major, minor, and trace elements with coal mineral and lithotypes can be used to possibly predict a given element's association and its fate or impact during utilization.

- 1. Altschuler, Z.S., Schnepfe, M.M., Silber, C.C., and Simon, F.O. (1983) Science, Vol. 221, No. 4607, pp. 221-227.
- 2. Cecil, C.B., Stanton, R.W., Dulong, F.T., and Renton, J.J. (1982) Atomic and Nuclear Methods in Fossil Energy Research; Edited by R.H. Rilby, pp. 323-335, Plenum press, New York.
- 3. Zygarlicke, C.J. (1983) University of North Dakota Energy Research Center, Internal Report UNDERC/IR-6, pp. 2-4.
- 4. Finkelman, R.B. (1980) University of Maryland, Ph.D. Dissertation, pp. 83.

VOLCANIC FEATURES ON JUPITER'S SATELLITE, IC

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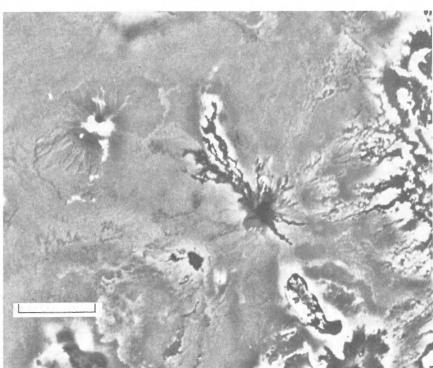
Study of volcanic features of Jupiter's satellite Io is part of a photogeologic mapping project supported by NASA through the Planetary Geology Program. Voyager spacecraft high-resolution imagery and geophysical data along with earth-based observations of chemistry and physics of Jupiter and its satellites have been used in a geologic mapping project of Kane Patera, a quarter-million square mile quadrangle in the southern hemisphere of Io.

Since the 1979 Voyager 1 and 2 encounters with Jupiter, Io, the innermost of its four planet-size satellites, has been singled out for special attention because of its volcanic activity. Io's reddish color and other spectral characteristics suggest a surface rich in several allotropes of elemental sulfur in black to red to brown lava flows, dust covered regions of yellow to brown sulfur and sulfates, and white sulfur dioxide frosted areas. Io's surface is young, free of meteorite impact craters, and probably renewed completely by volcanism every few million years. Eleven known volcanic eruptions occurred during, and between the four-month interval between Voyager 1 and 2 flights past Io. Huge sulfur volcanoes and calderas erupted large plumes shaped like umbrellas up to 170 miles high. Thermal centers up to 150°C hotter than the surrounding areas dot the surface of the planet corresponding generally with plumes or specific volcanic landforms. Io's intense volcanic activity results from the dissipation of internal heat produced by tidal flexing as alternating gravitational distortions change its shape while being pulled periodically out of circular orbit by sister satellites Europa and Ganymede.

Large calderas, two types of shield volcanoes, and isolated groups of flows are four major volcanic forms that occur within the Kane Patera quadrangle. Low-relief shield volcanoes typically have a central vent region 15-30 km in diameter and sulfur flows extending typically asymmetrically from the vents for 100-300 km. Talos Patera (Fig. 1) is an example. Initial study suggests that the volcano is a low broad cone with a shallow depression containing several craters at the top. Dome-shape shield volcanoes consist of a central vent 10-20 km in diameter surrounded symmetrically by sulfur-silicate (?) flows extending 50-150 km from the vent to a marginal scarp. Taw Patera (Fig. 1) is an example with nearly rounded form. Calderas are large depressions 50-200 km in diameter and are typically developed on low-relief plains and surrounded by thin surface deposits and low-relief flows. Vahagn Patera (Fig. 1) is an example. Isolated groups of flows are fresh-appearing elliptical regions of sulfur flow complexes about 350 km long and 150 km wide. These flows may have a fissure source or may have small or covered central vents.

Figure 1. The low-relief shield volcano, Talos Patera with related flows, is in the right-central part of the photo. Recent sulfur flows are darkest and have light margins or haloes of sulfur dioxide frost. The central vent region appears to consist of several irregular to rounded depressions consisting of elongate, black, filled fractured regions which may be lava. The dome-shaped volcano, Taw Patera, with related flows, is in the upper left part of the photo. The sulfur-silicate flows from Taw Patera appear to have steeper dips and higher relief than those of low-relief shields with sulfur flows. A scarp partly outlines the outer margins of the uppermost sulfur-silicate flows. The vent appears to be a small black crater with an asymmetrical bright halo of sulfur dioxide frost. A medium size caldera, Vahagn Patera, is partially shown on the lower margin of the photo. Bar equals 100 km.

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A TERTIARY SOURCE OF ARCHAEOLOGICAL RAW MATERIAL IN WESTERN NORTH DAKOTA

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An extensive lithic workshop and raw material procurement site was discovered near West Rainy Butte, Slope County, North Dakota in 1982. The site covers over 500,000 sq. m on a small ridgetop approximately 2 km southwest of West Rainy Butte. Pebble, cobble, and small boulder sized pieces of silicified material are scattered as probable erosional lag across the surface of the site. Numerous artifacts of the material include cores, flakes, pieces of shatter, and bifacially modified tools.

The material at the West Rainy Butte site is identified as silicified plant remains. Much of it appears to be silicified wood, although further specialized work is needed to determine accurately whether other types of organic precursors were involoved. The material is extremely variable in color, and to a lesser extent, in internal structure. Within a single square meter at the site, five different colors were present. These range from reddish brown (5YR 4/3) to dark reddish brown (5YR 2.5/2) to dusky red (10YR 3/2 and 2.5YR 3/2) to dark brown (7.5YR 3/2). In general, the specimens are waxy, aphanitic, and very dense, with the dominant colors being reddish brown and dark reddish brown. Many have visible yellowish brown streaks (10YR 5/8), which give the material a very "woody" appearance. Other specimens lack streaks and are macroscopically homogeneous in color.

The stone is opaque and very dense, especially compared to other common siliceous raw materials. Specific gravity was measured for 25 specimens of the West Rainy Butte material, and for 18 specimens from six additional material types. The samples from West Rain Butte had an average specific gravity of 3.09, which is noticeably higher than any of the others tested, which included Knife River flint, porcellanite, Antelope Chert, jasper/chert, and smooth grey Tongue River silicified sediment. All of the material other than West Rainy Butte exhibited a specific gravity of 2.50 or less (Table 1). Specific gravity may prove to be useful in identifying the material derived from West Rainy Butte.

Table 1. Specific gravity of selected raw materials.

Material Type	Number of Samples	Mean Specific Gravity
West Rainy Butte	25	3.09
Antelope Chert	10	2.48
Knife River Flint	3	2.44
Porcellanite	3	2.30
Jasper/Chert	1	2.47
Tongue River Silicified Sediment	1	2.50

Petrographic examination, utilizing thin sections, reveals relict plant structures replaced by chert, chalcedony, and iron oxide. The iron, revealed as the mineral goethite by x-ray diffraction, is dispersed as globules in a matrix of chert, while chalcedony fills growth ring fractures and vugs. Uniserate ray cells and tracheids, which are part of the plants vascular system, are also visible. Ting (1972:165) observed similiar woody plant structure in a silicified peat from the Sentinel Butte Formation in Billings County. However, the material identified by Ting was not suitable for stone tool manufacture (1).

1. Ting, F.T.C. (1972) Science 177, 165-166.

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Evidence of environmental changes since the last major glacial retreat of Wisconsinan, have been documented over much of North America. Antevs (1) presented a model based largely on his studies in western North America which represent what has been called an "indistinct transitional model". Bryson et al. (2) have introduced, in North America, the "episodic model" which uses terminology developed for northern Europe. In North Dakota several studies have recently helped further efforts at determining the nature of Holocene environmental change in the northern Great Plains (3,4). During 1981 and 1982, an Archeological site on the Knife River in North Dakota was excavated. This site has provided further evidence suggesting that environments have fluctuated between relatively xeric and mesic episodes over the latter half of the Holocene in this region.

The Emerson site (32DU285), an extensive scatter of chipped artifacts and fire-cracked rock, was recorded in August 1981, by University of North Dakota Archeological Research. Test excavation exposed several vertically stratified components and so in April 1982, the site was block-excavated. Presence of several buried A horizons, exposed in the excavations, indicates that soil development has been influenced by periods of relative stability and intervening episodes of sediment deposition. The buried mollic horizons appear to have developed under environmental conditions similar to those found in the region at present. However, sediments in which these soils developed may have been deposited under somewhat different prevailing environmental conditions. Laboratory and field analyses were conducted to determine the probable origin of sediments, genesis of soils, environmental conditions which prevailed during periods of sediment accumulation and development, and age of distinguishable buried soils.

The site is situated on a terrace remnant, 15m above the present flood plain of the Knife River and is bounded on its northern edge by a relatively steep scarp which slopes to the flood plain. Vegetation in the site vicinity is principally of mixed grass prairie type although the southern portion of the site is under cultivation. Climate in this region is presently continental, with temperatures which have ranged from -43° C to 41.7° C and average 4.5° C. Precipitation occurs mainly between April and September and averages 419mm per year.

Soil analyses (Table 1) show that sediments at the site are dominantly of fine-grained sand and silt in all horizons. Translocation of clay minerals and development of a B horizon is not readily evident within the profile.

Table 1. Res	ults of	soil an	alysis	
Horizon &	Size	Class	%	%
Depth (cm)	Sand	Silt	C1ay	CaCO ₃
A1 0-19	68.3	20.4	11.3	-
IIA1b 19-24	69.4	17.4	13.2	-
IIСЬ 24-26	69.1	17.4	13.5	-
IIIA11b 26-31	69.0	17.6	13.4	-
IIIA12b 31-38	70.4	19.1	10.5	-
IIICb 38-53	73.0	19.2	7.8	-
IVA1bca 53-71	58.3	28.8	12.9	1.87
IVCbca 71-110	63.1	26.1	10.8	1.99

Pairs of sediment samples were collected from each of the buried A horizons and were submitted for radiocarbon dating. Results of this analysis were overall unsupportive. However, one date (UCR-1582) was within the range of ages which had been estimated prior to submitting samples for analysis (3670 \pm 280 BP). In addition samples were also submitted for pollen analysis. Pollen preservation in general was poor but did support the contention that the buried A horizons were developed during relatively mesic episodes and the intervening deposits during relatively dry episodes.

While rather inconclusive by themselves, these results do correlate with other sites within the region. The lowest buried soil which yielded a date of 3670 BP corresponds quite well with dates of 3570 BP (32BI317) and 3030 BP (32BI249) taken on charcoal extracted from nearly identical soils. These results indicate that during the last 4000 years there have been three periods when climate was similar to present and at least two that were significantly drier.

^{1.} Antevs, E. (1955) Am. Antiquity 20., 317-335.

^{2.} Bryson, R.A., et al. (1970) <u>Dept. Geol. U. Kan., Spec. Publ.</u> 3.

^{3.} Clayton, L., et al. (1976) N.D. Geol. Surv., Misc. Ser. 54.

^{4.} Cvancara, A.M., et al. (1971) <u>Science</u> 171., 172-174.

ASTRONOMY of the INDIANS of the GREAT PLAINS PRIOR to THEIR CONTACT WITH EUROPEANS:
MONUMENTS, PICTOGRAPHS, STORIES, MYTHS

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At a Meeting of the Astronomical League in Peoria, Illinois, it was suggested that we in North Dakota take a serious interest in Indian Archaeological sites in our state and nearby regions for any archaeo-astronomical significance, as our contribution toward a better knowledge of the Archaeo-astronomy of the Indians of the Upper Great Plains. The Indians of the Upper Great Plains were essentially in a Stone-Age culture before the arrival of Europeans, and very little real evidence remains for their interest in, and knowledge of, the heavens above them.

We are well aware of the highly accurate scientific knowledge of Astronomy possessed by the Aztecs, Mayas, Toltecs, etc., of Mexico, and the Incas of Peru. By contrast, the Indian tribes of the Northern Great Plains were culturally far less developed. Writing was unknown and we must rely on structures and pictographs, the interpretation of which is often open to controversy.

Indian stories about the stars are numerous, but whether, and to what extent, they are of ancient Indian origin, or merely tales retold from what they heard from hunters, trappers, missionaries, and priests of European origin, is not at all clear. Unfortunately many of our immigrant-settler ancestors so completely destroyed Indian tradition and culture, or so completely converted the Indians to some form of Europeanization, that almost no suitable records exist of what these Indians or their forebears really knew or believed about the phenomena of the heavens a few hundred years ago.

The so-called <u>WOOD HENGE</u> near Kahokia, Illinois, dated as of presumably about 900-1000 A.D., is indeed an impressive structure or series of structures, well predating European influence. The various structures and petroglyphs, such as the Anasazi sighting rock opening, and the star-crescent carving-painting interpreted by some as a representation of the Super-Nova of 1054 A.D., are impressive, but subject to various kinds of interpretations and doubt. The great Indian MEDICINE WHEEL near Sheridan, Wyoming, at near 9000 ft. altitude, can be made out as impressive indeed, but has also been interpreted as a day or two of energetic fun by young Indian braves laying out a very crude twenty-eight spoke wheel with a few extra rock cairns, as possibly based on what they had heard from hunters or priests around 1700-1750. The large number of similar, though less detailed, Medicine Wheels, <u>supposedly</u> dated as of 2000 years ago, do however tend to support genuine Plains Indians archaeo-astronomical knowledge of solar and lunar risings and settings, and of the heliacal risings of several bright stars, such as Sirius, Aldebaran Capella, Arcturus.

Carvings on rocks, and configurations of rock-boulder placings at various locations in the Dakotas, Wyoming, Montana, Saskatchewan, and especially Southeastern Manitoba, offer intriguing questions as to their origin and meaning. Studies and conjectures made so far by amateurs, astronomers, historians, archaeologists, etc., should be subjected to scrutiny by astronomers, amateur or professional, but well versed in star patterns, apparent motions of the Sun, Moon and naked eye planets throughout the year and years, heliacal risings (and settings), novae and supernovae appearances, such as that of VELA thousands of years ago, and known to us only from present-day spectral evidence on the residual nebula, that of 1054 A.D. observed and recorded by the Chinese and Arabs, possibly observed by Europeans but perhaps suppressed for religious reasons, and also possibly by Indians in our own Southwest, and recorded as a star and crescent, and now remaining as an ever-expanding CRAB NEBULA. The Anasazi SUN-DAGGER has been cited as an archaeo-astronomical monument, but geologists consider it to be a formation of natural origin rather than a man-made structure.

Many sites are known, but the exact locations are kept secret in order to protect against vandalism. Serious and reputable investigators, can, however, obtain the exact locations, and permission to carry out suitable studies, by contacting the appropriate state historical and archaeological societies and museums.

We have had considerable correspondence with the Historical, Anthropological, and Archaeological Societies of North and South Dakota, Wyoming, Montana, Minnesota, Illinois, Saskatchewan, Manitoba, with respect to possible archaeo-astronomical sites in North Dakota and the immediately surrounding area. We and some others had intended to visit some of these sites during the past summer (1983) but did not get to do so. We hope for more success in our plans for 1984.

- 1. Probing the Mystery of the Indian Medicine Wheels, John A. Eddy, National Geographic 151, January 1977, pp. 140-146.
- 2. Native American Astronomy. Anthony Aveni.

THE RELATIONSHIP OF SOIL ORGANIC-C TO SOIL COLOR, SOIL TEXTURE, AND OTHER SOIL PROPERTIES OF SELECTED NORTH DAKOTA SOILS

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Organic carbon (OC) is widely regarded as a fundamental component of soil which influences plant growth. The present study began as an effort to relate the principal factors influencing the amount and distribution of organic carbon in the profile of a variety of dark-colored prairie soils.

Forty-five profiles of soils developed in glacio-lacustrine sediments were analyzed for OC with a corrected Walkley method; the data were evaluated using traditional statistical methods. The controlling independent variable for OC was depth ($R^2=0.51$, p=.01). The change with depth in a soil profile is nonlinear and is re-lated to some factor of soil genesis or soil management. In this study texture is important ($R^2=.24$, p=.01) but can be masked by other properties. Soil color in the surface horizons is related to a combination of texture and the amount of organic carbon - the darker the color the higher the OC.

The above study indicated that an analysis of OC with depth was needed for comparing the OC distribution among soils. A two parameter equation was developed and tested using the data from the 45 profiles. The parameters can be estimated or calculated from means or medians of profiles at several profile depths in certain soil classification groups and readily compared. The equation is % OC = Kl/(K₂D + 1) where % OC is the percent organic carbon and D is the profile depth in cm. Kl is the surface extrapolation of % OC and K₂ is the depth distribution parameter. Kl for the 45 profiles above is 4.0 and K₂ is 0.064.

Three textural groups of soils were further examined using this equation based on texture: 1)

Three textural groups of soils were further examined using this equation based on texture: 1) silty clay lacustrine soils of three soil series; 2) wind blown sandy sediments of two series; and 3) three loess derived soil series.

The silty clay soils had a high amount of surface organic carbon (Kl = 5.3) and fairly steep drop-off (K_2 = 0.09). We attribute this to the high clay content. No difference was observed among these three soil series but differences that can be attributed to salinity and soil management may be important.

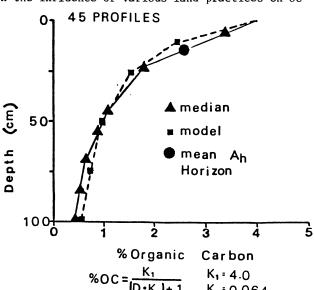
The two sandy textured series investigated consisting of an Entisol (Serden series) and a Mollisol (Maddock series). The low amount of OC in the Entisol, which was less than 1% in most of the soil profile, resulted in a Kl value of 1.1 and a K₂ value of 0.02, which means nearly no drop-off with depth. The Maddock series has greater than 2%OC in the surface horizon and a sharp drop-off (K₂ = 0.05). More data are needed to determine the variation and increase the confidence of the central tendency in the Kl and K₂ parameters on the sandy soils.

A third textural group of soils, all from loess parent material, had high amounts of silt and roughly 20% clay. These soils had a similar profile distribution of OC as the original study (K2 = 0.05) but had reduced surface OC (K1 = 2.4). Since the soil forming factors vary remarkably, the similarity was a surprise. The thick dark surface soils in concave landscape positions (Grassna series) were quite different (K1 = 2.2, $K_2 = 0.02$). Apparently the soil material carried to these soils by erosion created the lower surface OC even though more OC occurs at depths of 0.5 m.

Texture is a factor that influences soil OC distribution but is often masked by agricultural practices and landscape positions. Additional work on the influence of various land practices on OC specifically needs to be conducted.

Walkley, A. (1946) <u>Soil</u> <u>Sci.</u> 63:251-264.

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SOIL BULK DENSITY AND IRRIGATION WATER QUALITY: A GREENHOUSE STUDY

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The overall purpose of this research was to characterize the physical, chemical, and biological effects of irrigation water quality in some North Dakota soils with restricted permeability. The bulk density results presented here are a small part of the results from the study. Alteration of bulk density by irrigation water has received little attention in the literature. Bulk density changes generally have been studied in connection with mechanical disturbance, as in tillage, or compression from heavy loads, such as harvest machinery. Although bulk density can be measured with a relatively low coefficient of variation compared to some other soil properties (1), it also exhibits a relatively limited range of naturally occurring values. Rather precise experiments are therefore necessary to detect subtle treatment effects. We were able to measure some changes in bulk density caused by irrigation water quality when alfalfa was grown in undisturbed soil columns in the greenhouse.

Table 1 - Treatment water EC, SAR, and oven dry bulk density (DBOD) for the four soils (surface sample).

TRT	EC	SAR	Barnes	Will.	Pars.	Svea
	S/m			Мg·ш	-3	
Α	0.0	-	1.28(1)#	1.42(3)	1.40(2)	1.31(3)
В	0.1	3	1.33(4)	1.30(2)	1.36(2)	1.18(2)
С	0.1	3+	1.16(1)	1.30(2)	1.31(2)	
D	0.1	9†	1.30(4)	1.42(3)	1.49(2)	1.32(2)
E	0.3	8	1.42(1)	1.36(4)		1.04(1)
F	0.3	20	1.48(3)	1.45(3)	1.42(3)	1.31(2)
G	@	@	1.40(3)	1.47(3)	1.54(3)	1.45(2)

- @ Alternate TRT A and TRT F.
- † High residual sodium bicarbonate.
- # Numbers in parenthesis are the number of samples in the average.

Sampling sites were selected on four soil series (Barnes, Svea, Williams and Parshall) representing slowly to moderately permeable North Dakota soils. At each site 28 soil cylinders 20 cm in diameter and 60 cm long were obtained in plastic irrigation pipe. Alfalfa was planted and grown on the cylinders in the greenhouse. After the alfalfa stand was established, seven qualities of water were used to irrigate the alfalfa. Alfalfa growth with the treatment water continued for about 20 months. Treatment water electrical conductivity (EC) ranged from 0 to 0.3 S/m and sodium adsorption ratio (SAR) ranged up to 20 (Table 1). Further details are available in a project final report.

Upon completion of the alfalfa growth stage of the study, the 112 cylinders were cut apart and sampled from 9 depth increments. One sample was taken from the near-surface soil (0-2 cm). The eight remaining samples were obtained from depth increments of 7.5 cm. The sample from each depth was analyzed for bulk density by the saran-coated clod method. The data from the surface samples were analyzed separately while the data from all the other depths were incorporated into a common model. This was done for two reasons: the surface samples were from a smaller depth increment than the other samples; and a relatively large percentage (45%) of the surface sample data were missing. Difficulties in sampling and applying the clod method to the more friable surface soil were responsible for the excess of missing data.

Data from the surface samples (Table 1) were analyzed by a randomized factorial model (MODEL DBOD = SOIL TRT SOIL*TRT) using the SAS procedure GLM. The SOIL and TRT main effects were highly significant with a mean square error of 0.006. There was no interaction. Results from the samples obtained from the eight depth increments within the soil (i.e., excluding the surface layer) were analyzed in a grand model represented by the SAS statement:

MODEL DBOD = SOIL TRT SOIL*TRT CYLINDER(SOIL TRT) DEPTH DEPTH*SOIL DEPTH*TRT DEPTH*SOIL*TRT;

Our data set included 711 of the 896 possible observations for this model, with no missing cells. The results, requiring 42 minutes of CPU time on an IBM 4341 computer, indicated no 3 factor interaction, no main effect due to TRT and no SOIL*TRT interaction (using Type III SS). A regression model was used to examine the linear and quadratic effects of depth alone and the linear effect of depth for each soil and treatment. The SAS statements used for the GLM procedure were:

CLASSES SOIL TRT CYLINDER;

MODEL DBOD = SOIL CYLINDER(SOIL) DEPTH DEPTH*DEPTH DEPTH*SOIL DEPTH*TRT; All effects in the model (excluding the random error term CYLINDER(SOIL)) were highly significant. The least square means for the soils were calculated and were 1.38, 1.40, 1.47 and 1.50 Mg·m⁻³ for Barnes, Svea, Parshall and Williams soils, respectively. Bulk density, on the average, increased with depth. However, for treatments A, D, and G the rates of change were less than average. Treatments B, C, and F showed an average bulk density profile and treatment E resulted in the greatest changes.

1. Mader, Donald L. (1963) Soil Sci. Soc. Am. Proc. 27, 707-709.

WHEAT PRODUCTION IN THE USSR: SATELLITE DATA COLLECTION AND ANALYSIS

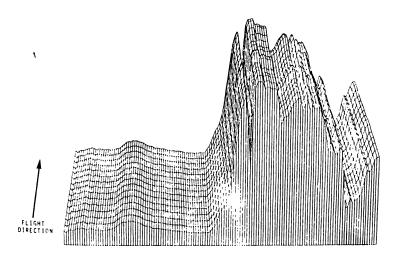
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The Soviet Union is one of the world's most important food producers, ranking first in the production of wheat, rye, oats, potatoes, sugarbeets, sunflower seeds and fiber flax. It is less outstanding in terms of overall farm output and yields, and since the socialization of agriculture the Soviet Union has had difficulty supplying quality food to an expanding population (1). In the past few decades, the Soviet Union imported grain to offset crop shortfalls and to avoid reducing livestock herds. Currently, the more dramatic rise in Soviet agricultural imports can be related to a consumer-oriented improved diet program. As a result the Soviet Union in 1981 was the world's leading importer of wheat and feed grains, and the second-ranked importer of wheat flour. The United States supplied nearly two-thirds of the total Soviet grain imports in 1979, but the 1980 partial agricultural embargo drastically reduced exports from the United States and the Soviet market was not regained when the embargo was lifted in April 1981. Soviet perception of the United States as a reliable and a dependable supplier of grain, Soviet import needs, and the ability of the United States to quickly respond to a Soviet agricultural shortfall will be a key determinant in future trade relationships. In order to gain insights into prospective Soviet grain needs, the United States Department of Agriculture has initiated a cooperative federal program to determine the usefulness, cost, and reliability of aerospace remote sensing agricultural data.

Agricultural and Resources Inventory Surveys Through Aerospace Remote Sensing (AgRISTARS) is a long-term program of research and predictive model development to satisfy federal information requirements on the Soviet Union's and other selected foreign nations' grain production, anticipated reserves, internal demands and possible competition for international sale of selected agricultural products. Topics currently researched include: 1) crop and land cover; 2) soil moisture; 3) early warning and crop condition assessment and 4) yield model development (2). Wheat production in the Soviet Union is influenced by numerous institutional and physical factors—a number of which can be monitored by satellites.

A CROP SPECTRA OF HEADED WHEAT

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Satellite secured land cover and hectares sown to wheat in the Soviet Union have provided sufficient basic information to develop crude predictive models. Remote sensed measurements of soil moisture, as input to models for predicting accurate wheat yields, are related to soil microwave emissions and backscattering properties; results gained are not at this time adequate for a superb predictive relationship. Early warning of wheat plant stress or disease and overall yield assessment using meteorological and satellite data input to simulation models have provided timely alerts of abnormal and of optimal conditions. Droughts and the impact of hot, dry "sukhovey" winds upon yields of spring and winter wheat have been detected and quantified; initial results are encouraging. Satellite measurements of environmental and wheat plant characteristics during the growth cycles

required the application of pertinent test criteria to spring wheat grown in North Dakota, a climatic and agricultural analogue to specific areas in the Soviet Union. Preliminary results indicate wheat yields are very sensitive to the soil moisture budget, and phenological accuracy correlated to soil moisture availability is critical. After three years of progress, it is apparent that aerospace remote sensing will contribute in a significant way to providing base-line data necessary to react and to respond to Soviet wheat needs.

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MONITORING AGRICULTURAL PRODUCTION IN TROPICAL ENVIRONMENTS: A COLOMBIAN EXAMPLE

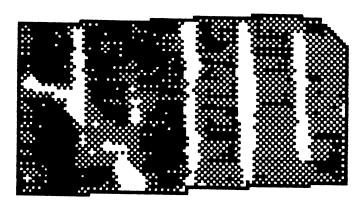
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The natural habitat of oil palm (Elaeis guineensis) is found in the transitional zones between rain forest and savanna regions of tropical Africa (1). Palm oil has been used as a source of fat and oil for centuries by persons living in those areas. Although export of palm nuts began in 1842, palm plantation techniques were not introduced until the present century (2). Today, oil palm has been successfully introduced to many other parts of the tropical world where rainfall ranges between 1000 and 1500 mm per annum and mean monthly temperatures exceed 21 degrees C. Oil palm has proven to be very competitive with other oil seed crops because of its high yield per acre and its high quality.

Cultivation of "Palma Africana" began in Colombia in 1960; by 1974 production had increased to over 50,000 metric tons per year (3). At that time, production was limited to portions of five "Departamentos" (States): Norte de Santander, Valle del Cauca, Meta, Santander, and Caqueta. Although Colombia ranked 7th in palm oil production in 1975, its 57,000 tons represented less than 2 percent of total world output (4). Since that time, palm oil production in Colombia has been extended to many new areas, and annual production increased to over 284,000 metric tons in 1979-1980 (5). Because of the high investment involved, both private investors and governmental agencies in Colombia are very interested in monitoring the extent of African palm plantings and their quality (i.e., production potential). The need for periodic monitoring of African palm plantations has promoted research by UNDIRS into possible remote sensing techniques that could be applied.

Airborne flights were conducted over the Rio Magdalena and adjacent lowlands between Barrancabermeja and Puerto Wilches, Colombia, on October 7, 1983. Small-format aerial photography and airborne video data were acquired at that time for oil palm plantations. Near infrared video data were analyzed using a Measuronics LMS system to determine the feasibility of monitoring African palm plantations in the test area. A recently planted area (see figure) shows the relationship between the planted areas (darker tones), and the rather large areas used for drainage (lightest tone). Additional information related to this area can be determined from the video imagery, e.g., spacing between plants, plant maturity and vigor, and number of plants in the test area. Our research demonstrated that airborne video data can be very useful for monitoring African oil palm plantations in Colombia.

CLASSIFIED VIDEO IMAGES OF AFRICAN PALM OIL PLANTATION



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THE CANADIAN WHEAT BOARD AS A DATA SOURCE FOR STUDIES IN AGRICULTURAL GEOGRAPHY

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The Canadian Wheat Board, a Crown Agency, is the sole marketing agency for wheat, oats, and barley grown on the Canadian Prairies for export or human consumption in Canada. The Board has on computer file at its headquarters in Winnipeg a considerable body of information about the some 145,000 Prairie producers of grain for sale. The information on file includes the specific location and acreage of each parcel of land owned and land rented from others by each producer, which information is brought up to date annually. These data are not published, but they are available at cost. For example, the author obtained for 900 Canadian dollars a complete listing of the location, acreage, and tenure of land operated by each of the some 26,000 producers in Manitoba. The names of producers are not released, but they can be determined by other methods.

The file also contains, for each producer, the seeded acreage and specific varieties of wheat, oats, barley, and canola (rapeseed), and acreages of rye, flaxseed, perennial forage, fallow, miscellaneous crops, new breaking, and uncultivated land, including natural pasture. Taken together, the acreages give the total area farmed by each producer. These land use statistics are published annually by the Wheat Board in summary form by province and grain delivery point, and can be obtained for individual producers. The Board also prepares, and upon request makes available, tables and computer maps of grain production and quality based on data provided by the more than 3,000 elevator operators across the Prairies.

All this information is used by the Wheat Board to set grain delivery quotas for individual producers, and to facilitate the movement and marketing of grain within Canada and abroad. The Board goes to considerable lengths to achieve fairness in setting quotas. To discourage false statements, producers are required under the Wheat Board Act to have declarations concerning the location, extent, and use of their land verified by a legally liable witness. The Wheat Board itself runs annual computer checks to detect instances of individual parcels of land being claimed by more than one producer or unusual locations of parcels. When such discrepancies occur, it contacts the producers involved for clarification. Questionable claims usually involve less than two percent in any one year. These considerations suggest that the Wheat Board data have a high degree of accuracy.

Statistics on file at the Wheat Board have several advantages over more conventional sources. The main advantages are that the Wheat Board collects information annually and for individual producers; in contrast, Statistics Canada collects agricultural data every five years, and publishes it only in aggregate form by census subdivision. Unpublished information is obtainable from Statistics Canada at a cost for areas smaller than census subdivisions, but no data are released on individual farms. Wheat Board records thus allow aggregation into any size or shape of areal unit, while census data do not. In addition, the census provides no information on grain production, but the Wheat Board does, even down to local geographic areas covered by single delivery points. On the other hand, the census provides information on all farms while the Wheat Board files pertain only to producers of grain for sale. Both sources suffer from deficiencies in studies where farm operating units as opposed to census farms or producer's holdings are being examined.

Surprisingly little use has been made by researchers of Wheat Board data, particularly that on individual producers, probably because they are unaware of its existence and availability. Some important exceptions are Weir (1), Swanson (2), and Carlyle (3,4).

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4. Carlyle, W.J. (1983) <u>Prairie Forum</u> 8., 1-23.

ECLIPSE of the SUN (GERHANA MATAHARI TOTAL) June 11, 1983, in JAVA, INDONESIA

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The total eclipse of June 11, 1983, was the longest lasting eclipse of this decade, up to five minutes ten seconds. The path for totality began in the South Indian at Long. 60°E, Lat. 37°S, passed over Christmas Island, and then, about 125 km. wide, over Central Java, including such million-population cities as Jogyakarta, then Papua-New Guinea, and ended, near the International Date Line at Lat. 18°S.

Our eclipse group consisted of Bart Bok, as scientific leader, Herbert Koenig, on his seventh eclipse, James Gall, as tour guide, and some fifteen other eclipse watchers. Mr. Gall, who had twice visited various possible eclipse sites in Java (on the basis of Indonesian Weather Office data and consultations with Prof. Hidayat at the Astronomical Observatory), selected as our observing site Salatiga in East Central Java, at Long. 110.5°E, Lat. 7°20'So., some 20 miles from the Central Line. There totality was about four and a half minutes. Other eclipse groups were at Tuban on the Java Sea, Jogyakarta, and Port Moresby, New Guinea.

On eclipse day, part of our group, including Dr. Bart Bok, decided to observe the eclipse from within Salatiga, at about 2000 ft., above Sea Level. They had a perfectly clear sky, and were able to observe not only the planet Venus but also Mercury, as well as several bright stars.

Most of us drove some fifteen miles to an altitude of about 5000 ft., near Kopeng, to make our observations from a military parade ground, scouted the day before. The sky was nearly clear blue, but after the partial eclipse began about an hour before totality, huge white cirrus clouds began to form. Venus became beautifully visible in a clear portion of the sky. About ten minutes before the onset of totality our scout reported that, because of the motion of a very huge cloud, another field, about 150 ft. lower, and an eighth of a mile away, would be more favorably located during totality. Most of our group decided to move whatever equipment they had to the lower site. Herbert Koenig, Klaus John, and I remained.

Using our Celestron-8 with a Pentax camera attached, as well as another Pentax camera for scenic views, we shot a series of photographs of the eclipse.

Table 1. Total Solar Eclipse of June 11, 1983. Film: Agfachrome 200 (speed: ASA 200). Optical System: Schmidt-Cassegrain-200 mm¢ focal length: 2000 mm (speed: f/10).

	Slide 83-8 D)	7	8	9	10	11	12	13	14	15	
	Exposure time ((sec)	1/250	1/125	1/60	1/30	1/15	1/8	1/4	1/2	1/4	
S	lide 83-8 D	16	17	18	19	20	21	22	2.	3	24	25
Expo	sure time (sec)	1/8	1/15	1/30	1/60	1/125	1/250	1/500	1/2	250	1/125	1/60

We started with very short exposures; then, in rapid succession, increased the exposure time up to one half second, then returned again to successively shorter exposure times, then again to longer.

The short exposure-time slides show excellent red solar flares or prominences; the longer exposures increasingly brighter and larger white coronas, with the red flares blotted out. The photographs taken just as totality began, and just as it ended, show a number of bright spots just at the edge of the dark lunar disk, the Diamond Ring, sunlight through the valleys of the Moon. The sky and scenery darkened in ominous fashion, reflections from cirrus clouds illuminate the panorama with an eerie light.

The Government issued an eclipse medallion; to prevent eye damage, ordered all persons to remain indoors during the eclipse, but local police did, using our filters, observe the phenomenon.

The next eclipses are a broken-ring annular eclipse, May 30, 1984, Mexico, U.S.; November 22/23, 1984, Papua, New Guinea, and July 31, 1991, seven minutes over Mexico City.

COMPUDOSE, RALGRO AND SYNOVEX IMPLANTS FOR YEARLING FINISHING STEERS W.E. Dinusson*, R.B. Danielson, L.J. Johnson and D.L. Feller Animal Science Department, North Dakota State University, Fargo, North Dakota 58105

Introduction

Implants have been used to increase gains and/or feed efficiency of beef cattle for over three decades. The first implant was stilbestrol. Since the banning of stilbestrol two other implants, Ralgro and Synovex, have been widely used. A new implant, Compudose, was shown to increase gains of yearling steers on pasture by 15 percent (1) over the nonimplanted controls. This research was conducted to evaluate Compudose in the feedlot and compare it to Ralgro and Ralgro-Synovex implants.

Procedure

Ninety yearling steers were purchased from one herd for this experiment. The steers were trucked to the Research Center at NDSU, allowed a 5 day rest and then were weighed on two consecutive days, allotted at random to 18 pens of five steers each. Five pens served as controls, five pens were implanted with Compudose and eight pens were implanted with Ralgro. Four of the Ralgro implanted pens were reimplanted with Synovex-S halfway through the trial period (at day 77).

The same ration was fed to all lots. Chopped, mixed hay and a supplement were fed at a constant level with cracked corn fed to appetite. A salt-mineral mixture was provided free choice. The supplement was formulated utilizing sunflower seed oil meal fortified with Vitamins A, D, E, limestone, Tylan and Rumensin. The steers were fed twice daily with the supplement top-dressed.

Weights were taken every 28 days with additional weights taken on days 70, 98 and 126 of the trial. Weights were taken on consecutive days at the termination of the experiment.

The summary and pertinent information is presented in table 1. The data for each treatment were averaged together for this summary. The steers gained very well for this 154 day trial. The Compudose and Ralgro-Synovex lots gained significantly faster (P<.05) than the control or Ralgro treatments.

The feed intake was very similar between treatments. However, because of faster gains, the Compudose and Ralgro-Synovex treatments required seven percent less feed per pound of gain.

Summary

Compudose implants improved gains by 9.5%, Ralgro implants by 2% and Ralgro-Synovex implants by 8.6% over a control with no implant in a 154 day feedlot experiment. Compudose and Ralgro-Synovex treatments required 7.1% less feed per pound of gain than the controls.

TABLE 1. COMPUDOSE, RALGRO AND SYNOVEX IMPLANTS FOR STEERS

Treatment	<u>Control</u>	Compudose	Ralgro	Ralgro + Synovex
No. Steers	231	251	202	202
Initial wt., 1b.	618.9	624.4	623.8	618.6
Final wt., 1b.	1088.1	1136.4	1100	1126.6
Avg. daily gain, 1b. 3,4	3.04 ^a ±.06	3.33 ^D ±.07	3.10 ^a ±.05	3.30 ^b ±.09
% increase over control	-	9.5%	2%	8.6%
Feed per day, total, 1b.	21.28	21.54	21.38	21.35
Feed per 1b. gain, total, 1b.	7.0	6.5	6.9	6.5
% less than control	-	7.1%	1.3%	7.1%

Averages of five lots.

³Averages of four lots.

 $^{^{\}pm}$ 0.06, etc. is standard error of mean which shows variation of gains.

Superscripts a significantly different (P<.05) from b.

⁽¹⁾ Dinusson, W.D., Johnson, L.J., Danielson, R.B. and Dunn, W.J. (1982) J. Anim. Sci., 417.

COMPARISON OF MICROVILLUS DENSITY OF BLASTODERM CELLS IN RESPONSE TO CALCIUM - BLOCKING DRUGS

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Calcium fluxes are suspected to be involved in a variety of cellular structural changes. Calcium Blocking agents include substances of a wide variety of drug classes (procaine, nitroprusside, verapamil, chlorpromazine, and nifedipine). All of these agents at high concentrations exhibit other pharmacologic actions such as receptor blocking, sodium channel blocking, and local anesthetic properties.

Microappendages are now recognized to be plasmalemmal extensions prevalent on the free surfaces of most cells indicating internal cell activity. Microvilli are small extensions of approximately 0.1 μm in diameter and of variable length found on various absorptive surfaces. The internal structure of microvilli has been reported to consist of microtubules, others have reported microfilament bundles of actin as the internal structure. The polymerized actin supported microvilli have been shown to dissolve back into the cell in the presence of high intracellular calcium concentrations. Workers in this laboratory have previously qualitatively observed procaine, a local anesthetic with some calcium-blocking action, to be able to induce microvillus formation indicating a possible association between the anesthetic action of procaine and the induction of microvilli. High atmospheric pressures are known to reverse the effects of anesthesia and Akers has previously noted blastodermal microvilli to become shorter and more bleb-like in response to high pressure.

The objectives of the current study are to observe the effects of calcium-blocking drugs on the microvillus density of chick embryo blastoderm. These effects were quantitated with respect to duration of exposure and drug concentration. With our results we hope to correlate our observations with current knowledge of actin polymerization, and a model of microvillus formation.

Our method involved the explantation of three day chick embryos to Hank's saline containing the calcium blocking drug preparations. Drug concentrations used were 10^{-6} M, 10^{-7} M, and 10^{-8} M. The drugs studied were verapamil, nifedipine, chlorpromazine, and nitroprusside. Control solutions containing no drug were included as well as solutions of 10^{-3} M procaine since its effect had previously been noted. The embryos were incubated at 35°C for 5 minutes, 15 minutes, and 30 minutes of exposure to the drugs. Viability of each embryo was monitored by the presence of embryonic heart contractions. At the appropriate times the embryos were fixed using a 4% glutaraldehyde solution, and further preserved by the addition of $0s0_4$ in cacodylate buffer. The embryos were then serially dehydrated in increasingly concentrated acetone solutions. Finally, critical point drying with liquid $0s0_4$, and sputter-coating the samples with gold-platinum completed the preparations for scanning electron microscopy.

Photographs of the embryos were taken at magifications of one thousand and ten thousand. Microvillus density was estimated by counting the number of microvillus appendages in a prescribed area in a number of photographs taken from each sample at the magnification of 10,000. Using an analysis of statistical variance test (ANOVA) comparing each drug to control samples we found significantly increased microvillus density with respect to both drug concentration and duration of exposure for procaine (p = 0.0258), nitroprusside (0.005), chlorpromazine (0.0001), calcium (0.0001), and cobalt (0.0001). Nifedipine was significantly different with respect to concentration (0.0185) and duration of exposure (0.0001). Verapamil was only significantly different with respect to exposure when compared to controls (p = 0.0014). Further, comparing the control mean density to the mean densities for each drug reveals an optimal concentration and duration of exposure for each drug. It appears the optimal point is related to molecular size of the blocking agent. It may be the larger molecules require a much smaller concentration and exposure time than smaller molecules.

Studies in other³ labs have shown the formation of microappendages associated directly with changes in concentration of extra mitochondrial calcium ions in hepatocytes as well as in gastro-intestinal mucosal cells. Our experiments appear to agree with these observations. By the application of calcium-blocking drugs in order to vary the intracellular calcium concentration our data shows mixed results depending on the drug. Although it suggests no significant increase in micro-villus density compared to control samples for any of the drugs, some drugs did appear to produce density trends with respect to concentration of drug and time of exposure.

¹Glenney, J., Matsudaira, P., Klaus, W. (1982) in Calcium and Cell Function (Wai Yiu Cheung, ed.), pp. 357-375. Academic Press, New York.

²Akers, T.K. Pressure Reversal of Anesthetic-induced Ultrastructure Changes in Cell Surface. Fed. Proc. 41: 1621, 1982.

³Glenney, J., Matsudaira, P., Klaus, W. (1982) in Calcium and Cell Function (Wai Yiu Cheung, ed.) pp. 357-375. Academic Press, New York.

GROWTH AND VIABILITY OF CULTURED CELLS UNDER OSMOTIC STRESS

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Water deficit is a form of stress which is responsible for numerous changes at the cellular and biochemical levels in plant tissues. These stress responses include ultrastructural disruption, reduced growth, and depressed protein levels. Soybean cell cultures were used to determine some of these stress responses.

Soybean cell suspension cultures were grown in the dark with continuous gyratory shaking on Gamborg's B5 medium, which was supplemented with 1 mg/ml of 2,4-dichlorophenoxy acetic acid. Mannitol was added to the medium at the concentrations of 0.32M, 0.48M, and 0.64M. These concentrations corresponded approximately to the osmotic potentials of -10b, -15b, and -20b respectively. All experiments consisted of 25 ml of growth medium and 5 ml or 500 mg of cell suspension culture. Growth was ascertained by settled cell volume and dry weight determinations after 1, 3, 5 and 7 days of treatment. Cell volume was obtained by pouring the culture into a graduated cylinder and determining the volume of cells after 30 min. The cells were then harvested by vacumm filtration, washed with water for 30 sec on preweighed miracloth, dried overnight in a 70 C oven, and weighed for dry matter accumulation(1). Protein was estimated by the Lowry method on a whole culture basis and on a per gram fresh weight basis. Cell viability of stressed and unstressed cultures was determined by phase-contrast microscopy of stained and unstained cells. Cells were stained with 0.1% (W/V) phenosafranin and 0.5% (W/V) Evan's blue in the growth medium (2).

The manitol-induced osmotic stress caused reduction in growth as measured by settled cell volume (Table 1) and dry weight accumulation. Protein also decreased with increasing stress when measured on a per culture basis. However, when protein was determined on a gram fresh weight basis, the stress treatments contained more protein than the control. This is probably a reflection of greater cell numbers in the stress treatments required for a 0.2 g sample, while the control sample contained fewer, but more hydrated cells (Table 2). Microscopic examination of phenosafranin and Evan's blue stained cells revealed plasmolysed cells in all treatments. Uptake of the dyes indicated cell death. Quantitative measurements of plant cell culture viability are different to obtain because of the size of cultured plant cells, but a greater number of cells appeared dead in the stress treatments.

Table 1

	Settled Cell Volum	ne (m1)	
Treatment	Control	0.32M	0.64M
Initial	less than 1.0		
Days 1	1.70	1.17	1.33
3	2.17	1.70	1.30
5	2.75	2.17	1.50
7	3.50	2.70	2.50

Table 2

	Protein Content (m	ng/g fr. wt.)	
Treatment	Control	0.32M	0.64M
Initial	3.90		
Days 1	6.45	5.78	4.78
3	4.30	5.96	6.17
5	4.10	5.23	6.67
7	3.40	4.73	5.21

⁽¹⁾ Cook, Deborah A. (1982) Mannitol - Induced Osmotic Stress of Cell Cultures. M.S. Thesis, NDSU. 63 pp.

⁽³⁾ Widholm, Jack M. (1972) Stain. Tech. 47., 189-194.

REPRODUCTIVE PHYSIOLOGY IN VITAMIN E DEFICIENCY

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Vitamin E, a fat soluble vitamin designated as alpha tocopherol and found in nature as a mixture of tocopherols including the isomers and homologs beta and gamma tocopherol, was discovered by Evans and Bishop in 1922. It was called Factor X by these investigators and over the ensuing years has also been described as the Antisterility Factor, Reproductive Vitamin, and Fertility Vitamin. Although it has been synthesized from alkylated hydroquinone it is found in nature in a variety of leafy plant foods such as lettuce and alfalfa, but in highest concentrations in vegetable oils. Wheat germ oil produces the greatest quantity of all plants, sunflower oil about one-fourth as much and olive oil none at all. No synthesis is known to occur in the animal organism.

The ubiquitous nature of the vitamin makes acute human clinical deficiencies difficult to find, but evidence is accumulating that alpha tocopherol deficiency is involved in some hemolytic anemias, in platelet physiology, in the immune process and, [with selenium] in protection against environmental pollutants. This latter mechanism is thought to be exemplary of an anti-oxident activity of the vitamin.

The first experiments with vitamin E deficiency involved reproduction. It has been known since the Evans and Bishop work that E-deficiency irreparably damaged the seminiferous epithelium of the rat testis. It was also shown at the same time that pregnant rats on E-deficient diets lost their conceptuses shortly after mid-pregnancy. During these early investigations purified diets were not as readily prepared as they are now and as a result, vitamin E content varied from one preparation to another. Accordingly, experimental results varied and so an experiment using successful pregnancy to study, for example, the effects of E-deficiency would produce variable results and the ratio of failed to successful pregnancies had to be carefully controlled with pregnancies of animals on E-supplemented diets. Data generated by such experiments were analyzed statistically.

We have studied the effects of vitamin E deficiency on female reproduction by the use of purified commercially available diets estimated to possess vitamin E in the following concentrations:

Diet I E-deficient diet 0.5 ppm

Diet II E-low diet 6.0-7.5 ppm

Diet III E-supplemented (control) diet 128 ppm

The diets were identical in all respects other than the quantity of vitamin E present. The animals were provided with diet and water ad libitum. Diet was provided in metal weighing cups for collection of food intake data. The rats were of the Long-Evans strain from a direct line to the original work in Berkeley. They were shipped as weanlings with a maximum of two days exposure in transit to the vitamin E in commercial diet. Upon arrival they were placed on purified diets and time of sexual maturation observed by changes in external genitalia. When reaching 200 gm weight, their estrus cycles were observed on a daily basis by vaginal smears. When in proestrus they were mated to normal males who were on commercial diet. The presence of sperm in the vaginal smears the following day signified day 1 of pregnancy.

The following reproductive events were observed for the three groups of animals on the three diets: 1) Sexual maturation, 2) Estrus cycle, 3) Insemination, 4) Implantation, 5) Parturition, 6) Lactation, and 7) Weaning.

For Diet III, all of these events occurred on schedule and were within normal limits of variation.

For Diet II, reproduction appeared to be normal through the implantation stage, after which reproductive performance varied between successful weaning of litters which appeared to be normal in number and weight to total resorbtion in utero.

For Diet I, no animals were successful beyond parturition and most resorbed their litters during the several days after mid-pregnancy.

	Implant	Implantation		ition	Lactation	
	Pregs	Rats	Pregs	Rats	Pregs	Rats
Diet I	20	12	2	2	0	0
Diet II	14	10	13	10	4	4
Diet III	4	4	4	4	4	4

It is apparent that vitamin E deficiency, in addition to disturbing gestation is also capable of disturbing lactation in the event that gestation progresses to term. Lactation is the delivery of nutritious milk to the gastro-intestinal tract of the young. The process starts with differentiation of the mammary gland and requires a number of successful nutritional as well as neural and humoral processes. Which of these events is disturbed by vitamin E deficiency is currently under study.

²Evans, H.M. and K.S. Bishop (1922) Science, Vol. LVI, pp. 650-651.

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In a study (1) of average annual age-adjusted death rates for multiple sclerosis (MS) per 100,000 population in Canada and the United States between 1959-1961, North Dakota (latitude $46^{\circ}-49^{\circ}$ N) was shown to have the highest mortality rate, namely, 1.4 per 100,000. Although North Dakota has been identified as having high MS mortality rates, statistics on the prevalence of this disease are not found. To estimate prevalence of MS in North Dakota, hospital admission data for MS patients who required treatment for their illness, or for conditions related to their illness, or for treatment of conditions other than their illness were examined for a period of five years.

Information on 1,535 admissions for 746 patients (255 males, 491 females; mean age 64.8 years, \underline{SD} = 14.5) who required hospitalization from January 1, 1977 through December 31, 1981 inclusive was obtained through survey of acute care hospital medical record departments throughout the state. Information obtained from these hospitals was as follows: (a) day, month and year of each MS patient hospital admission; (b) diagnosis as written on the patient's chart (including diagnoses other than MS); (c) age of patient; (d) patient's sex; (e) patient's zipcode at each admission; and (f) date of initial diagnosis of MS. Patient variables were matched with data from the same hospital and other hospitals throughout the state to identify patients with more than one hospital admission. Validity checks for the data were obtained by contacting directors of nursing services of 26 hospitals where patients had been admitted for diagnosis of MS, or treatment of exacerbation of MS symptoms to ascertain whether these patients would be seen by a neurologist. Eleven hospitals reported that a neurologist would see these patients; the remaining 15 reported that patients would be referred to other hospitals for neurologist consultation.

An analysis of the resultant 1,535 admissions via computerized frequency counts revealed that 578 patients were North Dakota residents and the rest (n = 168) were from out of state. Subjects in the North Dakota sample ranged in age from 14 to 81 years (overall mean = 47.3, $\underline{SD} = 14.4$). The female to male ratio (390 females and 188 males) was 2 to 1. Based on the 1980 Census population figure of 652,717, estimated prevalence of MS (excluding those diagnosed as having possible or probable MS) was 84 per 100,000 persons.

Using U.S. Geological Survey base maps, meridians and parallels that crossed county lines were used in conjunction with the 1980 Census statistics, to determine, population densities from which estimations of MS prevalence east and west of longitude 100° 30' W, and prevalence of MS by latitudinal bands within North Dakota could be made. The fact that MS prevalence increased with latitude progression toward the north of the state supports previous studies, in that, MS may be latitude-dependent (Figure 1A). As noted by Kurtze (2), in areas extending from about 43° to 65° latitude north high frequency prevalence rates for MS (between 30-80 per 100,00 population) is not an uncommon finding.

A finding of interest (Figure 1B) was that prevalence rates of MS were higher in the eastern than they were in the western half of North Dakota. This phenomenon may have occurred because of differences in climate. According to Koeppen's climate classification, the area located east of the 100th meridian has a subhumid climate, whereas, the area to the west is semi-arid. Another distinction between the two areas is population. Eastern North Dakota had a population density of 359,906 persons while western North Dakota had a population of 292,811. Therefore, differences in climate and an increase in

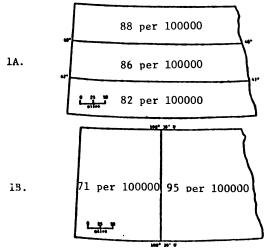


Figure 1A. Prevalence of MS in North Dakota by latitude. 1B. Prevalence of MS in North Dakota east and west of longitude 100° 30' W. as estimated from 1977 through 1981.

Therefore, differences in climate and an increase in persons living in high latitude locations, may have accounted for the increased prevalence of MS in the eastern half of the state.

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ISOLATION AND CHARACTERIZATION OF HEPATIC PHOSPHOENOLPYRUVATE CARBOXYKINASES FROM RABBIT

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Induced by starvation or diabetes, gluconeogenesis is responsible for the maintenance of blood glucose levels (1). A principal step in the pathway involves the enzyme phosphoenolpyruvate carboxy-kinase (PEPCK). In mammals, PEPCK activity is predominantly located in the liver and kidneys but its subcellular distribution in liver is species dependent (2). Nonetheless, PEPCK in both human and rabbit is present in hepatic mitochondria and cytoplasm (2). Hence, to better understand regulation gluconeogenesis in human, we have set out to isolate mitochondrial and cytoplasmic PEPCK activities from rabbit liver, to characterize their physical properties and to investigate their regulation in relationship to gluconeogenesis.

We have now purified the mitochondrial and cytoplasmic PEPCK activities to near-homogeneity. Mitochondrial PEPCK is purified as follows: after release by freeze-thawing, it is chromatographed successively on QAE-Sephadex, hydroxyapatite and Reactive Blue 2-agarose. Specific activities after these procedures are 0.10, 0.47, 3.01 and 13.6 respectively. A 134 fold purification of the enzyme is achieved with an overall yield of 14%. Purification of the cytoplasmic PEPCK involves preparation of a 100,000 X g supernatant, $(NH_4)_2SO_4$ fractionation, chromatography on Sephacryl 200, QAE-Sephadex, cellulose phosphate and Reactive Blue 2-agarose. Specific activities after these procedures are 0.007, 0.01, 0.06, 0.48, 3.34 and 11.2 respectively. A 1600 fold purification of the activity is achieved with an overall yield of 13.5%.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate indicates identical electrophoretic behaviors and molecular weights of about 64,000 for the most highly purified forms of both mitochondrial and cytoplasmic activities. However, other data indicate that physical differences do exist between the two forms of the enzyme. For example, the enzymes are separated by chromatography on QAE-Sephadex by elution with a salt gradient. At pH 7.7 in 50 mM Tris buffer, mitochondrial PEPCK elutes with 65 mM NaCl whereas cytoplasmic PEPCK elutes with 120 mM NaCl. Ionic charge differences of proteins may be determined by the use of chromatofocusing, a procedure in which a pH gradient is developed in the column allowing separation to occur according to the isoelectric point of the protein. With this technique, mitochondrial PEPCK elutes at a pH of 6.1 while cytoplasmic PEPCK elutes at a pH of 5.2 (see Fig. 1). Thus, data obtained with the above procedures suggest that cytoplasmic PEPCK is more negatively charged than mitochondrial PEPCK.

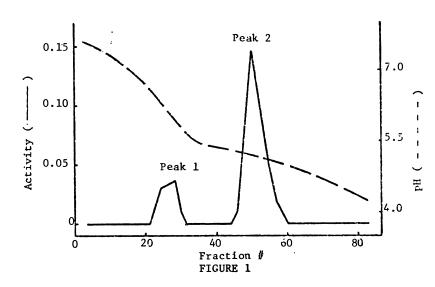


Figure 1: Elution profile of combined PEPCK activities during chromatofocusing. Peak 1 represents mitochondrial PEPCK; peak 2 represents cytoplasmic PEPCK. Enzyme activity is given in µmoles oxalacetate produced/minute/ml of column eluate.

We now plan to examine the molecular basis of the apparent ionic differences between the mitochondrial and cytoplasmic enzymes. (Supported by grants from the National Institutes of Health Grant 12705 and the North Dakota Affiliate of the American Diabetes Association)

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THE APPLICATION OF LUMINOMETRY TO THE STUDY OF THE EFFECTS OF ZINC NUTRITURE ON PHAGOCYTOSIS.

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Allen et al. (1) demonstrated in 1972 that polymorphonuclear neutrophils (PMNs) exhibit a chemiluminescence when presented with opsonized bacteria or latex particles. It was later shown that this chemiluminescence is directly associated with phagocytosis (2). This makes the measurement of chemiluminescence an ideal tool for studying the effects of zinc nutriture on phagocytosis. Lennard (3) and Beisel (4) have suggested that zinc status affects the ability of PMNs to phagocytize; however, their data are inconclusive. Thus, we have examined the effects of consumption of a zinc-deficient diet for varying lengths of time on phagocytosis in rats using the chemiluminescent response as an index of phagocytosis.

Male Long Evans rats (150-200g) were fed a zinc-deficient diet and zinc-supplemented pair-fed and ad libitum-fed controls were utilized for up to 58 days. Phagocytosis was measured in tail blood at 6, 10, 20, 30, 40 and 58 days. In addition, blood was taken from the heart of the experimental groups after 10 days, the buffy coat isolated by use of dextran (150-200,000 MW) (5) and the cells of the buffy coat were washed 2 times with saline. Any remaining red cells were lysed with water. The leukocytes were resuspended in HBSS containing 5% autologous rat serum at a concentration of 2 x 10⁶ PMNs/ml. Phagocytosis was also measured on peritoneal PMNs obtained from experimental groups 18 hours after the intraperitoneal injection of egg protein (10%). The peritoneal cells were washed once with saline and treated as the buffy coat cells above. Phagocytosis was quantitated using a luminometer equipped with automatic injectors (Picolite 6500, Packard Instrument Co.) (6). For analysis of chemiluminescence opsonized latex particles and luminol were injected into cuvettes containing the whole blood or isolated cells in a darkened chamber and the light given off was counted for 5 seconds. The counting procedure was repeated every 2 minutes for a 20 minute duration. Results were assessed as the area under the curve following injection of latex particles, and statistical significance was determined by analysis of variance.

There were no significant (p > 0.05) differences in the ability of PMNs in whole blood to phagocytize the opsonized particles at any duration of zinc deficiency tested. Furthermore, the PMNs isolated from the peritoneal cavities of rats from each group at day 50 did not exhibit any significant difference in chemiluminescence. Neither were there any significant differences between the groups in phagocytosis in PMNs from the buffy coat as separated from red cells by dextran on day 10. It should be noted, however, that the above data are expressed on a per cell (PMN) basis and that by 30 days of zinc deficiency the percentage of PMNs relative to lymphocytes and monocytes in whole blood is significantly increased and by 40 days the total white blood cell population is also increased.

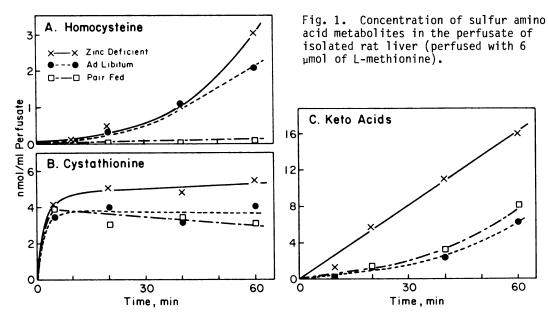
Thus it would appear that a) luminometry is a good tool for studying the effects of zinc nutriture on phagocytosis and b) the ability of PMNs to phagocytose does not seem to be significantly changed during experimental zinc deficiency.

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- 6. 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.

THE EFFECT OF ZINC DEFICIENCY AND FOOD RESTRICTION ON SULFUR AMINO ACID METABOLISM IN ISOLATED PERFUSED LIVERS

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Abnormal sulfur amino acid metabolism in zinc-deficient (ZD) rats has been reported (1). We examined sulfur amino acid metabolism in livers from ZD, pair-fed (PF) and <u>ad libitum</u>-fed (AL) rats. The livers were perfused with twice plasma levels of all essential amino acids containing 6 μ mol L-methionine (met) isotopically labeled in carboxyl (14 C) and methyl (3 H) positions (2). The rate of uptake of met from the perfusate was slower (p > 0.05) in the livers from the zinc-deficient rats than zinc-supplemented controls. Livers from the zinc-deficient rats utilized 35-40% of available met within 20 min, whereas zinc-supplemented controls used 60-70% of the met over the same period. Liver concentrations of S-adenosylmethionine (AdoMet) were similar in the ZD and AL rats (75 nmol/g), whereas levels of AdoMet in PF liver (46 nmol/g wet weight) were lower. Liver concentrations of S-adenosylhomocysteine (AdoHyc) (17 nmol/g) were not affected by zinc deficiency, which indicates that AdoHyc hydrolase was not impaired. The turnover rate of $[^{3}$ H-Methyl] AdoMet in the ZD perfused liver was 50% of that in the controls. This difference is probably a reflection of the depressed rate of methylation in the ZD livers (3), and in fact, the turnover rates of $[^{14}\text{C-carboxyl}]$ AdoMet were similar in the livers from the ZD and control rats. Free homocysteine was detected in perfusates from ZD and AL livers but not in PF controls (Fig 1A). However, if we increased the concentration of met in the perfusate, homocysteine could be detected in perfusate from PF liver. Free homocysteine could not be detected in tissue even at the high concentration of met employed. Intracellular homocysteine must be bound either to specific enzymes or other proteins. Cystathionine was excreted into the perfusate reaching levels of 3-4 nmol/ml within 10 min with all livers (Fig 1B). This level remained constant in ZD liver, whereas the concentration decreased with time in both PF and AL controls. Similar results were obtained when livers were perfused with higher concentrations of met (25 µmol), except the level of cystathionine reached 10-12 nmol/ml in the perfusate. α -Ketobutyrate, one of the products of the enzyme cystathionase, accumulated in the perfusate to a greater extent in ZD livers than PF or AL controls (Fig 1C). This buildup occurred at either the high or low level of met used. The accumulation in α -ketobutyrate and L-homocysteine in the perfusate of the isolated livers and differences in the rate of utilization of met between PF and ZD livers indicate an impairment in sulfur amino acid utilization in zinc deficiency.



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Unlike other animals, rats concentrate arsenic in red blood cells. This characteristic makes rats unsuitable animals for nutritional studies of arsenic. Thus, experiments were undertaken to determine the feasibility of using the hamster for arsenic deprivation studies. Initial experiments with hamsters showed that arsenic deprivation elevated plasma cholesterol (Chol) and depressed plasma glycine. Thus, the following study was done.

Male weanling Golden Syrian hamsters were assigned to groups of 6 in an experiment designed to see if high dietary cholesterol or benzoic acid (Benz) would affect arsenic deprivation. Arsenic was supplemented to the diet at levels of 0 and 1 μ g/g as Na₂HAsO₄·7H₂O₆. Cholesterol and benzoic acid were supplemented to the diet at levels of 0 and 10 g/kg. The basal diet, based on acid-washed ground corn and high protein casein, contained approximately 12 ng arsenic/g. The environmental conditions were similar to those used for rats (1). After 10 weeks the hamsters were decapitated subsequent to cardiac exsanguination with a heparin coated syringe. Several tissues were removed and weighed. Hematocrits (Hct) were determined on heart blood. The various parameters listed in the table or mentioned in the text were determined by described procedures (1).

Growth was not significantly affected by the dietary treatments. Dietary cholesterol significantly elevated hemoglobin, spleen wt/body wt ratio, plasma cholesterol, plasma urea and ceruloplasmin whereas benzoic acid significantly elevated heart wt/body wt ratio and significantly depressed plasma uric acid. Selected parameters are listed in the following table.

Treatment			Liver wt Body wt	Heart wt Body wt	Treats	nent		Kidney wt Body wt	Plasma
As	Cho1	Hct	(x 100)	(x 100)	As	Benz	Hct	(x 100)	Urea
μg/g	g/kg	<u>%</u>			μ g/g	g/kg	<u>%</u>		mg/100 m1
0	0	48.6	3.01	0.311	0	0	48.6	0.369	29.3
1	0	51.1	3.00	0.321	1	0	51.1	0.374	27.6
0	10	49.0	5.22	0.329	0	10	49.6	0.361	25.1
1	10	48.2	5.84	0.351	1	10	49.3	0.407	34.2
			Ana	alysis of Va	ariance - P Va	alues			
Arsenic e Cholester As x Chol	ol effect	NS 0.049 0.014	0.002 0.001 0.001	0.053 0.006 NS	Arsenic eff Benzoic act As x Benz		NS NS 0.040	0.008 NS 0.026	0.060 NS 0.009

Cholesterol supplementation depressed hematocrit in arsenic-supplemented hamsters but apparently did not affect hematocrit in arsenic-deprived hamsters. This explains the significant interaction between arsenic and cholesterol. An interaction between arsenic and cholesterol also affected liver wt/body wt ratio. Arsenic supplementation elevated liver wt/body wt ratio in hamsters fed supplemental cholesterol but not in hamsters fed no supplemental cholesterol. The meaning of the numerous interactions between arsenic and cholesterol are unclear. Further experiments are apparently needed to elucidate the significance of the present findings.

An interaction between arsenic and benzoic acid affected hematocrit. Arsenic deprivation depressed hematocrit in hamsters fed no supplemental benzoic acid. When benzoic acid was supplemented, arsenic deprivation had no effect on hematocrit. Kidney wt/body wt ratio and plasma urea were affected similarly by an interaction between arsenic and benzoic acid. When fed supplemental benzoic acid, arsenic supplementation elevated kidney wt/body wt ratio and plasma urea. Arsenic supplementation did not affect these parameters when the hamsters were fed no additional benzoic acid.

Regardless of the arsenic status, plasma uric acid was depressed in benzoic acid fed hamsters. Arsenic deprivation exacerbated this decrease. This indicates a decreased availability of glycine for the synthesis of uric acid since benzoic acid is excreted as hippuric acid, a conjugate of glycine and benzoic acid. Arsenic deprivation apparently decreases further the availability of glycine for uric acid synthesis. Studies with chicks indicate that arsenic affects methionine metabolism (2). An altered methionine metabolism probably affects glycine utilization because methionine together with glycine and arginine is utilized in the synthesis of creatine. The feeding of benzoic acid may prove to be a useful tool in the study of arsenic deprivation in animals.

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41 EFFECT OF BORON, MAGNESIUM, ALUMINUM AND THEIR INTERACTION ON KIDNEY MINERAL CONTENT

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Regulation of mineral metabolism via parathormone action, and regulation of erythropoiesis through erythropoietin synthesis, are two important functions of the kidney. Signs of boron deprivation in rats include anemia and altered calcium metabolism. This suggests that boron deprivation might alter kidney function or composition. Therefore we decided to see if boron nutriture influences the mineral content of the kidney. Because magnesium deficiency and aluminum toxicity signs apparently are influenced by renal function, we also decided to see if these treatments would influence any changes in kidney mineral composition induced by dietary boron or vice versa.

Male weanling Sprague-Dawley rats were assigned to groups of six or nine in a fully-crossed, three way, two-by-two-by-two design. The treatments were the supplementation of the basal diet (containing 0.3 to 0.4 μg boron/g) with boron (as boric acid) at 0 and 3 $\mu g/g$, with magnesium at 200 and 400 $\mu g/g$, and with aluminum (as aluminum chloride) at 0 and 1000 $\mu g/g$. Environmental conditions have been described (1). The rats were fed their respective diets for five weeks, weighed and decapitated subsequent to cardiac exsanguination with a heparin-coated needle and syringe. The kidneys were removed, weighed and quickly frozen for later mineral element analyses by our usual atomic absorption methodology (2). The findings are shown in the following table.

Trea	itment, μg	/g diet	Kidney wt, g		μg of element/g dry kidney					
В	Mg	A1	Body wt, 100 g	Zn	Cu	Fe	Mn	Ca	Mg	
0	200	0	0.327	91	17.9	159	4.93	152	749	
3	200	0	0.337	91	17.5	171	4.29	144	773	
0	400	0	0.327	91	17.4	164	4.28	153	791	
3	400	0	0.348	93	16.9	176	4.11	162	757	
0	200	1000	0.326	97	20.1	132	4.44	163	787	
3	200	1000	0.309	94	19.4	149	4.12	166	775	
0	400	1000	0.327	91	17.1	130	4.05	154	816	
3	400	1000	0.315	91	16.5	153	3.42	162	781	
			Analysis of	f Variance	- P Values	<u>.</u>				
Boro	n effect		NS	NS	NS	0.006	0.0001	NS	NS	
Magr	nesium eff	ect	NS	0.02	0.0004	NS	0.0001	NS	NS	
Boro	on x Magne	sium	NS	NS	NS	NS	NS	NS	NS	
	ninum		0.003	0.03	NS	0.0001	0.0001	0.02	NS	
Boro	on x Alumi	num	0.005	NS	NS	NS	NS	NS	NS	
Magr	nesium x A	luminum	NS	0.001	0.01	NS	NS	0.03	NS	
B x Mg x Al			NS	NS	NS	NS	0.04	NS	NS	

All dietary treatments affected the mineral profile of the kidney. Boron deprivation depressed iron and elevated manganese content. The difference in manganese was more marked in rats fed no aluminum and low magnesium, or high aluminum and adequate magnesium. Magnesium deprivation elevated both manganese and copper in the kidney. The elevation in manganese was more evident when dietary aluminum was high. High dietary aluminum depressed both manganese and iron in the kidney. High dietary aluminum also elevated kidney calcium and zinc when dietary magnesium was low.

In spite of the numerous significant findings, none of the changes seen were remarkably large. Although the changes themselves probably have little impact on kidney function, they do indicate that boron deprivation, magnesium deprivation, and aluminum toxicity affect kidney function.

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DIETARY COPPER, CYSTINE AND METHIONINE AFFECTS VANADIUM METABOLISM IN THE RAT

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42

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We have shown a relationship between vanadium and iron, influenced by cystine (Cys) that affects hematopoiesis in the rat (1). Vanadium deprivation exacerbated the depression in hematopoiesis exhibited by rats fed about 16 μ g iron and 4.65 mg Cys/g diet. When dietary Cys was about 10.15 mg/g, vanadium deprivation did not affect, or tended to elevate, hematocrit in moderately iron-deficient rats. These and other findings (2) indicated that vanadium indirectly affected the utilization of iron after absorption, and suggested that vanadium was more directly affecting another nutrient that affected iron metabolism. The present experiment was done to see if that nutrient was copper.

Male weanling Long-Evans rats were assigned to groups of six in a fully-crossed, three-way, two-by-three-by-three design. The levels of vanadium, sulfur amino acids (SAA) and copper in the basal diet were the variables (see table). The rats were fed their respective diets for seven weeks. Parameters listed in the table were determined by our usual methods (1).

Tr	eatm	ent, ug/	g diet			Plasma		Plasma
			1 SAA	Hematocrit,	Hemoglobin,	Cholesterol,	Heart wt, g	Ceruloplasmin,
V	Cu	Cys	Met	% 	g/100 m1	mg/100 mg	Body wt, 100 g	IU/ml
0	0	1,680	18,340	38.7	11.63	185	0.751	-0-
ĭ	Ō	1,680	18,340	40.4	12.30	163	0.595	-0-
ō	1	1,680	18,340	44.4	13.69	121	0.437	3.3
1	1	1,680	18,340	44.6	14.03	109	0.400	17.8
0	10	1,680	18,340	46.7	15.09	110	0.382	142.0
1	10	1,680	18,340	46.9	14.68	104	0.368	134.6
0	0	1,680	5,740	37.5	11.16	130	0.539	-0-
1	0	1,680	5,740	37.7	11.47	117	0.560	-0-
0	1	1,680	5,740	45.9	14.37	121	0.374	2.8
1	1	1,680	5,740	43.9	13.82	110	0.378	27.2
0	10	1,680	5,740	45.6	14.60	93	0.345	130.6
1	10	1,680	5,740	45.5	14.43	110	0.315	141.9
0	0	14,280	5,740	36.9	10.65	134	0.606	-0-
1	0	14,280	5,740	37.3	10.35	102	0.617	-0-
0	1	14,280	5,740	44.7	14.51	117	0.370	66.5
1	1	14,280	5,740	45.0	13.97	107	0.337	26.0
0	10	14,280	5,740	45.8	15.04	104	0.347	126.1
l	10	14,280	5,740	45.5	14.79	113	0.332	121.9
				An	alysis of Vari	ance - P Values		
۷a	nadi	um effec	t	NS	NS	0.05	0.01	NS
				0.0001	0.0001	0.0001	0.0001	0.0001
		fect		0.04	NS	0.0002	0.0001	0.005
۷a	nadi	um x Cop	per	NS	NS	0.03	NS	NS
۷a	nadi	um x SAA		NS	NS	NS	0.02	0.01
		x SAA		NS	0.002	0.0001	0.03	0.0001
۷a	nadi	um x Cop	per x SAA	A NS	NS	NS	0.01	0.002

The findings indicate that dietary vanadium affects copper metabolism, and/or vice versa. However, the direction and extent of the effects are markedly influenced by the sulfur amino acid content of the diet. For example, when the diet contained marginal copper (l μ g/g), and proportionally greater methionine (Met) than Cys, plasma ceruloplasmin was lower in vanadium-deprived than vanadium-supplemented rats. However, feeding high dietary Cys substantially elevated ceruloplasmin in vanadium-deprived, but not vanadium-supplemented, rats fed marginal copper. As a result, plasma ceruloplasmin was higher in vanadium-deprived than vanadium-supplemented rats when they were fed the diet containing marginal copper and proportionally greater Cys than Met. Similar changes occurred in hemoglobin but not in hematocrit. Regardless of dietary SAA, plasma cholesterol was higher in vanadium-deprived than vanadium-supplemented rats fed deficient or marginal copper. With luxuriant copper, the opposite was found at the lower level of dietary Met. Because copper and iron interact, these findings suggest that vanadium might affect iron metabolism through an effect on copper.

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THE CONTENT OF NITROGEN, PHOSPHORUS AND POTASSIUM IN NORTH DAKOTA RIVERS

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The content of NPK in the river water used for irrigation is important in considering a balanced fertilization program. Comparing the nutrient content in different rivers, some generalizations may be made about the contribution of the geology, erosion and cropping practices in the river basins.

NPK in the water of 83 North Dakota rivers were determined at irregular intervals between 1946 and 1982 by the U. S. Geological Survey (1). On only 14 rivers enough data are available to appreciate the nutrient content of river water for irrigation purposes (Table 1). Rivers with more than 40 NPK determinations are being used. When only 25-40 determinations of dissolved nitrogen are available, they are indicated in brackets. The data on each river are compared with the other rivers, with the average value of the two river basins and the average of 14 and 83 rivers(Table 1).

The waters in the Missouri River Basin have a lower average nitrogen content (all three forms) than the waters in the Red River Basin. Their average total phosphorus content is equal, but the dissolved phosphorus content is three times lower in Missouri River Basin than in Red River Basin. If the exceptionally high total phosphorus content in the Little Missouri River is not considered, the average total phosphorus content in Missouri River Basin is also about three times lower (0.11 mg/L) than in Red River Basin (0.29 mg/L).

Total nitrogen is lowest in the Missouri River, is low in all its tributaries (except the Little Missouri and the Timber), is high in the Red River and its tributaries (except the Sheyenne and the Baldhill Creek), and is very high in the Little Missouri. The content of dissolved nitrogen generally follows the concentration of total nitrogen.

Total phosphorus is lowest in the Missouri River and the majority of its tributaries, low in the Red River tributaries, high in the Red River and the James River, and maximum in the Little Missouri River. Dissolved phosphorus is lowest in the rivers of the Missouri River Basin (except the James River), and is high in the rivers of the Red River Basin (except the Baldhill Creek). The highest value is in Little South Pembina Creek.

Dissolved potassium is lowest in the Missouri River and the Red River, is low in the Pembina River and its tributary, is high in all the Missouri River tributaries, and very high in the Souris, Timber, James and Little Missouri rivers.

The high nutrient content of the Missouri tributaries in North Dakota does not significantly influence the nutrient content of the Missouri River water. This is because the Missouri River receives the main volume of water before entering the state.

Table 1. The nitrogen, phosphorus and potassium content in North Dakota rivers (mg/L).

		Nitrogen		Phosphorus		Potassium	
Rivers	total	dissolved	nitrate, dissolved	total	dissolved	dissolved	
	as N		as NO3	as P		as K	
MISSOURI RIVER BASIN							
l. Missouri	0.59	(0.56)	0.72	0.07	0.03	4.18	
2. Little Missouri	2.77	(1.72)	1.55	1.21	0.02	9.58	
3. Knife	1.35	(1.15)	2.12	0.10	0.04	8.75	
4. Brush Creek	1.31			0.08	0.04	9.39	
5. Heart	1.50	(0.97)	2.12	0.27	0.08	8.13	
6. Cannonball	1.45	(0.87)	2.15	0.10	0.03	8.58	
7. Timber	2.16			0.09	0.02	11.91	
8. James			1.70	0.37	0.19	10.88	
Average Missouri River Basin	1.59	(0.89)	1.73	0.29	0.06	8.92	
RED RIVER BASIN							
9. Red River	2.05		3.06	0.53	0.19	5.51	
10. Pembina	1.59	1.13	2.46	0.25	0.19	8.17	
ll. Little South Pembina			4.52		0.28	7.34	
12. Sheyenne	1.60	1.33	1.47	0.24	0.16	8.86	
 Baldhill Creek 	1.60	1.16	1.17	0.14	0.09	8.61	
14. Souris	2.15	(1.86)	3.86	0.27	0.21	12.80	
Average Red River Basin	1.80	1.37	2.76	0.29	0.19	8.55	
Average of the 14 rivers	1.48	1.22	2.35	0.25	0.15	8.71	
Average of the 83 rivers	1.49	1.24	2.33	0.22	0.15	9.87	

(1) U. S. Geological Survey. 1946-1982. Water Resources Data for North Dakota. Prepared in cooperation with the state of North Dakota and with other agencies.

HYDROGEOCHEMICAL CONTROLS ON THE MOBILITY OF RADIOGENIC CONSTITUENTS AT URANIFEROUS LIGNITE MINES IN SOUTHWESTERN NORTH DAKOTA

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Between 1955 and 1967, 595,288 pounds of uranium oxide was recovered from 85,138 tons of lignite mined from the Fort Union Formation in southwestern North Dakota (1). The lignite, which had a U₃O₈ content ranging from 0.001 to more than 1.8 percent (2,3,4) was strip mined in more than 12 pits. The lignite was burned in pit bottoms or ashing kilns nearby to concentrate the uranium in its ash by approximately a factor of 10. The resultant ash then was transported to uranium processing plants in other states. Stockpiles of ash remained in pit bottoms when the mines were abandoned, and reclamation has been initiated at only one site. Most pits have become flooded, because the lignite beds commonly serve as local aquifers and mines are located in recharge areas of the aquifers. Contact between mine wastes and ground water in the pits facilitates transport of radiogenic elements from the mine sites to regions where human exposure is more likely.

Uranium concentrations in pit waters range from 12 to 19,000 micrograms per liter, with accompanying radium-226 concentrations ranging from 1 to 73 picocuries per liter, commonly much greater than the interim maximum contaminant level (MCL) recommended for drinking water of 5 picocuries per liter radium-226 (5). The U.S. Environmental Protection Agency has proposed incorporating this MCL in the national revised primary drinking water regulations (6). Base flows in streams as distant as 3 miles down the hydrologic gradient from the nearest mine have uranium concentrations in excess of 200 micrograms per liter, approximately 10 times regional background concentrations.

Aerial measurements of gamma-ray exposure levels indicate that radiation from natural outcrops of the uraniferous lignite range from 10 to 30 microrems per hour, ash piles from 14 to 40 microrems per hour, and spoils piles from 10 to more than 200 microrems per hour. Investigation of the lignite in outcrops and on geophysical logs indicates most of the uranium occurs as a thin (1-2 foot thick) layer of hydrated uranium salts immediately above the lignite and is not adsorbed on organic materials. Apparently, most of this uranium was stripped and remained in the spoils during mining.

Accordingly, introduction of radiogenic species to the ground water is controlled principally by infiltration through the spoils and not by reaction between ground water and ash in pit bottoms. Preliminary analysis of saturated extracts and solutions from accelerated weathering experiments on spoils indicates that uranium concentrations in leachate are governed by the availability of dissolved organic compounds. Complexation of uranium on soluble hydroxybenzenes derived from oxidation of the lignite appears to be the principal mobilizing process. Solutions generated from ash contain uranium concentrations approximately 10-50 times less than those produced from spoils of identical initial uranium content, probably because soluble organic complexing agents were destroyed during the combustion process.

- 1. U.S. Atomic Energy Commission (1972) AEC Bull. AEC-72-U-4, 28 p.
- Denson, N. M., and Gill, J. R. (1956) U.S. Geological Survey Prof. Pap. 300, pp. 431-518.
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- 5. U.S. Environmental Protection Agency (1976) National Interim Primary Drinking Water Regulations, P. 7, U.S. Environmental Protection Agency EPA-570/9-76-003.
- 6. U.S. Environmental Protection Agency (1983) Fed. Reg. 48, 45502-45521.

CHARACTERISTICS OF PARTICULATE POLLUTANTS IN WESTERN NORTH DAKOTA

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Particulate pollutants, i.e. total suspended particulates (TSP), suspended nitrates (NO_3) and suspended sulfates (SO_4), were collected using high volume air samplers (Hi-Vols) at three rural locations in western North Dakota. Observed pollutant concentrations were analyzed for a three year period, 1980 through 1982, to determine differences between the years (trend analysis), differences between the sites (intersite analysis) and differences among the parameters monitored at the respective sites (intrasite analysis). The monitoring was conducted within the North and South Units of Theodore Roosevell National Park and near Dunn Center in central Dunn County. The parameters monitored at the respective stations, in addition to the particulate pollutants, included: wind direction (WD), wind speed (WS), and sulfur dioxide (SO_2) at all stations; hydrogen sulfide (WS) at the Park North Station; and temperature, nitrogen dioxide (SO_2), nitric oxide (SO_2) and ozone (SO_3) at the Dunn Center Station.

Initial analysis revealed that the particulate data was neither normal nor lognormal, hence, all analyses were performed on ranked values, an accepted nonparametric statistical method (Lehman, 1975).

Suspended nitrate was the only particulate pollutant that demonstrated a significant ($p \le 0.05$) trend over the three year period. Regression analysis showed a significant NO₃ reduction at all three stations over the three year period.

Intersite comparisons exhibited a strong regional characteristic between the sites for the respective particulate pollutants. Significant positive correlations were calculated between all sites for all particulate pollutants, Table 1. Suspended sulfate demonstrated weaker correlations than those demonstrated by the other particulate pollutants. The existence of less significant correlations between SO, observations suggests that sulfates are a more locally derived pollutant within the study area.

Intersite Correlation Coefficients for Particulate Pollutants

	<u>T:</u>	<u>SP</u>	<u>N</u> C	<u>)</u>	<u>50.</u>		
Station	Park South	Park North	Park South	Park North	Park South	Park North	
Dunn Center	0.87 F<0.0001	0.89 F<0.0001	0.80 p<0.0001	0.85 F<0.0001	0.21 F<0.03	0.41 r<0.0001	
Park North	0.84 F<0.0001	-	0.80 p<0.0001	-	0.40 F<0.0001	-	

Intrasite comparisons were performed for the individual stations and on data pooled for all stations. Because of the strong consistency between the stations, the presented analysis was based on pooled data. Particulate pollutants demonstrated a strong seasonal character; because of this characteristic, the analysis was performed on annually and seasonally stratified data.

Significant annual correlations were exhibited between SO₄ and NO₃ (r=0.44; p<0.0001) and SO₄ and SO₂ (r=0.48; p<0.0001) for the parameters monitored at all stations. Considering parameters monitored exclusively at one station, temperature demonstrated the strongest correlations with particulate pollutants; r=0.67, r=-0.41 and r=-0.19 for TSP, SO₄ and NO₃, respectively. The strong correlations between the particulate pollutants and temperature seems to support the contention of a strong seasonal nature to the particulate pollutants. Although additional significant correlations were found, small correlation coefficients suggest they were relatively unimportant.

Correlations performed on seasonally stratified data demonstrated the variability of the pollutant associations and strengths of the associations throughout the year. Although SO₄ and TSP displayed no significant correlations on an annual basis, seasonal relationships were found. Suspended sulfates had somewhat higher concentrations during the winter months, where they accounted for up to 50 percent of the observed TSP concentrations. On the other hand, TSP had peak concentrations during the spring and summer months, where SO₄ accounted for approximately 10 percent of the observed TSP concentration. As would be expected, SO₄ exhibited the strongest correlation to TSP during the winter months (r=0.35; p<0.0001). Suspended nitrates had a similar relationship with TSP, but, NO₃ also showed a strong correlation with TSP during the summer months (r=0.48; p<0.0001); SO₄ and NO₃ exhibited their weakest correlation during the summer months (r=0.17; p<0.1). The conflicting associations of NO₃ suggest that NO₃ is derived from two sources, one strongly related to SO₄ sources and dominant during winter months and the other strongly related to summertime TSP sources; a period when aeolien inputs would dominate. Other seasonal correlations worthy of note: WS and SO₄ demonstrated a negative correlation that was significant during all seasons except the winter, and NO₃ and temperature, which demonstrated a weak negative correlation annually, exhibited a strong positive correlation during the summer months (r=0.49; p<0.01).

To summarize: although three years is a relatively short period upon which to analyze trends, NO₃ appear to be decreasing while TSP and SO₄ are remaining relatively constant. Particulate pollutants demonstrated a strong regional character, with the exception of SO₄ which showed evidence of local influences. Interrelationships between the different parameters demonstrated the dynamic nature of the pollutants.

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STORM DRAINAGE SYSTEM DESIGN AND ANALYSIS USING A COMPUTER MODEL

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As urban areas continue to develop existing storm sewer systems are frequently overloaded causing environmental problems. The rapid urbanization of many areas results in larger volumes and higher rates of runoff leading to temporary failure of existing sewer systems. Therefore, a critical evaluation of existing sewer systems in such rapidly urbanizing areas is inevitable to assess the adequacy of existing systems and to propose changes to accommodate the future land use development in the area. Providing adequate in-system storage in the existing systems can effectively reduce peak flows and flooding and can also hold the first flush to be treated subsequently.

Many computer models have been developed in recent years for urban storm water runoff quantity and quality analysis. They vary widely in their capability to simulate the hydrologic and hydraulic processes in the system composed of drainage area with its sewer lines (1). Some of them are essentially planning models and can not be used in a design mode. Most of the planning models such as Storage, Treatment, Overflow Runoff Model (STORM) of Corps of Engineers and Storm Water Management Model (SWMM) of Environmental Protection Agency are continuous simulation models requiring a large memory computer. Use of these models is severely constrained by the budgeting limitations, data requirements and the need for specialized computer usage. However the model, Illinois Urban Drainage Area Simulator (ILLUDAS) has been gaining popularity among engineers and planners. It is a single event storm sewer evaluation or design model (2,3). The data requirements are moderate, practically the same as any other non-computer methods of sewer system evaluation and design. An important feature is the ability to compute detention storage desired in the system or storage accumulated because of an inadequate pipe. For moderate size areas with budget limitations and with no large-memory computer accessibility it is preferable that the model be 'easy-to-use' and has less memory requirements. In this study, the computer model ILLUDAS developed by the Illinois State Water Survey is used to evaluate the existing sewer system and to propose changes to accomodate the probable land use change in basin 12 of the City of Moorhead for a planning period of 20 years. For brevity only partial results are given in Table 1.

Table 1

Intersection Storage Volume (ft³) and Required pipe Diameter (inches)
(Basin No. 12 - 5-yr Storm)

	Intersection		Existing	New Design		
	Branch & reach	Allowable	System	New Design	(Land-Use Change)	
Storage	1-0	2,910	5,052	1,463	1,699	
Storage (ft ³)	1-5	3,880	4,776	3,880	3,880	
Pipe Size	1-0		15	18	21	
(inches)	1-5		27	36	36	

Main conclusions are 1. Although the accumulated storage in some intersections does exceed the corresponding allowable storage, the cost of reconstruction does not justify any system change at present. 2. Planned overland flow routes with proper street storage should be considered in storm drainage of urbanizing areas. It may often be necessary to redesign sewer branches that show no problems in the evaluation mode run of ILLUDAS to lower the overland flow that may be leaving one subbasin and contributing to storage problems in an adjacent subbasin. Such flow can be diverted overland wherever possible along properly planned routes without causing storage problems in the adjacent subbasin and thus eliminating the overdesign of sewers. 3. The computer model ILLUDAS is highly suited for this type of analysis of sewer systems. Data requirements are modest and the program is of manageable size in terms of memory requirements and complexity for the user.

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EFFECT OF PHOSPHOROUS ADDITION TO MODEL COOLING TOWERS USING COAL GASIFICATION WASTEWATER AS MAKEUP

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Cooling towers are an integral part of in-plant water reuse systems in industries such as the Greplains Gasification Plant being constructed at Beulah, North Dakota. Cooling towers can be used reduce water requirements, remove biodegradable organics, and concentrate non-biodegradable compount for ultimate disposal while accomplishing the basic water cooling function. The gasification wastewater used in cooling towers at coal gasification plants must be pretreated using solver extraction and ammonia stripping to produce stripped gas liquor (SGL). The SGL remains contaminate with organics, ammonia (NH₃), cyanides (CN), and thiocyanates (SCN) but at levels that microorganism can acclimate to in cooling towers (1). The objective of this investigation was to examine the effects of adding phosphorous to the nutrient deficient SGL in order to improve organics removal at to evaluate changes in biofouling and corrosion in the model cooling towers.

Two bench scale cooling towers were operated for 122 days with phosphorous being added to the maker water of cooling tower (A) while another tower (B) served as a control, without phosphorous addition Continuous basin water (BW) recirculation to the top of the tower packing at 121 l/day provided for constant wetting of packing surfaces while continuous makeup water addition at 2.8 l/day made losses due to evaporation and drift. Water from each basin was heated to 45°C, simulating a site of heat exchange, before being introduced back to the system. Two cycles of concentration were maintained by wasting approximately 1 l/day of blowdown. Carbon and stainless steel test specimen were suspended in air below the cooling tower packing for estimation of corrosion rates.

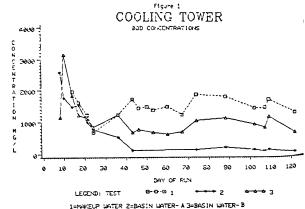
Heat dissipation in the bench scale cooling towers was calculated to be approximately 780 Btu/h based on a temperature drop of 21.5°C . Average operating conditions and summaries are shown in Tabl 1 and Figure 1. Results indicated that high reduction of bio-oxidizable organics can be achieved 1 cooling tower systems utilizing phosphorous enriched SGL as makeup. In tower A, the makeup water contained 1460 mg/s ROD and was reduced by over 95 percent to approximately 100 mg/s. BOD reduction were about 70 percent in B to 890 mg/s BOD. Phenol concentrations in the basin water in A and B were 0 and 47 mg/s, with an average makeup concentration of 126 mg/s. Maximum COD removal was about 4 percent in A. Hydantoins (5-5 dimethyl), which are not readily oxidizable, were a major portion of the refractory organics. Microorganism utilization of SCN was favored by the presence of phosphorous The thickness of the bio-film or slime on the cooling tower packing varied from about 0 to 5.0 mm in and 0 to 0.5 mm in B. Microoscopic examination of the BW and packing slime exhibited disting differences between the two towers. A greater variety of microbs were observed in A including higher level feeders from the phylum Protozoa such as amebas and paramecium. Corrosion rates for carbot steel test specimens were considered unacceptable at 7.0 mpy in A and 6.3 mpy in B. The values for stainless steel samples were insignificant at factors of 10^{-6} to 10^{-6} mpy.

The removal of contaminants from SGL was shown to be enhanced by the addition of phosphorous to makeup water for use in cooling towers. Unfortunately, the attached and suspended deposits became a increasing problem in terms of clogging the tower packing and tubing.

Table 1
Operating Condition Summary

Tower	BOD		COD		Phone?		Evdantoin		SCN	
	MW	BW	MW	BW	P'4	BW	MM	БN	MM	ρŅ
А	1460	100	5830	5380	126	0	1730	1900	245	84
Е	1460	890	5830	6170	126	47	1730	2000	245	390

Concentrations in mg/1



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LONG-TERM BIOCHEMICAL OXYGEN DEMAND OF COAL LIQUEFACTION WASTEWATERS

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The dwindling oil supply all over the world has caused an increasing interest in the production of synthetic fuel products from coal liquefaction processes. Coal liquefaction processes yield heavily contaminated wastewaters, which contain high level of BOD (bilchemical oxygen demand), COD (chemical oxygen demand), Phenol and ammonia. The objective of this study was to determine the long-term BOD values and factors affecting BOD exertion for the coal liquefaction wastewaters.

The wastewater used in this study was obtained from the project lignite process development unit in the Department of Chemical Engineering at the University of North Dakota, Grand Forks, North Dakota. Raw wastewater without any pretreatment was used in the long-term BOD study. The seed used in the test was obtained from the aerated lagoon of the City of Grand Forks wastewater treatment plant in Grand Forks, North Dakota, and from soil water extrat. The seed was acclimated for a period of two months prior to the BOD study. Factors affecting BOD exertion were investigated. These included the duration of incubation, the sample and size and the addition of methylene blue. Time of incubation included 0,1,2,3,5,7,10, and 20 days to determine the long term BOD exertion. Four different sample sizes of 50, 100, 150, and 200 ul were selected to determine the effect of dilution on the BOD results. Methylene blue addition was used to determine the effect of the suppression of nitrification on the BOD exertion. The experiment procedures followed the Standard Methods (1).

Results of long-term BOD exertion are shown in Table 1. The project lignite wastewaters were found to contain high concentration of BOD in the amount of 9,525, and 10,455 mg/l, for the 5-day, and 20-day BOD, respectively. A lag phase was observed, since there was no BOD exertion for the first day of incubation. For all four different sample sizes tested, BOD values increased with increases in the incubation periods, and a high percentage of BOD exerted at the end of five days incubation period. Sample size also had an important effect on the long-term BOD data. Long-term BOD values increased when sample size decreased or when dilution factor increased. For the BOD run without methylene blue addition, the 20-day BOD was 10,455 mg/l for the 200 ul sample (or a 1500-fold dilution), and was 28,500 mg/l for the 50 μ l sample size (or a 6000-fold dilution). Λ similar trend was noted for the BOD run with the methylene blue addition. The corresponding values were 10,080, and 21,540 mg/l. This indicated that the coal liquefaction wastewaters contained inhibitory compounds, and were toxic to microorganisms. Its toxicity decreased as the dilution factor increased or as the sample size decreased. With regard to the methylene blue addition, its effect on the BOD exertion was not consistent with respect to the sample sizes. For the 50 al samples, methylene blue effectively suppressed nitrification and reduced BOD values. For the 200 ul samples, methylene blue addition did not have any significant effect on the BOD exertion.

In conclusion, coal liquefaction wastewaters contained high level of BOD. The long-term BOD values increased as incubation period increased and as the dilution factor increased. Methylene blue was effective in suppressing the nitrification process at a high dilution factor but not at low dilution factors.

Time of BOD Values (mg/1) BOD Values (mg/1) incubation w/o Methylene Blue with Methylene Blue (days) for Sample size for sample size 200 ո1 50 Al لايم 200 1يه 50 100 Au1 150a1 100 Jul 150u1 0 0 0 7 C 0 0 0 0 1 0 0 0 0 0 0 0 0 2 5,280 9,420 9.900 5,940 0 5,100 4,395 4,460 3 11,160 11,520 8,260 6,615 5,700 8,020 7,175 540 5 9,060 11,640 12,030 14,400 10,060 9,525 11,160 9,750 7 11,800 14,100 9,900 12,420 13,740 9,720 20,340 9,960 10 16,320 28,820 15,480 12,120 10,095 14,970 11,700 10,020 20 <u>28,5</u>00 15,720 12,940 10,080 13,940 10,455 21,540 18,450

Table 1

Americal Public Health Association (1976) Standard Methods for the Examination of Water and Wastewater, 15th ed., New York.

PRETREATMENT OF COAL GASIFICATION WASTEWATERS BY THE COAGULATION PROCESS

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One approach to satisfy current energy needs is to convert coal to synthetic gaseous fuels using coal gasification processes, but all of the conversion techniques have serious environmental pollution potential. The wastewaters normally contain high levels of total organic carbon (TOC), chemical oxygen demand (COD), phenols and ammonia. The objective of this research was to determine optimum dosages of coagulants in the pretreatment of gas liquors.

The wastewaters used in this investigation was obtained from the slagging fixed-bed gasifier at the U.S. Department of Energy Grand Forks Energy Technology Center. The raw wastewater COD, phenol and ammonia concentration can reach 30,000, 7,300 and 7,000 mg/l, respectively. Gas liquors of three degrees of pretreatment were used in the coagulation study. These included lime precipitation followed by ammonia stripping, lime precipitation followed by ammonia stripping and one month exposure to air, and lime procipitation. Jar test was employed to determine the effectiveness of coagulation. The procedures included the addition of gas liquors, addition of coagulants to the gas liquors at predetermined dosages, rapid mix for 3 minutes at 100 rpm, slow mix for 30 minutes at 20 rand settling for 30 minutes. Transmittance was measured on the supernatant with a Spectronic 20. Coagulants investigated consisted of aluminum sulfate, ferrous sulfate, ferric sulfate, ferric chloride lime and ferric nitrite.

Table 1 presents the results of the coagulation study. For the lime precipitated and ammonia stripped samples, the optimum dosage for alum was 10 g/1 to give the best clarity. For ferric chloride the optimum dosage was also 10 g/1. The optimum dosages for both ferric nitrate and ferrous sulfate were 15 g/1. Ferric chloride presented the highest clarity of the supernatants at the optimum dosage of 10 g/1. Ferric nitrate also produced a clear supernatant. The alum coagulated samples possessed a yellowish supernatant, while the ferrous sulfate coagulated samples really did no clear much. A phenol test was conducted on this set of samples. The results proved that ferric chloride was the best coagulant. At a dosage of 10 g/1, the ferric chloride sample had a 43.9% reduction in phenol concentration. Ferrous sulfate removed only 20% of the initial phenol concentration of 8000 mg/1. For the lime precipitated has liquor followed by ammonia stripping and one month air exposure, ferrous sulfate and ferric nitrate were used as coagulants and yield similar results as in the previous run. For the samples which only received lime precipitation pretreatment, alum provided a very clear supernatant for all dosages exceeding 5 g/1. Freeic chloride and ferric nitrate coagulated samples did clear to some degree at higher dosages but did not possess as much clarity as the alum samples. The ferrous sulfate samples did not clear at all.

Some conclusions can be drawn from this study. The best coagulant was ferric chloride at a dosage of 10 g/l. At such high dosage the process may be uneconomical. Pretreatment should include lime precipitation and ammonia stripping prior to coagulation.

Sample	Coagulant	% Transmittance For							
Description	used	Dosage (g/l) at							
		0	5	10	15	20	25		
Lime + NH3	Fe(NO3)3.9H2O	82	91	95	100	86	77		
	FeSO4.7H2O	83	96	98	100	94	96		
	FeC13.6H2O	81	93	100	82	77	77		
	A12(S04)3.18H2)	93	97	100	97	97	99		
Lime + NH3	FeS04.&H20	86	100	87	89	88	87		
+ Air Expos.	Fe(NO3)3.9H2O	81	92	100	76	76	76		
	Fe(NO3)3.9H2O	91	77	78	79	100	98		
	FeS04.7H20	100	76	76	77	90	100		

86

81

76

97

78

99

90

100

97

99

100

99

FeC13.6H20

A13(SO4)3.18H2O

TABLE 1 Coagulation of Coal Gasification Wastewaters

PRESENCE OF ALTERNATE OPTIMAL SOLUTIONS IN ENGINEERING OPTIMIZATION PROBLEMS

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For engineering optimization problems falling into the general operations research area of mathematical programming and specifically linear and nonlinear programming problems, the solution of a multiple decision variable problem is specified by an optimal value for the objective function and the corresponding decision variables values. While the optimal solution (objective function) value does not change, there may exist alternate optimal solutions for the same objective function value by having different values for the decision variables (1). Although alternate optimal solutions may exist to the solution indicated, typically a computer program solution does not indicate them (2).

While in terms of the optimal result (objective function value), there is no advantage of these alternate solutions over the one indicated, the possible decision variable values of the alternate optimal solutions may allow the decision maker to pick a variable value for other than optimization considerations (such as cost or level) while having the optimal solution. Also a physical change to implement the decision variable values may be a major undertaking in the field so the existence and knowledge of alternate optimal decision variable values can be important in the decision process (3).

As an illustration, the following nonlinear example is used. An engineering optimization problem deals with optimal selection of four combinations of stages for a system. There are three possible sizes and levels that can be considered for the problem. Considering the several different sizes for the optimization problem and the number of levels, the following number of alternate optimal solutions were obtained for each combination (out of a total of 256 possible solutions, i.e., four things taken four at a time) as shown in Table 1.

Table 1
Number of Alternate Optimal Solutions

	Level 1	Level 2	Level 3					
Stage 1	1	16	21					
Stage 2	9	4	12					
Stage 3	1	3	4					

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The sizes represent three increasing allocations of time to be split among the levels. The levels represent three different flow arrangements for the stages. There are four sequential stages for the problem.

Generally, as the level is increased in value, the problem is less tightly bound. Thus, the less restrictive (tight) the bounds are there appears to be more likelihood of alternate optimal solutions. However, the combinatorial nature of the problem precludes total generality, as is shown with the effect of the value of size on the number of alternate optimal solutions. In terms of this illustration, the presence of no alternate optimal solutions for some combinations and the presence of a number (21) of alternate optimal solution gives an indication of what might occur in an engineering optimization problem. Also the decision variable values may not necessarily be close together.

A computer routine was developed to provide the alternate optimal solutions that exist for a linear programming (LP) problem. The program provides a listing of the alternate optimal solutions for LP problems in standard form, While the program is not capable of considering nonlinear programming problems, the presence of alternate optimal solutions has been shown by enumerations of the solutions. The program permits the decision maker to select the alternate optimal solution that he desires in terms of the decision variables values or for other nonquantifiable reasons. By having the alternate solutions available to the decision maker may permit secondary goals or objectives to be considered after the optimal objective function value has been determined. This permits a pseudo-sensitivity analysis to be done on the decision variable values to permit the decision maker to consider the effect of the decision variable values range on his selection and optimal choices.

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A NEW APPROACH TO DETERMINE
COUPLED MICROSTRIP LINE PARAMETERS USING GREEN'S FUNCTIONS

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The solution of electrostatic field distribution caused by point or line charges in the presence of a simple conductor or dielectric surface can be obtained by the method of electrical images [1]. This concept of locating imaginary charges together with the original charges helps in satisfying the bound ary conditions in the region. For a microstrip line, the correct evaluation of capacitance C per unil length of the line would specify the characteristic impedance Z_0 and velocity of propagation \vee . To determine C, the potential distribution around the line charges constituting the strip conductor placed at the interface of air and dielectric is evaluated by the method of electrical images using Green's functions.

In the present method the microstrip is assumed to be divided into substrips. The charge is considered to be concentrated at the center of each substrip and the field points are taken on the edge of each substrip. This approach of considering charge and field points at different locations takes into account the effect of all the substrips resulting in more accurate values of microstrip parameters [2].

For a coupled microstrip line the Green's functions for even and odd-mode after substituting the proper boundary conditions are given as [2],

$$G_{e}(o,y/o,y_{1}) = \frac{1}{2\pi\epsilon_{o}} \left[2a+b \ln\{(y^{2}-y_{1}^{2})/4h^{2}\} - c(y^{2}+y_{1}^{2})/2h^{2} \right] ------(1)$$
for even mode
$$G_{o}(o,y/o,y_{1}) = \frac{1}{2\pi\epsilon_{o}} \left[b \ln\{(y-y_{1})/(y+y_{1})\} + c yy_{1}/h^{2} \right] ------(2)$$
for odd-mode

where a,b,c are constants depending on the dielectric constant ϵ_r of the substrate, y and y₁, are field and charge point locations respectively. For N substrips of the coupled microstrip line the N simultaneous matrix equations are obtained as,

$$[V_1] = [G_{ij}][Q_j]$$
 -----(3)

where V_i , G_{ij} and Q_j are potentials, Green's functions and charges respectively with i,j=1,2,3,---N. These simultaneous equations are solved for given potential, usually 1V, for known separation between the strips and width of each substrip resulting in charge distribution as,

$$[Q] = [G]^{-1}[V], Q_{total} = \sum_{j=1}^{N} Q_{j}$$

From eqn. (4) the coupled microstrip parameters for even and odd-mode are determined by matrix inversion method as,

$$C_{Ki} = Q_{total}/V$$
, $v_i = 3x10^8/\sqrt{\epsilon_{reff}}$ and $Z_{oi} = 1/v_i C_{Ki}$

where i = e or o depending upon even or odd mode respectively and ϵ_{reff} = $C_{K\, \text{i}}/C_{K\, \text{A}}$ is the effective dielectric constant of the substrate with $C_{K\, \text{A}}$ as capacitance for air dielectric.

The results for $\epsilon_r=2.55$ are shown in Fig. 1. The accuracy of the results depends on the number of substrips. By increasing the number of substrips, more accurate results are obtained as exact charge distribution is approached but the computational work becomes complicated.

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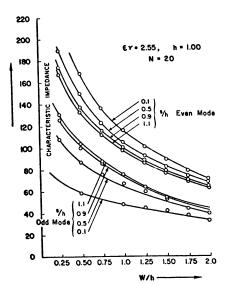


Fig. 1. Z_o of Coupled Microstrips

A 29-CHANNEL, 12 BIT, RS232 ANALOG-TO-DIGITAL CONVERTER

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Data acquisition of seasonal climatological factors and multispectral radiometric data are important for development of models for prediction of loss from foliar disease in cereal crops. The advent of the portable computer such as the Radio Shack Model 100 has made data acquisition and analysis in the field or laboratory feasible at relatively low cost. A 29 channel, 12 bit analog-todigital converter was built to interface with the computer via the RS232 serial port. (Fig. 1). Basic operation of the converter is as follows: When the UART Receiver receives a character from the serial input, it outputs it as the channel selection address to the Analog multiplexer. After a brief delay to allow the analog voltage to settle at the input of the Analog-to Digital Converter, the converter is signaled to begin the coversion. When the conversion is complete, the low byte of the conversion is selected via the Digital Multiplexer, and the UART Transmitter is signaled to send a character. After the low byte is transmitted, the last nibble is selected via the Digital Multiplexer and the UART is signaled to transmit it on the serial output. After the last transmission the logic is reset and ready for another channel selection and conversion. The UART is set up to operate at 19,200 BAUD with 8 data bits, no parity, and 1 stop bit. The Analog to Digital Converter is a 12-bit successive approximation converter which requires 13 clock periods to complete a conversion. Conversion time is 170 micro seconds with a clock frequency of 76800 Hz. The time required for a channel is:

Receive time=521 μ sec + Settle time=50 μ sec + Conversion time=170 μ sec + Send time=521 μ sec + Send time=521 μ sec = 1.78 milliseconds. (=560 channels per second maximum).

Power supply is sourced by 2 six volt gel-cell lead acid batteries connected in series. The total operating current is about 150 milliamps. Software written for the Model 100 enables choice of channels and storage of digitized voltage values in RAM. With appropriate transducers, the system has been configured to record light, temperature, humidity and dew. Operation modes are either automatic with cycle times triggered by the internal clock or manual.

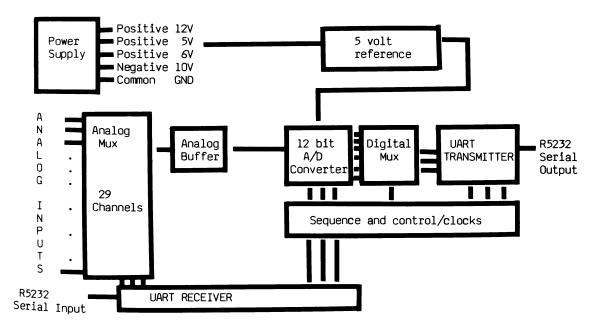


Fig. 1. Block Diagram of Analog-to-Digital Converter.

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CHEMICALLY MODIFIED VEGETABLE OIL DIESEL FUELS

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Vegetable oils have been under consideration as diesel fuels for some time. Early work goes bato Gautier (1928) when vegetable oils were determined to be satisfactory diesel fuels. Early studing (1) determined that from purely technical considerations, the use of vegetable oils as diesel fuel promising. There are, however, a number of technical problems which require solutions. The use modified vegetable oils has reduced or eliminated most of the problems reported earlier. The problems, as described by Bruwer et al., include injector fouling and ring sticking. The same studing also concluded that ester formation has resolved these problems in a 100 hour test when vegetable of are used as fuels in direct injection diesel engines.

The process of alkaline transesterification of fatty glycerides has been described in earliterature. This process, as described by Trent, (2) is essentially what we are currently using prepare sunflower oil methyl ester. Results of this study indicate that a very satisfactory fuel

obtained by >90 percent conversion to methyl ester.

Sufficient other work has been done on actual engine performance with respect to power and ful efficiency. These studies all seem to suggest the same things.

1. Vegetable oils foul injectors on direct injection engines and cause sticking of the ring. There is also a problem with vegetable oil which ends up in the crankcase polymerizing. A but the crankcase problem can be greatly minimized or eliminated by the use of engines wi precombustion chambers.

2. The thermal efficiency of vegetable oil esters is slightly higher than petroleum bas

diesel fuels.

Ester formation is a possible way to overcome all of the problems associated with vegetab oil diesel fuels.

The conversion of sunflower oil to sunflower oil methyl ester was accomplished by transeste ification with methyl alcohol using sodium methoxide as a catalyst. Five moles of methanol was us for every mole of sunflower oil and .93 grams of sodium catalyst was used for every liter of sunflow oil.

Engine testing on chemically modified vegetable oils is being done on a Lister Model LT-1 dire injection diesel engine. Engine testing was done at 2000 RPM with a loading of about 4.5 foot lbs. the dynamometer. Since, it has been found in our research that testing in this manner fouled engin in under 100 hours when pure vegetable oils were used. This allowed comparative testing to be done a fairly short time and with a minimum of fuel use.

It was noted during our work that sunflower oil methyl ester formed crystals at approximately 40° This is extremely unfortunate, as farm work requires diesel engines to perform below this temperatur A mechanical (i.e. fuel pre-heating) rather than chemical solution to this situation would seem mo

practical at this point.

The success of our research on sunflower oil methyl ester as a replacement diesel fuel has prompt ideas for complimentary research on chemically modified vegetable oils. In our case at the Universi of North Dakota, sunflower oil is still the obvious choice since the sunflowers are a major crop this area, however, our research is applicable to a group of oleic - linoleic oils among which a sunflower, cottonseed, peanut, corn, safflower, olive and palm. Primary research goals and objectiv are:

1. Utilization of renewable resources

2. Improving fuel low temperature properties

Developing fuel specifications which are applicable to vegetable oil fuels

The use of ethyl ester is a step toward a totally renewable fuel and at the same time, offers slight improvement in fuel low temperature properties. Methyl ester forms crystals at about 40-45 while ethyl ester is suitable for fuel use at about 30-35°F. It is expected that engine testing, wh completed, will demonstrate that ethyl ester performs as well as methyl ester from a fouli standpoint.

Ozonolysis as a means to improve low temperature properties of sunflower oil methyl ester currently under investigation. Ozone can be used to cleave double bonds with subsequent formation acids or aldehydes, depending on whether oxidative or reductive work-up of reaction products followed. The current plan is to use ozonolysis reaction products as additives to improve fuel l temperature properties.

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GAS CHROMATOGRAPHIC TECHNIQUES FOR THE ANALYSIS OF HYDROCARBONS
IN LOW-RANK COAL LIQUEFACTION PRODUCTS: PART I. TREATMENT OF THE DATA

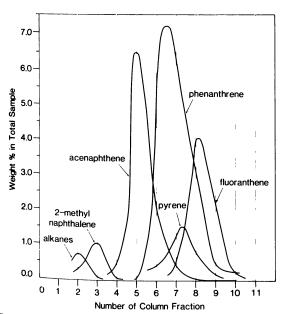
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The project that provided the impetus for this research involved the conversion of coal into refinable oils. The goals of the project were two-fold. First was to study the effects of two very different start-up solvents on the composition of the coal liquefaction product at line-out. Second was to characterize the changes that occurred during the line-out process. A major effort was on the analysis of the hydrocarbon oils by capillary gas chromatography.

The distillate oil samples were first separated by silica gel column chromatography into 11 fractions (1,2) to simplify the complex mixtures. Fractions were combined into four groups so that similar polarities resulted: 1) alkanes, 2) light aromatics, 3) di, tri, and tetra aromatics, and 4) heavy aromatics. A disadvantage of this approach was that it caused an increase in the variability of compound concentrations. Figure 1 demonstrates the wide range of concentrations of selected components in any given fraction (vertical lines).

Calibration standards, containing up to 30 standard compounds, were prepared and diluted to several different concentrations. An aliquot of the appropriate internal standard was added to each calibration standard as well as to the diluted column fractions. Because the relative response of the internal standard may vary at different concentrations, it was important that the same amount be added every time.

To calibrate the GC, the calibration standard dilutions were each injected twice and run with the same temperature program as the samples to be analyzed. Given the information from these runs, the Hewlett-Packard** (HP) calibration program calculated a response ratio for each compound with respect to the internal standard. Within the calibrated range, the HP program used the slope of the line between the two nearest points to calculate the concentration of a compound with a given response ratio. Out of the calibrated range, the line between the origin and the nearest calibrated point was used. Before any samples were run with a calibration, the calibration values were plotted by hand to find any errors or irregularities that might have affected the results. Once a reasonable calibration curve was obtained, it was possible to begin collecting data. If the curve was linear, and it passed through the origin, we found that it was more accurate to calculate the line by linear regression and enter only one value from that line into the program.



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FIGURE 1. Distribution of selected components from AO4 start-up solvent in column chromatography fractions.

There are some limitations to this technique: preparatory separations are time consuming; 2) it is necessary to choose instrument conditions reproducibility; 3) lack of standards limit the number of compounds that can be quantified; and 4) long temperature programs are sometimes required to obtain sufficient resolution of the numerous peaks. If the proper conditions are selected, however (3), the capillary GC is a reliable and powerful tool for the quantification of compounds in complex coal-derived mixtures.

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UNDERC carried out this work for the U.S. Department of Energy under Cooperative Agreement No. DE-FC21-83FE-60181.

**Reference to specific brand names and models is done to facilitate understanding and neither constitutes nor implies endorsement by the UND Energy Research Center.

GAS CHROMATOGRAPHIC TECHNIQUES FOR THE ANALYSIS OF HYDROCARBONS IN LOW-RANK COAL LIQUEFACTION PRODUCTS: PART II. INSTRUMENTAL ASPECTS

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Two long Continuous Processing Unit (CPU) runs were carried out to: 1) study the effect of two different start-up solvents on the composition of the recycle product; and 2) thoroughly characterized any change caused by the start-up solvent during the recycle process. Capillary gas chromatograph (g.c.) has been chosen as the major analytical tool at the University of North Dakota Energy Research Center in these line-out studies of coal liquefaction products.

Initial separations of distillate oils from CPU passes were carried out by the silica chromatographic method previously reported (1,2). The resulting hydrocarbon fractions were combined into four groups for gas chromatographic analysis (3). The four groups were chromatographed against the appropriate calibration mixture. Some components of the distillate oil were identified but not quantified due to insufficient amounts of some standards. Over 300 samples also necessitated the use of up to 30 components in a calibration standard.

The internal standard method was chosen for the quantification of compounds of interest. ability of the method to compensate for changes in conditions between sample runs and the variability of sample size were advantages of this method. A suitable internal standard (important to avoid coeluting peaks) was chosen after screening 16 potential internal standards. Known sample components were used to adjust for drift in elution time.

Selection of the proper chromatographic system was important in this analysis. A temperatureprogrammed capillary g.c. with flame ionization detection was used. The columns used were 60m DB-5 bonded-phase fused silica. These columns were chosen because of performance over a wide range of linear flow rates (4). In similar instances on-column injection seemed appropriate. However, in our case, splitless injection was chosen as a compromise to allow the use of an autosampler while maintaining adequate sample loading. Flame ionization detection was chosen because of its high sensitivity for organic compounds. The instruments used were a Hewlett-Packard (Palo Alto, CA) model 5880A gas chromatograph and a Varian (Walnut Creek, CA) model 3700 gas chromatograph. Each of these instruments is capable of accommodating an autosampler, cryogenic cooling to speed temperture equilibration, and multi-level temperature programming. Temperature programming allowed good resolution.

Resulting chromatograms showed near-ideal peak shapes. Peak areas were integrated, ratioed to the internal standard and compared to the appropriate calibration curve (3). Components were identified by comparing retention times and were confirmed by gas chromatography/mass spectroscopy. Parameters such as threshold, peak width, and baseline construction mode were adjusted for optimum sensitivity. For valid comparisons to be made conditions were carefully reproduced.

Although chromatography is not often thought of as an exact science, chromatographic systems can be optimized for a given analytical situation. In this case, we have successfully used capillary gas chromatography for the automated identification and quantification of up to 30 species in a single coal liquefaction fraction. We have quantified 87 compounds in the distillate oil. This method may also serve as the basis for analysis of other complex samples.

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- 2.
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The authors would like to acknowledge Edward F. Bitzan, Teresa J. Lechner, and David J. Miller for their contributions to this research.

Reference to specific brand names and models is done to facilitate understanding and neither constitutes nor implies endorsement by the University of North Dakota Energy Research Center.

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Lignite Liquefaction in Aqueus-H₂S Media

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So far anthracene oil (AO4) and SRCII middle distillate (SRMD) had been used to effect lignite liquefaction at 420° using H₂ and synthesis gas with and without H₂S (1).

At temperatures above 374.2°K and 218.3 atm, respectively, water is suggested to promote low-rank

coal liquefaction (2). The elucidation of such relationship is just being begun.

Three lignites: North Dakota Beulah no. 3 (B3), Texas Big Brown no. 1 (BB1) and North Dakota Indianhead (ZAP I) which have rather widely differing ash and water compositions were tested in a 12-ml stainless steel autoclave (3). The reaction temperature was 420°C and gas pressures were: $H_2S = 250$ psi, $H_2 = 490$ psi, CO = 490 psi, and water 1.0 g. The reaction time was 1 hour at temperature.

Volatile product yields of three low-rank coals using the H₂O-H₂S system are given in Table 1. By referring to the conversion in AO4 and SRCII mixtures, it is evident that the water effect is most pronounced in all lignites. Synthesis gas with H₂S is clearly outperforming synthesis gas alone for the conversion of lignites into volatile products. The H₂O-H₂S solvent matches the

performance of the organic solvent for the promotion of lignite liquefaction.

Table 1 Analytical Data for Low-rank Coal Conversion, %

Lignite	Conversi	on, %	Elementa		
	In AO4 and SR11 media (1)	In water media	Ash, %	н ₂ 0,%	Composition of gases
В3	30 ± 1.5 40 ± 1.5	14.7 34 ± 1 38 ± 4	11.35	28.61	Ar* H ₂ + CO H ₂ + CO + H ₂ S
BB1	42 ± 0.5 48 ± 3	15.5 45.8 ± 1 48.8 ± 1.2	9.26	26.15	Ar* H ₂ + CO H ₂ + CO + H ₂ S
ZAP I		16.0 37.4 ± 2.0 42.9 ± 0.4	7.667	34.292	Ar* H ₂ + CO H ₂ + CO + H ₂ S

^{*}Pressure = 1.000 psi

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IDENTIFICATION OF MONO METHYL-DIBENZOTHIOPHENES IN COAL LIQUEFACTION PRODUCTS

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Previous work at the University of North Dakota Energy Research Center (UNDERC) has involved investigations into the effects of H₂S on the liquefaction of low-rank coal. Addition of H₂S to the process increases the yield and improves the quality of distillable oils, but may be incorporated into the product oil. We are therefore interested in the detection and identification of sulfur compound in liquefaction products; especially as this pertains to sulfur incorporation (1).

The liquefaction process is typically conducted in the presence of a hydrogen donor solvent. The samples analyzed in this work were portions of the products of two runs which employed two distinctly different startup solvents. These two will be referred to as Runs 101 and 103. The startup solvent used in Run 101 was a commercial anthracene oil, AO4, derived from bituminous coal, while Run 103 used a lignite-derived solvent obtained from previous work at the University under Project Lignite to produce solvent refined lignite. In the runs, Indian Head lignite (Mercer County, ND) was liquefied in a continuous process unit (CPU) operating in a bottoms recycle mode (2). The runs were carried out at 400°C under 4000 psig 95% H₂-5% CO atmosphere, with addition of H₂S (5 wt % maf coal). The sample analyzed in this work were separated by open column chromatography on neutral silica gel (3).

Authentic samples of the four isomeric methyldibenzothiophenes were synthesized according to published procedures and characterized by correlation of $^{1}\mathrm{H}$ NMR chemical shifts. $^{13}\mathrm{C}$ NMR and mass spectroscopy were used for further characterization. 2-Methyldibenzothiophene, (2-Me-DBT), prepared by sequential lithiation and methylation of 2-bromodibenzothiophene (4). Methyldibenzothiophene, (4-ME-DBT), was prepared by condensation chlorocyclohexanone, and subsequent aromatization (5). An isomer. of o-thiocresol and An isomeric mixture of 1- and 3methyldibenzothiophenes was similarly prepared using m-thiocresol. A separate sample of (1-Me-DBT) was synthesized by appropriate ring closure of 2-allylbenzothiophene (6) and used to differentiate it from (3-Me-DBT) in the synthetic isomeric mixture. Mass spectra were obtained on an HP5985 GC/MS** with an SE-54 capillary column while gas chromatographic analyses were performed on an HP5730A GC with a wide bore DB-5 capillary column using a temperature profile of 40° to 320°C at 2° per minute. 2 Methyl-tridecane was used as an internal GC standard.

Dibenzothiophene and the methyldibenzothiophenes in the samples from Runs 101 and 103 were initially identified by GC/MS. Unfortunately, the individual isomers could not be distinguished since each exhibited the same fragmentation pattern. The isomers were subsequently identified by matching their GC retention times with those of the authentic samples. Analysis of the startung solvents showed that certain sulfur compounds were present; therefore, without quantification of all the volatile products we are unable to conclude whether any sulfur incorporation had occurred.

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UNDERC carried out this work for the U.S. Department of Energy under Cooperative Agreement No. DF FC21-83FE-60181.

** Reference to specific brand names and models is done to facilitate understanding and neither constitutes nor implies endorsement by UNDERC.

SILYLATION OF COAL LIQUEFACTION PRODUCTS AS AN AID TO PHENOL IDENTIFICATION

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At the University of North Dakota Energy Research Center (UNDERC) we have an ongoing program to identify phenols that are present in low-rank coal-derived liquids. We have developed a method for the conversion of the phenols to their trimethylsilyl ethers that facilitates the study of the phenols in liquefaction products.

It has been reported (1) that trimethylsilylation increases the volatility of phenols. This increase in volatility is very useful for gas chromatography (GC), permitting analysis of otherwise non-volatile phenols. An additional advantage of trimethylsilylation is that intermolecular hydrogen bonding of the phenols is diminished, thus reducing tailing on the gas chromatograms.

Before attempting to silylate phenols in liquefaction products, we optimized the silylation conditions for a series of model compounds, which had previously been identified in our liquefaction products (2). They include phenol, o-, m-, and p-cresol, 1- and 2-naphthol, p-phenylphenol, and several other substituted phenols. As shown in equation 1, the phenol is heated with chlorotrimethylsilane (TMSC1), hexamethyldisilazane (HMDS), and pyridine to yield the trimethylsilyl ether. All of the model compounds have been fully characterized by IR, ¹H, ¹³C and ²⁹Si NMR, and mass spectroscopy.

$$R \xrightarrow{OH} + TMSCI + HMDS \xrightarrow{pyridine} R \xrightarrow{OSiMe_3} [1]$$

Our liquefaction process yields three major liquid product types: a water fraction, a light oil, and a heavy oil. All three fractions have phenols present, but differ in the relative amounts of each type.

The polar fraction of a pentane-soluble oil, obtained from silica gel column chromatography of a heavy oil, was silylated as shown in equation 1. Gas chromatographic analysis of the silylated product indicates an increase in volatility, as expected. The silylation appeared to be quantitative because there was no evidence of any underivatized phenols. Use of GC/MS has enabled us to identify the trimethylsilyl ethers of phenol, methylethylphenol (two isomers), cresol (three isomers), tetrahydronaphthol, indanol (two isomers), and dimethylphenol (four isomers).

We have found that trimethylsilylation of low-rank coal-derived liquids is a valuable addition to our present methods of phenol identification. We are currently exploring the limitations of this technique, and are expanding our collection of silylated phenol standards.

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The Influence of Yttrium on Adherence and Plasticity of Alumina Scale on FeCrAl Alloy During Cyclic Oxidation at High Temperature

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Small amounts of yttrium (about 1%) in FeCrAl alloys produces a marked improvement in alloy behavior, particularly, in cyclic oxidation conditions (1). Results of morphologic studies and data of electron-probe microanalyses indicate on the formation of clusters of smaller yttrium oxide in interior layer of alumina which interact with scales and with host alloy (2). This effect is known as a mechanical keing or pegging of the scale to the alloy. Although mechanical keing of the scale to an alloy has a profound effect on a good adhesion, the electrostatic interaction between yttrium-oxygen-aluminum ions cannot be neglected.

The defect structure of alumina scales and the nature of grain/grain interface was studied by X-ray spectroscopy. Making reasonable assumptions it has been postulated that Al-OʻʻY complexes could be formed between two alumina grains and alloy-alumina grains when in contact. measurements of integral intensity of AlK α_1 2 line indicated that non-equilibrium aluminum vacancies (V_{A1}) were created above 1373K probably by yttrium diffusion along the α -Al $_2$ O $_3$ grains boundary. Typical isothermal oxidation kinetics made by gravimetric method show less oxidation resistance of FeCrAlY alloy above this temperature than that of the yttrium-free alloy, while at temperatures below 1373K the oxidation resistance of FeCrAlY alloy is considerably greater.

The random impurities and vacancies distribution in alumina scale have a considerable effect on non-homogeneous change of elastic constants (C;) and lead to change in distribution of plane stress (σ_{yy} + σ_{yy}) (3) and diffusional flow. The variable stress exhibited in the alumina scales could be involved in characteristic yield stress. For a small applied stress, only a small fraction of all the contact point will be stressed to the yield point resulting in low creep rate. As the stress is increased by the gradient temperature during cooling samples down there are also circumstances to increase stress in grains to the yield point.

Compressive stress in the scales formed on FeCrAl and FeCrAlY alloys was measured by X-ray diffraction using the sin-P method. It was found that compressive stress generated in the scale formed on EeCrAlY alloy increased by about two orders of magnitude $-(\sigma_{\chi\chi} + \sigma_{\gamma\gamma}) = 5.10^{\circ}$ N/m² ± 1.010⁻¹⁸ N/m² due to yttrium embedded at the alumina grain boundaries.

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IMMUNOGENICITY OF AN AMMONIUM SULFATE-PRECIPITATED EXTRACT OF TOXOPLASMA GONDII EXOTOXIN

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Toxoplasma gondii is an intracellular parasite that has both sexual and asexual reproductive capacity. In felids (cats) T. gondii is capable of sexual reproduction. This capacity allows the organism to maintain its life cycle both within and outside the host. Since the oocysts shed by cats into the environment may serve as a source of infection to numerous other animals, cats can be a continuous source for environmental contamination (2). However, in other animals, mammals, birds, reptiles, etc., T. gondii appears to only utilize the asexual means of reproduction. This means that these animals are then terminal hosts since the organisms are not shed into the environment.

Although $\underline{\mathbf{T}}$. $\underline{\mathbf{gondii}}$ may infect a wide variety of visceral and neurological tissues, the organisms infrequently cause severe disease symptoms in adult animals. Immunological surveys among human volunteers indicate that at least 50% of the United States adult population has been exposed to toxoplasmosis (4). There have also been several reports which have indicated a high $\underline{\mathbf{T}}$. $\underline{\mathbf{gondii}}$ infection rate among domestic animals (3).

Since the capacity for vertical transmission of this infection exists between a female and her off-spring, a serious epidemiological problem exists (2). Most adult infections of <u>T. gondii</u> are asymptomatic (4). Because of the insidious nature of this infection in adult animals, severe outbreaks of toxoplasmosis may occur in neonatal animals before the disease is diagnosed, and the infected animal(s) removed from the uninfected population. An effective vaccine could be used to immunize existing animal populations and thereby dramatically reduce the chances of an epidemic outbreak of toxoplasmosis.

To date, all efforts to manufacture a vaccine to be used against \underline{T} . \underline{gondii} have met with failure. It is possible, however, that an effective vaccine could be made from a specific subunit of the parasite. This is a reasonable expectation in light of the fact that adult animals usually develop life-long immunity to this parasite (4). Recent work in our laboratory has found that a toxic protein, administered at sublethal doses (20 mg protein), can be used to protect mice against a 10 x LD₅₀ challenge with \underline{T} . \underline{gondii} tachyzoites. Other studies have shown that certain agents can be added to \underline{in} \underline{vitro} cultures of lymphocytes or macrophages making them more active against \underline{T} . \underline{gondii} infections (1).

By using differential ammonium sulfate precipitation and electrophoretic purification we have been able to develop a preparation from <u>T. gondii</u> that provides significant protection when only 0.6 mg of protein are injected into a mouse. See Table 1. By comparing the different banding patterns of the 35% and 55% ammonium sulfate-precipitated proteins we hope to identify a protein or group of protein subunits that could be used for effective vaccine development.

Immunogen	<u>T</u> . <u>gondii</u> Tachyzoite Challenge Dose	No. of Mice	Percent Survival	Mean Time to Death
35% ppt 0.1 mg	40 x LD ₅₀	10	30%	11.86 ± 2.19 days
0.3 mg	$40 \times LD_{50}$	10	40%	12.67 ± 2.07 days
0.6 mg	$40 \times LD_{50}$	10	70%	15.67 ± 1.15 days
1.0 mg	40 x LD ₅₀	10	50%	16.60 ± 1.82 days
2.0 mg	$40 \times LD_{50}$	10	30%	13.20 ± 1.60 days
55% ppt 0.1 mg	40 x LD ₅₀	10	0%	11.40 ± 1.50 days
0.3 mg	$40 \times LD_{50}$	10	10%	12.00 ± 1,87 days
0.6 mg	40 x LD ₅₀	10	10%	12.33 ± 1.80 days
1.0 mg	40 x LD ₅₀	10	0%	13.10 ± 1.60 days
2.0 mg	$40 \times LD_{50}$	10	0%	12.60 ± 2.59 days

Table 1. Immunogenicity of <u>T</u>. gondii Exotoxin

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CHARACTERIZATION AND RELATIONSHIP OF BACTERIA ASSOCIATED WITH SUGARBEET (BETA VULGARIS L.) ROOT TISSUE

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It is widely known that sucrose content of sugarbeets (Beta vulgaris L.) decreases during storage. Several possibilities may account for this observed decrease in sugar content. Sucrose breakdown may be due to activity of endogenous invertase, microbial degradation or other undetermined biological mechanisms. Since parasitic bacteria are known to inhabit a wide range of higher plants (2), it was decided to determine if microbial parasites were responsible for a significant portion of the sucrose. loss. Twenty-two sugarbeet roots were harvested during the growing season (July to October) to deter mine the quantity and species of bacteria initially infecting sugarbeet roots. Beets were harvested from untreated border rows at a North Dakota State University (NDSU) research plot. Soil adhering to the harvested beets was collected and evaluated for bacterial content. Sugarbeets were then washed with tap water, to remove the remaining debris, rinsed in 95% ethanol and flamed to minimize bacteria carryover during subsequent cross-sectioning. Roots were cut into thirds; top, middle and bottom, using a sterile knife. Samples of internal beet tissue were added to sterile saline and blended for § minutes in a sterilized blender. These samples were diluted and spread onto nutrient agar plates to count and isolate bacteria. Table 1 summarizes the data obtained during this portion of the experi- 2 mentation. Bacterial populations have been shown to increase to an average of 4 x 105 cells per grammatic contract to the contract of the cont after 100 days of storage at 4° to 6° celsius (1). However, a high bacterial population does not always cause visible disease symptoms and thereby beet loss leading indirectly to decreased sucrose yield. It was, therfore, decided to evaluate the possible invertase activity of bacterial species associated with the sugarbeet roots. Table 2 lists the most common bacteria isolated from beet tissue Previous work at NDSU indicates that some bacteria may be highly invasive of sugarbeet tissue. Bacter ins were made from five of the most frequent isolates. These bacterins were used to immunize New Zealand white rabbits. Antisera obtained from these rabbits are being used with both fluorescene or ferritin labled anti-rabbit immunoglobulin to locate and identify these microbes within beet tissue. Simultaneous studies are underway to immunologically evaluate the levels of bacterial and endogenous sugarbeet invertase activity within the tissue of stored beets. Antibody against Psuedomonas fluorescens invertase has been prepared by immunizing BALB/c mice with electrophoretically purified bacterial invertase. This antibody has been used to successfully block invertase activity by Psuedomonas fluorescens. Once other monospecific antibodies have been prepared against other bacteria invertase enzymes and the endogenous sugarbeet invertase, an accurate estimate of the microbial contribution to sucrose loss during sugarbeet storage can be made.

<u>Table 1</u>

Average Quantity of Bacteria Isolated from Sugarbeet Tissue

<u>-</u>	rorage guaranty or	Dacteria iborated in	om Sugarbeet 113	<u> </u>
Cross-Section of Root	Adhering Soil	Peripheral Tissue	Core Tissue	Secondary Root Emergence Zone
Top Third	$2.17 \times 10^9/g$	15.9 x 10 ⁴ /g	13.64 x 10 ⁴ /g	2.95 x 10 ⁶ /g
Middle Third	1.79 x 10 ⁹ /g	13.71 x 10 ⁴ /g	0.85 x 10 ⁴ /g	
Bottom Third	1.57 x 10 ⁹ /g	$92.46 \times 10^4/g$	0.81 x 10 ⁴ /g	$1.80 \times 10^6/g$

<u>Table 2</u>
Bacterial Isolates from Sugarbeet Roots

Bacillus subtilis*	Corynebacterium sp.*		
Erwinia herbicola	Lactobacillus sp.		
Psuedomonas aeruginosa* Psuedomonas fluorescens*	Xanthomonas sp.*		

^{*} Bacterins made

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DEVELOPMENT OF SPECIAL BARLEY LINES FOR RESEARCH ON USTILAGO HORDEI (PERS.) LAGERH.

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Inoculation of barley seedlings with teliospores of <u>Ustilago hordei</u> requires removal of persistent lemmas, paleas, and pericarps or the use of vacuum to insure infection of susceptible cultivars (2,3). The removal of these seed structures or the use of vacuum requires time and labor. pisease expression occurs on maturing plants and takes from 70 to 90 days in the greenhouse. Therefore, cultivars that are late or tiller profusely lengthen the time plants are grown to obtain symptom expression. Special non-tillering, naked, short season barley lines are needed to reduce the amount of time and labor required to obtain disease expression.

Odesssa, C.I. 934, a covered, six row, thin pericarp barley is susceptible to all collections of $\underline{\text{U.}}$ $\underline{\text{hordei}}$. Among the F₂ progeny of a cross, Odessa X Excelsior, a plant susceptible to $\underline{\text{U.}}$ $\underline{\text{hordei}}$ race 6 produced a few naked seeds. Progeny from these seeds were backcrossed through two generations to an Odessa line containing the male sterile one gene, msg. A barley line, 934N3-3, was selected that is naked, six row with a thin pericarp and susceptible to all isolates of U. hordei tested to date.

The percentage of ruptured coleoptiles of 934N3-3 in comparison with three other naked barley cultivars are presented in Table 1. The interference with infection by <u>U. hordei</u> caused by unruptured pericarps has been reported (1). Line 934N3-3 is susceptible to all 17 races of <u>U. hordei</u> (Table 2).

Table 1 Percentage of pericarps ruptured by elongating coleotpiles of barley kernels germinated for 48 hours at 20C.

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Barley Line	Ruptured Pericarps
C.I. 595, Nepal C.I. 967, Jet	31 3
C.I. 1248, Excelsion	18
934N3-3 Derived Naked	93

Table 2 Reaction of a naked, susceptible, male sterile barley line (934N3-3) to 17 races of Ustilago hordei.

Race	Smut Infection %	Race	Smut Infection %
0	100	9	50
1	50	10	25
2	50	11	75
3	100	12	75
4	50	13	75
5	100	14	50
6	75	15	100
/	100	16	100
8	100		

Since the coleoptiles of this susceptible line rupture the pericarps during germination, inoculations may be made directly onto germinating seedlings without additional manipulations. This results in considerable time and labor savings. Furthermore, the male sterile one gene eliminates the necessity for emasculation; and since all the florets of the sterile six row head are used, large populations of hybrid seed are obtained per cross.

Line 934N3-3 was crossed to C.I. 6885 which is a dwarf barley (about 20cm). Lines were selected from this cross that are dwarf, six row, naked with thin pericarp and susceptible to U. hordei races 0, 4, 6, 9, 10, 11, and 12. Inoculated seedlings produced infected heads in 40 days which reduced the time required to obtain head sori by about one half. Their short stature allowed the plant density per M^2 of bench to be increased from a maximum of 90 for conventional height plants to 252.

Line 934N3-3 was crossed to barley genetic stock 253 carrying the recessive gene for uniculm, uc2. Lines were selected from this cross that are uniculm, six row, naked and susceptible to all the races of U. hordei except race 2. One line, U9, has large, six row type inflorescences that produce large quantities of teliospores when infected. The plants were headed 63 days and mature enough for harvest in 84 days. The uniculm feature allowed plant density per M² to be increased from a maximum of 90 for conventional tillering cultivars to 252 for uniculm plants.

The dwarf plants allow for quick development of smut sori and dense populations of the plants on the

greenhouse bench. Uniculm line U 9 also allows for increase in density of plant populations in greenhouse benches but produces larger volumes of teliospores per infection. Both the short growth period of the dwarf line and the large head development of the uniculm will aid the study of the genetics of <u>U</u>. <u>hordei</u>.

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ERWINIA RHAPONTICI, A BACTERIUM CAUSING PINK WHEAT KERNELS

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Durum wheat (Triticum turgidum L.) is a tetraploid wheat used in pasta products such as macaronià High quality durum grains are amber colored and translucent (vitreous). Any off-color or opacity lowers the grade and price of the grain. One discoloration reported on durum has been called . seed". Several fungi in the genus Fusarium are known to turn grain pink. Fusarium blighted (scabby grains are generally shrivelled and chalky in appearance. Among 1980 – 1981 samples of durum received by the USDA Wheat Quality Laboratory at North Dakota State University, 1–6% of the kernels were distinctly pink. The pink color extended into the endosperm but plumpness and vitreousness of the kernel were generally not affected. However, the pink color was readily visible in semolina milled from the kernels, making such semolina unsuitable for pasta production. This study was initiated to determine the cause of this pink discoloration in the durum grains.

In 1981 seeds from each of 2 durum cultivars showing pink or no discoloration were washed under running tap water for 30 min, blotted dry, then plated on potato dextrose agar (PDA, general purpose medium), and on two Fusarium selective media, 5 seeds/medium/kernel color. After incubation at C for 1 week, a bacterium that produced a diffusible pink pigment in the agar was recovered from 7 % of pink seeds plated on PDA. No bacteria were isolated from the selective media, which con tained antibiotics. Fusarium spp. were isolated from 23% of the pink seeds from all agars; 7% of the normal colored seeds yielded Fusarium spp., but none yielded bacteria.

Pathogenicity studies using the pink-diffusate producing bacterium were conducted in 1982. At flowering or post-flowering, $10~\mu l$ of washed bacteria cell suspension was syringe injected into the spikelets of three durum cvs. Checks were syringe inoculated with sterile distilled water. In another test, detached heads at the soft dough stage were dipped into a bacterial cell suspension, & covered or not covered with plastic bags, and then allowed to mature with the stem-ends in water. At maturity all heads were threshed and examined for pink kernels. Syringe injection was successful in producing pink seed at the site of inoculation. Pink seeds were not visible above or below the site of wounding. Dipped spikelets yielded pink seed only when the heads were kept moist.

To complete Koch's postulates, isolations from these artificially inoculated seeds were attempted. Seeds were surface sterilized in 1% NaOCL for 3 minutes, rinsed in sterile distilled water, then cut in half. One half was plated directly on PDA; the other half was placed in sterile distilled water, soaked for 30 minutes, macerated by grinding, then this solution was streaked on PDA. Plates were incubated for 5 days at $20-24^{\circ}$ C. All of the artificially inoculated pink seeds yielded a bacterium that produced a diffusible pink pigment. In addition, 14% of the normal colored seeds from inoculated heads yielded the bacterium. No seeds from checks yielded bacteria.

This bacterium was characterized as gram negative, facultative anaerobic, and oxidase negative. On nutrient agar it produced small white colonies, on MacConkey agar - pink with purple nucleated colonies, and on sucrose-peptone agar - a diffusible pink pigment. API 20E and MicroScan bacterial identification systems indicated the bacterium was positive for glucose, sucrose, sorbitol, arabinose, melibiose and rhamnose fermentations, citrate utilization and beta-galactosidase, and was negative for nitrate reduction, HoS, urease, tryptophan deaminase and arginine dehydrolase. The API 20E system indicated the bacterium to be either Enterobacter agglomerans or Serratia phymuthica. The MicroScan system indicated a 60.7% chance of it being E. agglomerans. Enterobacter and Serratia are distinguished with a DNAse test. The DNAase test was negative, indicating it was Enterobacter rather than Serratia. The API and MicroScan system classify bacteria on the basis of their occurrence in animals, the API 20E system being specific for Enterics. The plant pathogenic genus most closely corresponding to Enterobacter is Erwinia. Two species, Erwinia rubrifaciens and E. rhapontici, produce a pink diffusible pigment on sucrose-peptone agar, as does E. agglomerans. E. rubrifaciens is pectolytic while E. rhapontici is not. Our bacterium was not pectolytic on pectate calcium medium. We concluded that the pink-diffusate producing bacterium was E. rhapontici (Millard) Burkholder. Scientists in Europe (3,4) reported seed contamination of hexaploid wheat seeds by a similar bacterium. Roberts (4) identified the bacterium as Erwinia rhapontici, a known pathogen of rhubarb.

Our recovery of this bacterium is the first report of its presence in the United States and the second report of such a bacterium in durum. Howe and Simmonds (2) isolated a pink diffusate producing bacterium from kernels of a Canadian spring wheat and a durum. Campbell (1) found non-scabby pink seed in samples of Canadian spring wheat that was swathed early and accumulated moisture for several weeks prior to harvest. Based on our results and those of others (2, 3, 4), the bacterium appears to infect primarily wounds and the disease is associated with long periods of high humidity.

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Recently, a disease resembling bean common mosaic affected popular commercial varieties of dry edible beans, Phaseolus vulgaris L., in variety trials in North Dakota. The pinto bean cultivars Olathe and Pindak showed disease symptoms about one month after planting. Leaves developed interspersed regions of dark green and chlorotic tissue, were slightly malformed, and became brittle with age. Infected plants were stunted and set few pods. The bean cultivars had been bred with resistance to the most prevalent "Type" and New York 15 strains of Bean Common Mosaic Virus (BCMV) (3). A new strain of the virus might threaten the bean crop in North Dakota.

Electron microscopy of negatively stained leaf dip preparations from infected plants showed

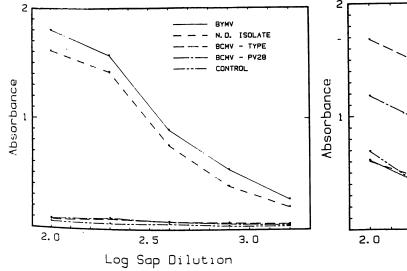
flexuous filamentous virus particles resembling BCMV. Measurements from photographs of 50 particles in infective sap showed particle lengths between 600 and 650 nm with the majority of the particles measuring 625 nm. The virus particles were few in number and tended to clump. Sixty particles in partially purified sap preparation were photographed and measured. The majority were 675 nm long.

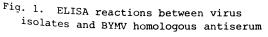
The average virus particle length was 657 nm.

Dilution end point of 10^{-3} - 10^{-4} was determined on sap from infected kidney beans extracted in 0.01 M phosphate buffer--pH 7.2, expressed through cheesecloth, serially diluted in 10-fold steps to 10-6 and inoculated onto susceptible kidney beans in the greenhouse. Thermal inactivation point of 50-55C was determined by heating 2 ml of sap for 10 min at temperatures ranging from 45-80 C with 5 C intervals. The sap was rapidly cooled after heating then inoculated onto plants in the greenhouse. Longevity of 3-4days was determined by storing the sap in stoppered flasks at room temperature, then testing for infectivity every 24 hrs. Tests were made from 24-144 hr. These tests did not differentiate the virus as being either BCMV or Bean Yellow Mosaic Virus (BYMV).

Purification of the unknown virus with standard techniques vas generally unsuccessful(2). Electron photomicrographs of the purified sap showed the particles had clumped and appeared to be disintegrating. Because yield of purified unknown virus was low, immunological properties were not studied. Rather, the serological relationships to known isolates of BCMV and BYMV were determined with antiserum to the known viruses. Nonspecific interactions occurred in Ouchterlony double diffusion agar gel tests. The unknown virus interacted strongly with antibody to BYMV (Fig. 1) and only weakly with antibody to the Type strain of BCMV (Fig. 2) in enzyme linked-immunosorbent assay (ELISA).

Based on these results and on other studies that showed the unknown virus capable of attacking cultivars of beans resistant to BCMV, we have concluded the unknown viral pathogen was BYMV originating possibly from infected weed clovers(1). This virus had not been reported previously in North Dakota.





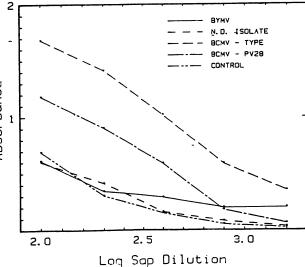


Fig. 2. ELISA reactions between virus isolates and BCMV homologous antiserum

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ENCAPSULATION OF VEGETATIVE CELLS OF BACILLUS POPILLIAE WITH VEGETABLE OIL USING THE WURSTER AIR SUSPENSION COATING PROCESS

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The Japanese beetle, <u>Popilliae japonica</u> Newman, attacks many economically important plants. May _Q June beetles, <u>Phyllophaga</u> spp., feed on trees, shrubs, grasses and crops (4). Some species occur in North Dakota (N. Dak.) and damage lawns, pastures and row crops, particularly corn in southeastern N. Dak. (D. McBride, personal communication).

Milky disease of the Japanese beetle was discovered in New Jersey in 1933 when a few abnormally white grubs were found in the field, and microscopic examination showed the hemolymph of these grubs was teeming with bacterial spores (2). The presence of these bacteria accounted for the white appear ance of the grubs and led to the name milky disease. In 1940, Dutky described two species of bacteria as causative agents of the disease (1). Bacillus popilliae is the better known, and production and distribution of this bacterium has resulted in one of the classic examples of microbial control. However, the preparations available for use are produced in living larvae and have been expensive and in short supply. Attempts to develop a method for economical production of infective spores in vitro have been unsuccessful. Since vegetative cells of B. popilliae can be produced in large numbers on artificial media, there has been interest in investigating the possibility of using them for control. Klein and McMahon (unpublished results) found that vegetative cells contained in coated tung-oil pellets gave up to 93% milky larvae when injected. This observation encouraged us to encapsulate vegetative cells with a commercially available vegetable oil that could be applied using an establish process for coating particles.

B. popilliae NRRL B 2-309 was grown 12 hours (h) in standard medium, centrifuged, resuspended in 5.0% monosodium glutamate plus 0.5% gum tragacanth, frozen in acetone at -70 to -80°C and lyophilized The lyophilized preparation to be encapsulated contained 5.3 x 10¹⁰ viable cells per gram (g). Cells were encapsulated by H. Hall, Coating Place Inc., Verona, Wisconsin using the Wurster Air Suspension Coating Process (3). This process applies and dries encapsulating materials onto particles supported by an upward moving airstream resulting in intimate contact between the particles being coated and the drying air. The movement of particles within the coating chamber is controlled by the size and distribution of perforations in a plate producing a cyclic flow pattern into which the coating material is atomized. The moving particles cycle past a nozzle every 4 to 6 seconds, receiving an increment of coating on each pass. The particles exhibit uniform build-up of coating as the run progresses. The coating used was Kaorich (Durkee Industrial Foods, Cleveland, Ohio). Kaorich is a hydrogenated vegetable oil that is used in the food industry to add solids to shortening systems to improve physical stability and product life. The ratio of Kaorich to cells was 70:30 (wt/wt).

The Wurster Process has been used to coat pharmaceuticals, agricultural chemicals and food ingredients. Results of the present study indicate that the process is also suitable for coating bacteria. After coating, the product was dry and granular and showed a viable cell count of 2.0 x 10^7 per g. After 14 weeks of storage in a desiccator with $CaSO_4$ at 4°C, the count was 3.0 x 10^5 viable cells per g. Coated cells stored 7 weeks under these conditions followed by storage 2 months in a petri dish exposed to the ambient relative humidity (RH) and temperature in the laboratory showe a count of 1.0 x 10^5 viable cells per g. The coating provided some protection from the destructive effect of moisture on lyophilized bacteria. After 24 h of storage in distilled water at room temperature, 17% of the coated cells were still viable. Some cells survived exposure to 42% RH at room temperature for 1 month.

Dr. M. Klein, United States Department of Agriculture, Horticultural Insects Research Laboratory, Wooster, Ohio will test encapsulated cells for their ability to produce typical milky disease in Japanese beetle larvae.

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98 MYCORRHIZAE OF NATIVE GRASSES IN THE SHEYENNE NATIONAL GRASSLANDS OF NORTH DAKOTA.
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When plant roots are colonized by certain specialized fungi a symbiotic organ called 'mycorrhiza' is produced. Several types of mycorrhizae are known, each having its own particular morphology and plant and fungal symbionts. The most common type, called 'Vesicular-Arbuscular Mycorrhiza' (VAM) in reference to certain peculiar fungal structures within the root, is found on most grasses, native forbs and shrubs, and many crop plants (2).

The fungi forming VAM are a highly specialized group, classified among several genera within the family Endogonaceae (Zygomycetes). About 60 species are known. Mycorrhizae have been shown to provide several advantages to their host plants. These include enhanced uptake of phosphorus and some minor elements, increased tolerance to environmental stress, and sometimes protection from pathogens attempting to invade the roots. Additionally, many perennial plants appear to require mycorrhizal status to achieve normal growth past the seedling stage. Much of the research on mycorrhizae concerns forest trees or crop plants. Much less is known about VAM in native grassland communities. Spores of VAM-forming fungi occur within or near plant roots in the soil (1,2).

As a benchmark for further studies on crop or range plants or revegetation of disturbed sites, it is desirable to know what mycorrhizal fungi originally occurred in an area. This study was undertaken to provide that information for a northern mixed-grass prairie site typical of that which originally covered most

of North Dakota.

Collection sites were chosen within the Sheyenne National Grasslands along the Richland County - Ransom County border. In July and again in October 1983, grass plants were dug up with as much soil from the 0-20 cm depth as possible. Spores were extracted from this soil by washing and decanting in water followed by wet sieving and density gradient centrifugation. Roots were cleared and stained and examined for colonization (3). Enhancement cultures using soybean trap plants were also established for six of the grass species (3).

Of the eight grasses examined, all showed a substantial proportion of their root system colonized by VAM (Table 1). The greatest levels of colonization were in little bluestem (A. scoparius), blue grama (B. gracilis) and Junegrass (K. cristata). The lowest colonization was found in needle-and-thread grass (S.

comata) but even this species had nearly one third of its root length colonized (Table 1).

We identified 10 species of mycorrhizal fungi from spores found in soil from the grass collections. The total spore numbers recovered were 300 to 700 per kilogram of soil. There were six species of Glomus, three of Gigaspora and a Sclerocystis. Spore numbers ranged from 400 per kg of soil for a few Glomus species to less than 10 per kg for some Gigaspora species. Several other unidentified Glomus species were present but their spore populations were too low to yield a sample adequate for determination. In addition to species identified from spores extracted directly from the field soil, several species were also identified in the enhancement trap plant cultures.

We found a diverse population of fungi forming VAM in this native grassland soil. Spore numbers were low despite the high levels of root colonization in the grasses. This agrees with findings for other grassland sites (1). Studies on enhancement of plant growth, vegetation establishment, or root protection need to allow for the great native diversity of fungi forming VAM. This may be especially true where disturbed sites are being returned to mixed grassland.

Table 1. Mycorrhizal colonization of eight native grasses.

Species	Percent root colonization		
Stipa comata Trin. & Rupr.	32.4		
Koeleria cristata (L) Pers.	70.0		
Poa pratensis L.	34.5		
Agropyron smithii Rydb.	46.7		
Andropogon scoparius Michx.	71.6		
Bouteloua gracilis (HBK) Lag. ex Steud.	79.3		
Andropogon hallii Hack.	52.0		
Muhlenbergia sp.	56.9		

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CLASSIFICATION OF GRASSLANDS AND SHRUBLANDS IN SOUTHWESTERN NORTH DAKOTA

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Naturally occurring grassland and shrubland vegetation associations were quantitatively analyzed for three consecutive years (1981-1983) as a NDSU Botany Department/U.S. Forest Service project. Fifteen habitat types (h.t.) were recognized on the basis of floristic composition, topography and soils. Classification into h.t. allows field recognition of types and provides information for subsequent management. Vegetation sampling included canopy and basal cover, forb and shrub density, and aboveground biomass. Soil cores were taken and selected chemical and physical properties were measured.

Andropogon scoparius was found as a major component of three h.t. The Andropogon scoparius h.t. occurs on steep upland slopes 18-28%. This h.t. had great species diversity. Grasses made up 11.1% of the basal cover with Andropogon scoparius comprising 56%. Forb density was 5.1/0.1 m. Shrubs were common but grew scattered. Soils were shallow clays (25-50 cm) with exposed scoria common. Production was 2082 kg/ha in 1982 and 2871 kg/ha 1983. The Andropogon scoparius-Juniperus horizontalis h.t. was prevalent upslope on more shallow soils where 58% bareground and scoria was common and only 3-9% vegetation cover. Although basal cover was low, species diversity was high. Forb density was 5.2/0.1 m. Juniperus horizontalis was always abundant while other shrub species varied with exposure and geographic location. Production was 2459 kg/ha in 1982 and 2900 kg/ha in 1983. The Potentilla fruticosa-Andropogon scoparius h.t. is similar to the preceding type, occurring on steep upland slopes (36-38%) with northern exposures. The two types had many species in common, however, the canopy of Potentilla fruticosa contributed 25%. Production varied from 2850-4000 kg/ha in two years.

The Artemisia cana h.t. was common on floodplains and low terraces. Artemisia cana dominated and varied in size (average 48 cm tall) and cover (20-50%). Understory grasses were somewhat sparse (8.2 basal cover) consisting largely of Agropyron smithii and Stipa viridula. Forb density was low (2.4/0.1 m). Production was 3800 and 3970 kg/ha of which 32-65% was shrubs. This h.t. graded into the Agropyron smithii-Stipa viridula h.t. where Artemisia cana was less common (0-1% cover) and shorter (average 26 cm). Basal cover of grasses (11.4%) and forb density (7.1/0.1 m) however, were higher. Agropyron smithii and Stipa viridula were the most dominant grasses, however, Bouteloua gracilis occurred in drier inclusions. Production ranged from 3200 to 3385 kg/ha.

Artemisia tridentata is limited to the extreme southwestern part of the state, but dominates three h.t. The Artemisia tridentata-Bouteloua gracilis h.t. was found on upper slopes and terraces (2-20% slope). Artemisia tridentata varied in size (29-67 cm) and canopy (6-25% cover). A considerable amount of ground cover was present (65%). Opuntia polycantha and O. fragilis occurred frequently. Production ranged from 2328 kg/ha in 1982 to 2875 kg/ha in 1983. The Artemisia tridentata-Atriplex confertifolia h.t. occurred on barren clay areas (46-81% bare soil). Shrubs dominated (18% cover) and forbs and grasses typically occurred in patchy stands. Production was low, 1665 kg/ha in 1982 and 270 kg/ha in 1983. Atriplex canescens was common. The Sarcobatus vermiculatus-Artemisia tridentata h.t. was similar, occurring on old road cuts and stream beds. The two h.t. share all dominant species, however, Sarcobatus vermiculatus was much more abundant making a total shrub cover of 56%. Production was 2577 kg/ha in 1982 and 3890 kg/ha in 1983 with 50% attributable to shrubs.

Calamovilfa longifolia was common on sandy uplands. In the Calamovilfa longifolia h.t. it comprised 18% of the graminoids. Carex filifolia, Carex heliophila and Bouteloua gracilis were also important components. Production was determined as 2215 kg/ha in 1982 and 3200 kg/ha in 1983. The Andropogon hallii-Calamovilfa longifolia h.t. occupied more easily eroded fine sands. The looseness of the soil and abundance of Andropogon hallii (5-20% basal cover) made the types distinguishable. Production was lower, 1709 kg/ha in 1982 and 2057 kg/ha in 1983.

The Stipa comata-Bouteloua gracilis h.t. also occurred on sandy uplands. Stipa comata often appeared to dominate with a large canopy (33%) however, Bouteloua gracilis often had a higher basal cover. Carex filifolia was also prominent. Production was 2277 kg/ha in 1982 and 2386 kg/ha in 1983. On more silty soils, Agropyron smithii became more abundant. The Agropyron smithii-Stipa comata h.t. occurred on level to rolling uplands (0-12% slope). Cool season graminoids dominated with carices contributing 25% of the basal cover. Production was 2416 kg/ha in 1982 and 3138 kg/ha in 1983.

On mesic sites which receive additional moisture from runoff or snowcatch the Andropogon gerardii h.t. occurred. Andropogon gerardii comprised 54% basal cover. Symphoricarpos occidentalis was typical especially in misused areas. Stands were generally small (less than 10 sq m) and infrequent. Production was high, 4323 kg/ha in 1982 and 4630 kg/ha in 1983.

The Agropyron smithii-Bouteloua gracilis-Distichlis spicata h.t. occupied clay soils underlain by a hard pan. Vegetation was patchy with large bare areas ("pan-spots") common. Although the soil restricts root growth, species diversity was high (48 species/stand). Production was 1966 kg/ha in 1982 and 2173 kg/ha in 1983. Vegetation growth was also restricted on the Distichlis spicata-Puccinellia nuttalliana h.t. which occurred on saline floodplains. Distichlis spicata comprised 40% and Puccinellia nuttalliana made up 6% of the basal cover. Agropyron smithii appeared on drier areas while Hordeum jubatum occurred on hydric inclusions. Species diversity was low (27 species/stand) as was ground cover (56% bareground). Production averaged 1570 kg/ha.

VEGETATION OF ABANDONED MINES IN WESTERN NORTH DAKOTA

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The work reported here is part of an extensive study conducted in 1982-83 to assess vegetation-environment relationships on abandoned mines representing a wide range of environmental conditions. Vegetation cover by species was quantified on a total of over 1700 0.5 x 0.5 m quadrats and 1130 point-frame pin-drops at 35 abandoned mines in 18 counties ranging from Canadian to South Dakota border in western North Dakota. Cover was highly variable, not only among mine sites, but also between locations within mine sites. The vegetation cover ranged from 0 to 100% in very proximal areas within the same mine sites. Much of the variation reflects spoil and topographic influences.

Species composition was also highly variable. About 120 genera (115 species) were identified; a majority belonged to the two families Asteraceae and Poaceae. Melilotus officinalis, Agropyron cristatum, Bromus inermis, Agropyron smithii, Poa compressa and Stipa viridula were dominant species at different locations having relatively better plant growth conditions. Grindelia squarrosa, Salsola spp., Lactuca oblongifolia and Kochia scoparia were the most frequent species on different locations having poorer plant growth conditions.

The Columbus mine (1280 acres) in Burke County had a number of interesting localized areas demonstrating differences in plant growth conditions. These include spoil slopes and valleys which had relatively good plant cover, generally with Agropyron cristatum as the dominant species. Other areas had relatively barren spoils with scattered individuals of Kochia scoparia and occasional small patches of vegetation in which Distichlis stricta was the dominant. In some valleys at the base of spoil piles could be found patches of the halophyte Salicornia rubra which reflects the saline nature of the spoil material.

The Velva mine in Ward County had relatively good plant-suitable growth material which supported diverse vegetation including several species of Aster and Solidago. Most of the species found here were perennials, which, along with the diversity of species, is an indication of more advanced ecological succession at this site.

Vegetation at the Lovejoy mine in Williams County and the McKenzie mine in McKenzie County consisted primarily of scattered individuals of *Chrysothamnus nauseosus*, a species common to the Badlands of the Little Missouri River. Other species included *Hordeum jubatum*, *Kochia scoparia* and *Grindelia squarrosa*.

Dakota Star mine in Mercer County had two major sections; the northern section of the mine had poorer vegetation growth and lower species diversity than the southern section. However, *Melilotus officinalis* was the dominant species on both sections. An interesting species, *Silene cseri*, was found only on the southern section of this mine.

The influence of topography was particularly evident on the relatively good plant-suitable growth materials at Wilton mine in Burleigh County. Here *Melilotus officinalis* was dominant with high cover on the relatively cool-moist north-facing slopes while *Agropyron cristatum* provided the most cover on the relatively hot-dry southern exposures. Patches of *Agropyron smithii* and *Stipa viridula* were also found on the south-facing slopes.

Vegetation cover at New Salem mine in Morton County was very low consisting mainly of scattered individuals of Kochia scoparia, Grindelia squarrosa and Hordeum jubatum. This small mine had some of the most unsuitable plant-growth materials.

The Davenport mine in Grant County was found to support a very high vegetation cover with relatively high species diversity. However, the ratio of perennials to annuals and biennials was about 1 as compared to about 6 for the Velva mine, indicating a somewhat earlier stage of ecological succession at the Davenport mine. Again, Melilotus officinalis was dominant on most of this mine. Bromus inermis was found to be dominant on some north-facing slopes while Bouteloua gracilis was dominant on some south exposures.

The Fritz mine in Slope County represents an unusual type of site in the Northern Great Plains in that the spoils were found to be very acidic and hence very unsuitable plant growth materials. Vegetation at this site consisted mainly of small scattered patches of Calamovilfa longifolia and scattered individuals of Artemisia longifolia, Grindelia squarrosa, and Hordeum jubatum. In a localized section at this mine, a relatively well vegetated area (Lotus pursianus dominant) was found adjacent to a poorly vegetated area (Polygonum aviculare present) reflecting chemical differences in the spoils.

The examples presented here illustrate the importance of topography and spoil properties as factors influencing the vegetation cover and composition at these abandoned mine sites. Spoil chemical and physical properties are reported elsewhere in these proceedings.

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ECOLOGY OF WESTERN SNOWBERRY IN THE MISSOURI COTEAU

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The ecology of western snowberry (<u>Symphoricarpos</u> <u>occidentalis</u> <u>Hook.</u>) was studied at the Central Grasslands Research Station near Streeter in south-central North Dakota. The station is located in the Missouri Coteau. The Coteau is a band of morainic hills averaging 30 miles wide and extending northeastward from east-central South Dakota through North Dakota and into southwestern Saskatchewan The most outstanding feature of the Coteau is its hilly, rolling topography which is almost entirely glacial in origin. It consists of closely spaced "hummocks" or knobs alternating with marshy slough or prairie potholes. Water is saline due to evaporative losses in a closed system. There is an almost complete absence of streams or stream-cut valleys on the Coteau.

Western snowberry is a characteristic and abundant shrub species of the Northern Great Plains(1). Within much of its range, it is a characteristic component of the shrub layer of open woods, the forest-prairie ecotone, of chaparral, tame pasture, disturbed areas, and of open prairie and plains. However, it is considered a problem species when occupying large portions of pasture.

Western snowberry is an erect, woody shrub, 30 to 100 cm. tall, spreading from rhizomes. The rhizomes account for the plants aggressive, weedy nature. It often forms dense colonies ranging in size from a meter or two to 50 or 75 meters diameter. Pink, spicate clusters of flowers usually appear in late June. The species rarely becomes established as seedlings, depending on vegetative reproduction almost entirely(1).

Concern over the relative abundance of the species on the Central Grasslands Research Station prompted a study which was initiated in 1982. The objectives were to determine the distribution, density, canopy cover, annual growth, and production of western snowberry communities and their associated graminoid, forb, and shrub species. Distribution was determined from infrared aerial photographs. Approximately 400 hectares of the stations 1336 hectares native range is occupied by western snowberry.

In 1982, fifteen permanent sites were analyzed for density, canopy cover, and production along four, 25 meter perpendicular transects. In 1983, five additional sites were added to the study and annual growth measurements were taken.

Density was obtained by counting the number of stems by age class within a one-eighth meter 2 frame. The five age classes were young, sucker, mature, resprout, and decadent. Western snowberry averaged 74 stems/meter 2 in 1982 and 54 stems/meter 2 in 1983. Mature aged plants dominated both years but a young age structure was exhibited within the community.

Canopy cover was measured utilizing the line intercept method. Intercept and frequency of associated shrub species was also determined. Western snowberry intercept was 44 and 34% in 1982 and 1983, respectively, and frequency was 100% both years.

Production of western snowberry was obtained by removing all annual growth from selected plants. The species contributed 1620 and 1928 kg/ha to production in 1982 and 1983, respectively, which was 97 and 98% of total shrub production. Total herbaceous production in western snowberry communities averaged over 2000 kg/ha both years. Kentucky bluegrass (\underline{Poa} pratensis) alone averaged 60% of total graminoid production, and over 40% of total herbaceous production.

Accumulated growth of western snowberry was measured for four age classes. Measurements were taken at two week intervals five times throughout the growing season. All age classes displayed a similar growth pattern with 90% of all growth having occurred by the middle of June.

This project has provided the baseline data for a long term study on the impact of rangeland grazing systems and associated livestock behavior on western snowberry communities. Research indicates a need for some type of control, as current abundance of the species is unacceptable. However, acceptable levels are as yet unknown. Western snowberry does provide valuable browse and cover for a variety of wildlife species. Cattle graze forage species within the communities and the shrub is utilized as browse to a certain extent in the diet.

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RANGE PLANT COMMUNITIES OF THE CENTRAL GRASSLANDS RESEARCH STATION, STREETER, NORTH DAKOTA

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The Central Grasslands Research Station was established by the Legislature in 1979 as a branch station of North Dakota Agricultural Experiment Station to be used as a range management research facility. The station is located 11.3 kilometers northwest of Streeter, ND, and consists of approximately 2144 hectares, most of which is rangeland. It is located on the glaciated Coteau du Missouri within the mixed grass prairie (1). Climate is continental, with a mean annual precipitation of 43 centimeters and a 120 day freeze-free period (2). In May 1981 a study was initiated to describe and delineate the range plant communities of the research station, to provide information which will aid in establishing stocking rates and provide a reference point upon which the effects of management techniques on vegetation may be measured.

Twenty-two stands were selected for study based on plant-soil relationships. Species composition data were collected during the summer of 1981, and repeated in 1982. Within each stand graminoid cover was determined by the point frame method, with 1000 points per stand. All culms intercepted at ground level were identified and recorded. Forb and shrub composition was determined by density counts in twenty 0.1 square meter (m²) quadrats. Peak standing crop (mid August) was determined from 1981-1983 by clipping 5 randomly located 0.25 m² quadrats at ground level on each site. Clippings were oven dried to an even weight and weighed to the nearest 0.1 gram. Soils of each site were classified to Soil Series.

Nine community types have been delineated based on species composition, production, topographical location and soils. Community types and their relative basal cover values, range sites and yield are presented in Table 1. The Poa pratensis-Agropyron repens-Solidago rigida community is a seral stage resulting from plowing which probably occurred during the 1930's. The other communities are more natural and have not been plowed in the past. Although the Bouteloua gracilis-Agropyron smithii-Poa pratensis community occupies a shallow to gravel range site it is receiving supplemental subsurface water which in part accounts for the atypically high production.

Table 1. Community types and relative basal cover values (%), range sites and yield (Kilograms/hectare † Standard error) of Central Grasslands Research Station.

10 Redeare	Beatron:	
Stands	Range Site	Yield
5	Very Shallow	2384.4 ± 183.6
1	Thin Upland	2665.4 ± 347.5
	•	
5	Silty	3599.3 ± 147.2
	Š	
1	Thin Upland	3807.8 ± 288.8
1	Saline Lowland	3842.7 ± 282.4
1	Shallow to Gravel	3951.8 ± 303.0
2	Silty	4118.8 ± 13.4
	•	
3	Subirrigated	4579.4 ± 148.1
2	Wet Meadow	4769.3 ± 278.8
-		1,0,13 = 2,010
	Stands 5 1 5 1 1 2	5 Very Shallow 1 Thin Upland 5 Silty 1 Thin Upland 1 Saline Lowland 1 Shallow to Gravel 2 Silty 3 Subirrigated

^{1.} Whitman, W. C. and M. K. Wali (1975) in <u>Prairie: A Multiple View</u>, 1st edition (M. K. Wali, ed.), pp. 53-73. University of North Dakota Press, Grand Forks, N.D.

^{2.} Jensen, R. E. (1972) Climate of North Dakota, National Weather Service, Fargo, N.D. 48 pp.

CONTROL OF THE PREOVULATORY SURGE WITH AN ORAL PROGESTOGEN AND GONADOTROPIN

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Strict control of mating behavior has been a long standing goal among livestock researchers and producers. Our objective was to study the effect of a combination treatment of an oral progestogen with different times of administration of a gonadotropin on time of onset of estrus and the preovulatory LH surge. Thirty-two cycling virgin gilts, individually penned, were orally administered 15 mg altrenogest (Allyl-trenbolone, AT) per head per day in their daily ration for 15 consecutive days. Three days prior to AT withdrawal all gilts were surgically fitted with an indwelling tygon cannula in the anterior vena cava via the cephalic vein for repetitive plasma sample collection. The gilts were randomly divided into four groups for purposes of evaluating the proper time to administer PMSG.

Group	No.	Treatment Imposed			
PMS-1	8	750 IU PMSG 1 day prior to AT removal			
PMS-0	8	750 IU PMSG day of AT removal			
Cont.	8	l ml saline day of AT removal			
PMS+1	8	750 IU PMSG l day after AT removal			

All injections were given subcutaneously. Blood samples were drawn every four hours beginning either at the time of PMSG injection or removal of AT. Approximately 24 hrs prior to standing estrus, blood samples were taken at 2 hr intervals until 24 hrs following onset of estrus. Blood samples were centrifuged (2500xg, 4°C, 10 min) and stored at -20°C until analyzed for LH by radioimmunoassay procedures.

TABLE 1. EFFECT OF ALTRENOGEST AND PMSG ON ESTROUS BEHAVIOR AND THE PREOVULATORY SURGE (X+SEM)

AT removal to:	PMS-1	PMS-0	Cont.	PMS+1
Onset of	105.0	97.5	108.6	107.3
Estrus (hr)	±5.2	±4.2	±5.5	±3.9
Onset of	97.4	92.0	97.5	100.8
LH surge (hr)	±6.7	±3.1	±5.2	±4.0
Peak LH	106.6	101.3	106.8	109.3
Conc. (hr)	±7. 2	±3.1	±5.2	±4.7
Maximum	4.3*	9.5	9.3	8.7
LH (ng/ml)	±0.4	±1.1	±0.7	±1.0

^{*}P<.05

All gilts exhibited onset of estrus between 86 and 132 hr following removal of AT with means to SEM reported in table 1. Those gilts which received PMSG on day of AT removal had the shortest interval from removal of AT to onset of heat, onset of the LH surge and time to reach peak LH concentrations. This group also demonstrated the least variation in all three of the previously mentioned parameters. This indirectly indicates that the time of ovulation may have been more concisely synchronized in this group than the other treatment groups. Peak levels of LH measured in the blood was significantly (P<.05) attenuated by PMSG treatment one day prior to AT removal. These results indicate that PMSG may serve to enhance the synchronization properties of AT provided it is administered at the proper time during the treatment sequence. Injections of PMSG too early or late in relation to AT removal may adversely alter the release of the pituitary hormone affecting exhibition of estrus or ovulatory patterns.

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Small pockets of native woodlands occur among the rolling mixed grass prairies of southwestern North Dakota. Their distribution is limited to areas with increased moisture, such as: draws, stream drainageways, springs, floodplains and north-facing slopes. Woodlands compose less than 1% of the vegetation of North Dakota (1), however, their value, both ecologically and economically, is great. They are important for wildlife and livestock habitats, species diversity, soil stabilization, firewood, aesthetics and watershed maintenance. Their value on the northern Great Plains has been estimated at \$10 million annually (2).

As pressure on rangeland resources continues to increase, the need for multiple use management becomes more urgent. Research was conducted during the summers of 1981-1983 to establish a habitat type classification system for these woodlands as a framework for future management. A habitat type is defined as a collective area composed of edaphic, climatic and topographical variables capable of supporting a relatively homogeneous plant community (3). The classification scheme is based on vegetation, soils and topography of relatively undisturbed stands considered to be in good to excellent condition. The vegetation was divided into 4 strata: herbaceous, shrub, sapling and tree. Species composition, species canopy coverage and production were sampled.

A total of 47 stands were sampled and tentative habitat types have been postulated based on the relative cover of the dominant species of each stratum (Table 1). Herbaceous data have not been included in the habitat types at this time due to the high species diversity and interstand variability. Average height and total number of species for each strata in each habitat type are shown in Table 2.

Native woodlands form unique, diverse, productive and important communities which warrant further study and may warrant special management considerations. This study was concerned with undisturbed stands, but the greatest proportion of the woodlands have been impacted in various ways. It will be important to learn more about their composition and productivity in order to compare them to higher condition stands so comprehensive management plans can be formulated.

Table 1. Tentative woodland habitat types of southwestern North Dakota based on relative cover of the dominant species in the tree, sapling and shrub strata.

- 1. Betula papyrifera/Betula papyrifera/Corylus cornuta
- 2. Fraxinus pennsylvanica/Fraxinus pennsylvanica/Symphoricarpos occidentalis
- 3. Fraxinus pennsylvanica/Prunus virginiana/Prunus virginiana
- 4. Fraxinus pennsylvanica/Ulmus americana/Ulmus americana
- 5. Juniperus scopulorum/Juniperus scopulorum/Juniperus communis
- 6. Pinus flexilis/Pinus flexilis
- 7. Pinus ponderosa/Pinus ponderosa/Symphoricarpos occidentalis
- 8. Populus deltoides/Fraxinus pennsylvanica/Symphoricarpos occidentalis
- 9. Populus deltoides/Juniperus scopulorum/Symphoricarpos occidentalis
- 10. Populus tremuloides/Fraxinus pennsylvanica/Prunus virginiana
- 11. Populus tremuloides/Populus tremuloides/Prunus virginiana
- 12. Quercus macrocarpa/Corylus cornuta/Prunus virginiana
- 13. Quercus macrocarpa/Quercus macrocarpa/Symphoricarpos occidentalis

Table 2. Average tree, sapling and shrub height in meters, and the total number of species found in each stratum for each habitat type.

Habitat	Tree	#	Sapling	#	Shrub	#
Type	Height	spp.	Height	spp.	Height	spp.
1.	10.7	4	3.3	10	0.7	17
2.	6.8	4	3.0	9	0.7	9
3.	10.2	4	2.4	7	0.6	9
4.	7.1	4	2.7	9	0.7	14
5.	5.7	2	3.6	2	0.6	9
6.	4.8	2	3.1	2	0.6	4
7.	8.6	2	3.1	3	0.5	8
8.	9.3	3	4.1	5	0.6	11
9.	10.9	3	3.0	4	0.6	8
10.	9.2	4	3.0	15	0.6	15
11.	8.3	5	3.3	9	0.7	15
12.	10.2	4	2.9	11	0.7	12
13.	8.2	3	3.2	7	0.6	9

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THE EFFECT OF LIQUID-SOLID SEPARATION OF DAIRY MANURE ON ANEROBIC DIGESTION EFFICIENCY

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Methane production from agriculture waste has received attention as one alternate energy source to help the United States achieve energy independence. Separating agricultural manures into a liquid and solid fraction has gained acceptance as a waste handling method to ease handling of the waste as well as provide valuable by-products (bedding, mulch, etc.). Combining these two processes could enhance both biogas production and waste handling. (1)

To test the biogas production potential of separated manure and unseparated manure, an experiment of 8 treatments with two replications was constructed. The sixteen 400 ml digesters were batch loaded with 300 ml of influent and 100 ml of "seed" (effluent from a working biogas digester). Dairy manure used for the digester was obtained from the NDSU Dairy Barn. The separated manure was processed with a perforated roller press. The separated and unseparated manure was corrected to 4, 8, 10, 12% total solid (TS) \pm 0.4%. The digesters were maintained at 35°C \pm 0.5°C. Biogas was measured in 330 ml \pm 5 ml water displacement gas collectors. The experiment lasted for 40 days until biogas production dropped to < 4 ml/hour.

The data was analyzed using the Statistical Analysis System (SAS) available at the NDSU Computer Science Department. Cumulative gas production versus time was plotted for each treatment. The production curves were similar, with the magnitude of total biogas produced increasing as influent TS increased from 4 to 12%. There was somewhat greater variation between treatments for the rate plots (ml/hr vs time) compared to cumulative gas production. However, good replication was also obtained for each treatment. There were two digesters that failed to continue to produce gas because of an equipment failure.

A plot of total gas production versus TS of the unseparated and separated manure indicated a possible linear relationship with distinct intercepts but common slope. A general linear model (GLM), a regression analysis program of SAS, evaluated this possibility. The GLM indicated that the relationship of the separated and unseparated manure could best be described as two distinct and parallel lines (95% confidence level).

The correlation coefficient (R^2) for the linear regression was: .963 and .954 for total gas production versus TS and volatile solids (VS) of the unseparated manure and similarly .962 and .945 for separated manure. The two failed digesters were not included in this analysis.

Biogas from each treatment was analyzed once a week for four weeks for a ${\rm CH_4/CO_2}$ ratio to determine if biogas quality had been affected. No significant differences in ${\rm CH_4/CO_2}$ ratio was determined. An average 60% ${\rm CH_4}$ to 30% ${\rm CO_2}$ ratio was observed for both separated and unseparated manure.

The table shows total gas production increasing with increasing TS content for both separated and unseparated manure. This data, coupled with the GLM output, indicate unseparated manure produces more biogas (of a similar $\mathrm{CH_2/CO_2}$ ratio), at a similar rate, then separated manure. For this test, an average of 1850 ml more biogas was observed for unseparated versus separated manure. Combining biogas production with liquid-solid separation needs careful consideration as to economic impact. Reduction of biogas production must be offset with a corresponding economic gain from the solid byproduct of liquid-solid separation.

	Separat	ed Manure (ml)	Unseparated Manure (ml)		
TS%	<u>R1</u>	<u>R2</u>	<u>R1</u>	<u>R2</u>	
4	4,660	1,470*	5,960	5,820	
8	20*	7,750	9.410	9,345	
10	8,700	8,965	11,970	10,950	
12	11,389	11,115	12,745	12,670	

Table 1. Total Gas Production

^{*}Failed Digesters

⁽¹⁾ Holmberg, R.D., D.I. Hill, T. J. Prince, N.J. Van Dyke (1983) Transactions of the ASAE 1803-

High Temperature Reactions of Bibenzyl with Sulfur Robert D. Hei* and Virgil I. Stenberg Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

As part of an ongoing project to understand how sulfur in its various forms catalyzes coal liquefaction (1,2), the coal related model compound bibenzyl (3,4) has been reacted with sulfur, hydrogen sulfide, hydrogen and combinations thereof under coal liquefaction conditions.

Table 1 illustrates the effect that H₂S, H₂, and H₂S-H₂ systems have on the 30-minute thermolysis of bibenzyl at 450°C. The results show that H₂S has no effect on the pyrolysis product, toluene or the hydrocracked products, benzene and ethylbenzene (4). Instead, a rearrangement forming

1,1-diphenylethane (1,1-DPE) is promoted. Within experimental error, the toluene yield is unaffected by the addition of molecular hydrogen. but the yield of hydrocracked products increases dramatically as is expected from the Vernon-Govek

mechanism (3,4). Conversion to hydrocracked products is highest for this system.

The addition of H₂S to the H₂ system produces two prominent conclusions: conversion of bibenzy) to hydrocracked products is lowered while the pyrolysis product is essentially unaffected, and the

yield of rearranged product, 1,1-DPE, increases from two to thirteen mole percent.

A temperature study of the bibenzyl-sulfur system reveals that in 15 minutes at 425°C, bibenzyl is converted over ninety percent to yield nine products together with polymer and gases. Beta cleavage to toluene is dramatically accelerated. Sulfur incorporation is found in two products: 2-phenylbenzothiophene (2-PBT) and 1,2,3,4-tetraphenylthiophene (4-PT). No hydrocracked products are formed.

At 425°C, 15 minutes, the data in Figure 1 illustrates that beta cleavage to toluene is maximized with a So:bibenzyl mole ratio of one. Trans-stilbene is a probable precursor for the formation of toluene, 2-PBT, and 4-PT. Toluene goes on to other products at higher sulfur concentrations.

At a S°:bibenzyl mole ratio equal to one, substantial conversion was found to occur during the

heatup time (2 minutes) with little change occurring after thirty minutes at 425° C. Table 2 illustrates the effect of H₂S and H₂ on the bibenzyl-sulfur system. Selectivity to toluene increases with H₂S concentration while the 2-PBT yield is not influenced. The 4-PT yield, Selectivity to and overall mass recovery decrease. Hydrogen does not effect the toluene yield, but the yields of 2-PBT and 4-PT decrease along with conversion. In separate studies, the main advantage of hydrogen is found to be in converting 2-PBT and 4-PT into starting material and hydrocracked products.

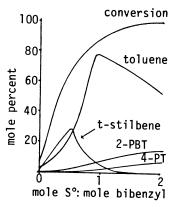
Table 1^a Thermolysis of Bibenzyl Under Ar, Has, Ha, Has-Ha

۲.	<u>د</u> ـ		<i>L</i> .
Ar	H ₂ S ^D	H ₂ ^c	H ₂ SD-H ₂ C
2	-	46	21
51	51	55	49
1	1	24	15
2	11	2	13
45	41	33	42
17	13	-	
55	59	67	58
91	93	94	98
	2 51 1 2 45 17 55	51 51 1 1 2 11 45 41 17 13 55 59	2 - 46 51 51 55 1 1 24 2 11 2 45 41 33 17 13 - 55 59 67

aconditions: 450°C, 30 min, 2.8 mmol bibenzyl, $1.8 \text{ mmol } H_2S$, and

c35 mmol H₂

Figure 1^a Thermolysis of Bibenzyl with Sulfur



^aconditions: 425°C, 15 min, 2.8 mmol bibenzyl

Table 2ª The Effect of H₂S, H₂ on the Bibenzyl-S System

System	S	s-H ₂ s ^b	S-H ₂ ^C
products			
(mole %)			
benzene	-	1	1
toluene	77	93	79
ethylbenzene	-	_	1
tibenzyl	8	1	30
t-stilbene	4	-	-
2-PBT	9	9	4
4-PT	7	3	3_
% conversion	92	9 9	70
% mass recovery	78	63	83

aconditions: 425°C, 15 min, 2.8 mmol bibenzyl, 8.5 mmol H₂S, and

c35 mmo1 H₂

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EFFECTS OF THE WHITE MUSCARDINE FUNGUS BEAUVERIA BASSIANA (BALSAMO) VULLEMIN ON A STEM WEEVIL CYLINDROCOPTURUS ADSPERSUS LECONTE.

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A stem weevil $\underline{\text{Cylindrocopturus}}$ $\underline{\text{adspersus}}$ LeConte has periodially caused economic losses in yield on cultivat ed sunflower in North Dakota. Damage consists of pre-harvest lodging of the plant.

The weevil lays eggs singly beneath the epidermis of the basal portion of sunflower stalks beginning about the middle of July and continuing until the middle of August. Eggs hatch in 7 - 14 days. Initially larval feeding is confined to the subepidermal tissue but progresses upward and inward as the larvae mature. Larvae form small chambers in which they overwinter as well as pupate (in June).

 T_{0} date no single control measure has proven entirely adequate. Due to the inaccessibility of immature stages, the use of pathogens has not been extensively researched. Use of a mechanical shredder could alter field conditions such that pathogens may be considered as a potential control measure. An investigation was conducted to establish the pathogenicity of $\underline{Beauvria}$ $\underline{bassiana}$ (Balsamo) Vullemin toward \underline{C} . $\underline{adspersus}$, and to observe the effects of a high and a low temperature regime on fungal growth and infectivity.

Two spore suspensions were made, each consisting of 0.6 gm. of spores added to 50 ml. distilled water. From each suspension 25 ul. were viewed at 400X and spores counted from 10 random microscope fields. A solution containing 35.5 gm. Sabauroid's dextrose agar (1) and 100 ml. distilled water was used to pour 28 culture plates. Half of the plates were each innoculated with 25 ul. of spore suspension one. Seven innoculated and 7 control plates were kept at 13° C. (56° F.) and 29° C. (85° F.) respectively, and were checked every other day for 14 days.

Overwintering stem weevil larvae were placed in two, 96 chambered microwell plates. From each group 48 larvae were dipped (with a fine brush) into spore suspension two and a microwell plate placed in each temperature regime. From both weevil larvae and culture plates, microscope preparations were stained with a 1% eosin dye solution (2) and examined.

Colony morphology and microsopic structure of conidia and conidiophores were in agreement with the taxonomic description of \underline{B} . $\underline{bassiana}$ (3). Colony growth at 29° C. ranged from 7 - 10 days ahead of colonies held at 13° C.

Stem weevil larvae held at 29° C. were able to complete development to adults in two weeks. In both the control and the innoculated group no mortality was noted.

Innoculated stem weevil larvae held at 13° C. became sluggish in 5 - 6 days. Hyphal growth was observed by the 14th day. Larvae developed a rose color (4), which is consistent with the ability of <u>B</u>. <u>bassiana</u> to produce different pigments when utilizing differing nutrient sources (5). At the lower temperature there was 100% mortality of innoculated larvae. The control group at 13° C. completed development about 14 days later than those at 29° C.

Under laboratory conditions white muscardine fungus will kill stem weevil larvae. So far as could be ascertained, this was the first trial of \underline{B} . $\underline{bassiana}$ on a sunflower pest. Two interdependent areas of investigation are suggested: testing of \underline{B} . $\underline{bassiana}$ on other sunfower pests, and studying the feasibility of using this pathogen as \underline{part} of an integrated pest management program for sunflower.

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THE EFFECTS OF METALS AND IONS ON HIGH AFFINITY DIGOXIN BINDING

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Digoxin is the most widely used cardiac glycoside and is used extensively to treat inotropic abnormalities and arrhythmic disorders of the heart. Digoxin and its allied glycosides are the treatment of choice for congestive heart failure, and various atrial and nodal dysrhythmias.

The main effects of digoxin are mediated through the sarcolenmal membrane at the heart, but this drug has been shown to bind with specificity and high affinity to receptor sites located in the central nervous system. The binding to the central receptors could possibly account for the hallucinogenic or behavioral changes associated with the prolonged use of the drug. These investigations are focused to quantify the role of neural control of cardiovascular function and specificially, to determine how digoxin may affect this control.

[3H]-digoxin has been shown to bind in a specific, saturable manner, dependent on time, temperature, and the presence of Mg and Tris-ATP. Utilizing male albino Sprague-Dawley rats, high affinity in vitro binding, and rapid suction filtration techniques, a concentration dependent ion-induced (e.g. Na) enhancement or inhibition of [3H]-digoxin binding by metals (e.g. Hg, Cd, Zn) has been demonstrated.

The study has been divided into three parts: 1) the first stage deals with the <u>in vitro</u> effects of these metals or ions on [H]-digoxin binding in whole brain homogenates; 2) the second part deals with the <u>in vitro</u> effects of metals and ions on [H]-digoxin binding in seven different areas of the rat brain; 3) finally the <u>in vitro</u> effects of metals on [H]-digoxin binding in developing rat brains were determined. In each phase of the study, three distinct sets of homogenates obtained from different animals were used. For the second phase the seven brain areas were isolated and each separate area pooled from several rats. [H]-digoxin binding is reported as nanomoles bound per gram protein; the protein estimation is by the method of Lowry.

These investigations have demonstrated the specific high affinity binding of $[^3H]$ -digoxin to preparations obtained from seven different areas of the rat brain. The binding of $[^3H]$ -digoxin was significantly increased in the presence of added NaCl to the incubation medium and such an increase was dose dependent. A comparative evaluation of metals' effects on $[^3H]$ -digoxin binding in whole brain homogenates was undertaken and the results indicate an order of potency (micromolar range): $Hg \rightarrow Cd \rightarrow Ag \rightarrow ZD \rightarrow Pb$ toward inhibition of digoxin receptor binding. The Group IIB metals ($Cd \rightarrow ZD \rightarrow Ag \rightarrow ZD \rightarrow Pb$ toward inhibition of $[^3H]$ -digoxin binding in the seven separate brain areas. All brain areas were inhibited, however the sensitivity of the metal ions towards stimulatory effects of NaCl on $[^3H]$ -digoxin binding was also demonstrated to a greater extent by observing selective inhibition of the binding in these studies. Finally, a developmental aspect of the study was conducted to ascertain whether changes in receptor populations occur as a function of age. The binding characteristics of the receptors increased with age and similarly to adult rat populations, addition of NaCl significantly enhanced $[^3H]$ -digoxin binding.

These results have provided evidence for the binding of [3H]-digoxin to brain preparations and the stimulatory effects by added NaCl, which is more sensitive to the actions of Cd , Zn , and Hg ions and could presumably account for the neural control of cardiovascular functions and the involvement of metal ions in heart disorders. Further, these studies may help towards understanding the long term neurological effects that accompany chronic digitalis use in patients suffering from congestive heart failure as well as other cardiac disorders.

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PORCINE PROLACTIN SECRETION ASSOCIATED WITH TWO DIFFERENT MATING TREATMENTS

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The stimulus of mating at short intervals has been reported to produce endocrine changes favoring greater ovulation rates and increased fertility (1). Our purpose was to examine the effect of mating at fifteen minute intervals on prolactin concentrations at varying intervals related to time of mating. Virgin cycling gilts which had expressed at least one prior estrous period were exposed to two matings to fertile males at 12 h intervals (N=9). The treatment animals (N=10) were mated twice at 12 h intervals with fertile males, then exposed 15 min after each fertile mating to a vasectomized male for a subsequent mating stimulus. All females were cannulated via the cephalic vein five days prior to mating. Blood samples were drawn hourly from the onset of estrus until one hour before mating. Beginning one hour before mating, samples were collected every 15 min until five h after mating. This same 15 min sampling procedure was repeated at the second mating. Blood samples were immediately centrifuged (2500 x g, 4°C, 10 min) and the plasma aliquots stored at -20°C until analyzed for prolactin by radioimmunoassay procedures (2).

plasma prolactin concentrations before and after mating are presented in table 1. Prolactin was significantly (P<.0005) elevated following mating in both treatment and control females. Two - six hours following the mating experience prolactin plasma concentrations had returned to the baseline values observed prior to mating. Although differences between the control and treatment gilts were large, statistical significance was not observed because of the large individual variation which existed following mating. Plasma prolactin differences (table 2) after first and second mating were significantly different (P<.02). Mating experience tended to result in less of a stress reaction at the second mating than at the first.

TABLE 1. Plasma Prolactin Concentrations Before and After Single or Repeated Matings

	Tı	Treatment		ontrol
	<u>N</u>	Ng/ml	<u>N</u>	Ng/ml
One hour before mating a One hour after mating	10 10	3.89± .71 ^b 6.49±1.24 _b	9	2.66±.36 ^b 3.27±.58 ^c
2-6 hours post-mating	10	4.27±1.15 ^b	9	2.31±.42 ^b

b, cDifferent superscripts within columns represent P<.0005.

TABLE 2. Plasma Prolactin Mean Concentrations for One Hour After First or Second Mating
_____ (± SEM)

	First Mating		Seco	nd Mating
	N	Ng/ml	<u>N</u>	Ng/ml
Fertile male only	9	3.12±.39 ^a 5.14±.94 ^c	9	2.38±.37 ^b 4.63±.81 ^d
Fertile male + vasectomized male	10	5.14±.94°	10	4.63±.81 ^d

a,b,c,d Different superscripts within rows significantly different at P<.02.

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THE EFFECT OF LEAF PUBESCENCE ON WHEAT LEAF RUST INFECTION

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Wheat leaf rust infection, caused by Puccinia recondita f. sp. tritici, is dependent upon the amount of moisture available during the incubation period (3). The more free moisture available during the incubation period, the more penetration and infections are likely to occur. Previous sta dies with leaf glaucousness have shown that the leaf surface plays a role in the amount of moisture retained on the leaf surface and in the number of rust pustules formed. The objective of this study was to look at the role of pubescence on the leaf surface in relation to available moisture and the number of rust pustules formed on the leaf surface.

Three cultivars of wheat (Triticum aestivum L.) were used in the study. The two non-pubescent cultivars were Olaf and Thatcher. The pubescent cultivar was Olaf*4//Polk/CI8515 (Olaf*). Plants were grown in the greenhouse at 210 ± 30 C under a 12 hour photoperiod, in 4 inch clay pots in an autoclaved mixture of peat, sand and clay soil. The primary leaves of 10-day old seedlings were im culated with 1 mg of urediospores (culture X65-virulent to all three cultivars) in 3cc of Soltrol 170 oil. Plants were misted by a hand held mist sprayer and incubated in moist chambers for 24 hours. The number of rust pustules were counted 10 days following incubation on 10 leaf sections each 5 cm long 1 cm from the apical end. Data were analyzed using a factorial design and Duncan's multiple range test.

To measure wettability the contact angles of water droplets on the external surface of the leaf were determined using the method described by Fogg (2). A Baush and Lomb microprojector with its apical axis horizontal was used to project the image of a water droplet resting on a horizontal leaf surface. Six different treatments were used involving a 0.5% crystal violet solution in distilled water and a 0.5% crystal violet solution with 1% Tween-20. The treatments also included an undamage leaf surface, a dry rubbed leaf surface and a wet rubbed leaf surface. A droplet about 3 mm in diameter was placed on the leaf surface with a glass pipet and the projected image traced. Five contact angle measurements were taken for each treatment. Contact angles were computed by use of Ebelings equation (1). Data were analyzed with a factorial design and Duncan's multiple range test.

In the three cultivars studied significantly more pustules were formed on the non-pubescent cultivars than pubescent cultivar (Table 1). Data from the water droplet contact angle demonstrated that an undamaged leaf surface had a higher contact angle than a damaged leaf surface. It also

Table 1 Average number of rust pustules for three cultivars of wheat

cultivals of wilcut.	
Cultivar	Rust Pustules
Thatcher	60 a ¹
01af	57 a
01af*	36 b

Table 2 Water droplet contact angles under 6 epidermal treatments (averaged over 3 cultivars).

o carcivars,		
	age cont	act
Epidermal Treatment	angle	_
1. undamaged; 0.5% crystal	128.53	al
violet		
2. dry rub; 0.5% crystal violet	114.97	b
3. wet rub; 0.5% crystal violet	111.72	b
4. undamaged; 0.5% crystal	83.84	С
violet + 1% Tween-20		
5. dry rub; 0.5% crystal violet	65.74	d
+ 1% Tween-20		
6. wet rub; 0.5% crystal violet	56.53	d
+ 1% Tween-20		

 $^{^{}m I}$ Values with the same letter not significantly different according to Duncan's multiple range test.

demonstrated that use of a surfactant such as Tween-20 reduced the contact angle of a water droplet even further. There was no significant difference between damaging the leaf surface with a wet or dry rub (Table 2). Significant differences did exist between the water droplet contact angle on pubescent and nonpubescent cultivars. The contact angles were higher the pubescent cultivar.

The study indicates that pubescence has an effect on the number of rust pustules formed on the leaf surface. This occurrence may be due to higher water droplet contact angle at the leaf surface which increases the possibility of the droplet rolling off the leaf surface. Rubbing the leaf surface, which simulated weathering in the field, decreased the water droplet contact angle significantly in both the pubescent and non-pubescent cultivars. Leaf pubescence may provide a passive, but effective means of resistance to wheat leaf rust infection.

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EFFECT OF pH ON SELECT METAL SOLUBILITY IN BOVINE AND HUMAN MILK

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Absorption of zinc is more efficient from human milk than from bovine milk (1). This is critical for an infant with acrodermatitis enteropathica, a rare hereditary disease in zinc malabsorption. for an incompanie the state of bovine milk; human milk can keep the disease in remission. Such in human milk components has yielded a number of zinc-binding ligands which may or may not affect intestinal absorption of zinc (2,3). Regardless of the ligand(s) responsible for metal absorption, a metal must be soluble in order to be absorbed; and solubility is strongly influenced by pH (4). Therefore, we have examined the solubilities of calcium, magnesium, and zinc over a pH-range of 2 to 8 in solutions containing these metals and several low-molecular-weight metal-binding ligands.

Aqueous solutions were prepared that mimicked bovine and human milks in concentrations of calcium, magnesium, zinc, citrate, phosphate, glutamate, and glutamine. These solutions are called milk models. The pH of aliquots of these solutions were adjusted, and any precipitated material was removed by centrifugation. Supernatant solution was analyzed for the three metals by atomic absorption spectrophotometry, metal in this fraction being taken as a measure of that metal in a soluble form. Unprocessed bovine milk and fresh human milk were defatted by centrifugation, and aliquots were pH-adjusted. Precipitate in the milks was removed by centrifugation and the supernatant fraction was ultrafiltered through an Amicon PM10 membrane. Ultrafiltrates were analyzed by atomic absorption spectrophotometry as a measure of soluble metal.

The solubilities of the metals in the bovine milk model are plotted in Fig. 1. The solubilities of all metals decrease as the pH increases. Calcium solubility declines markedly above pH 4.5. The

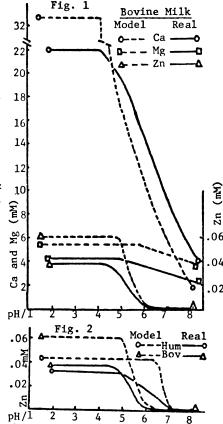
zinc solubility drops at pH's above 5, so that by pH 6 essentially all zinc in the bovine milk model is insoluble. The solubilities of the metals from real bovine milk are plotted on the same graph. It is apparent that the milk model solution, containing only a limited number of components, is able to mimic closely the solubilities of metals in real milk, although the solubilities of the three metals decline at lower pH's in the real milks. The magnesium solubility curve begins to drop at pH 5 in real milk, yet is otherwise parallel to the magnesium curve of the model. Calcium solubility drops at a slightly lower pH as well, yet also follows the decline of calcium solubility in the model. Zinc in real milk precipitates as early as pH 4.5, but remains partially soluble up to pH 6, as in the model. A similar comparison between a human milk model and real human milk shows that a milk model is also able to mimic the solubilities of calcium, magnesium, and zinc in real human milk. Comparing metal solubilities of the two milks, there are greater amounts of soluble calcium and magnesium in the bovine milk than in human milk over a pH-range of 2 to 8 (data not shown). This is not the case for zinc.

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The solubility curves of zinc in both of the milk models and both of the real milks are plotted in Fig. 2. Zinc in real human milk, although exhibiting diminished solubility above pH 5, is still soluble up to pH 7, as in the model. This is a full pH unit greater than the highest pH at which zinc is soluble in bovine milk. The differences in zinc solubility between the milks have been reproduced in subsequent experiments.

From the data presented, zinc is soluble over a wider pH-range in human milk than in bovine milk, and should, therefore, be more available for absorption.

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FORB AND BROWSE UTILIZATION AS INFLUENCED BY RANGE SITES AND GRAZING TREATMENT

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Since 1981, research studies have been implemented at the Dickinson Experiment Station Ranch to compare short duration grazing and repeated seasonlong grazing. This particular study, spanning 1982 and 1983, determined the effects of these two grazing treatments on livestock use of forb and browse species.

The study area (located 5 miles southwest of Manning, North Dakota) is typical of the mixed grass prairie. Precipitation averages 38 cm a year, with 80% of that falling between April and September. Average monthly temperature ranges from a low of $9^{\circ}F$ for January to a high of $80^{\circ}F$ for August.

A section of land, 640 acres, was divided into two 320 acre grazing treatments. The short duration treatment consisted of 8-40 acre pastures, each pasture being grazed 5 days and then rested for 35 days before being grazed again. Cows were grazed from the beginning of June to the middle and latter portion of October on both treatments for 1982 and 1983, respectively. Twenty cow-calf pairs were stocked on the seasonlong treatment while 75% more pairs, 35, were allocated to the short duration treatment.

Five range sites comprise most of this section of land, hence they were chosen for further investigation. Representative areas of each were delineated on both grazing treatments and production and utilization of grass by class, and forb and browse by species was estimated using caged and uncaged paired plots, double sampling and regression.

For the two years of study, grass utilization was similar between the two grazing treatments (Table 1). Forb utilization showed a three-fold increase on the short duration treatment over the seasonlong treatment, and was more evenly distributed over the five sites. It seems forb utilization per site is negatively correlated with grass productivity for that site regardless of the grazing treatment. Browse utilization showed a slight increase on the short duration treatment.

Forbs have the ability to provide a considerable quantity of forage as well as high quality forage; especially early in the grazing season. This study indicates that short duration grazing may have the potential to take advantage of this important forage producing class.

Table 1
Production and utilization by site on two grazing treatments

C+.					Sites				
31.	lty	Sar	ndy	Shal	Llow	Thin (Claypan	Clay	yey
SD1	SL1	SD	SL	SD	SL	SD	SL	SD	SL
509	655	320	304	377	347	264	425	181	183
0	0	7	0	10	7	19	12	25	5
1489	2422	1442	1468	1090	1121	658	635	1200	1657
40	63	52	52	19	41	31	11	78	26
97	29	151	173	41	99	122	48	19	14
0	0	12	0	0	0	0	0	24	0
	509 0 1489 40	509 655 0 0 1489 2422 40 63	509 655 320 0 0 7 1489 2422 1442 40 63 52 97 29 151	509 655 320 304 0 0 7 0 1489 2422 1442 1468 40 63 52 52 97 29 151 173	509 655 320 304 377 0 0 7 0 10 1489 2422 1442 1468 1090 40 63 52 52 19 97 29 151 173 41	509 655 320 304 377 347 0 0 7 0 10 7 1489 2422 1442 1468 1090 1121 40 63 52 52 19 41 97 29 151 173 41 99	509 655 320 304 377 347 264 0 0 7 0 10 7 19 1489 2422 1442 1468 1090 1121 658 40 63 52 52 19 41 31 97 29 151 173 41 99 122	509 655 320 304 377 347 264 425 0 0 7 0 10 7 19 12 1489 2422 1442 1468 1090 1121 658 635 40 63 52 52 19 41 31 11 97 29 151 173 41 99 122 48	509 655 320 304 377 347 264 425 181 0 0 7 0 10 7 19 12 25 1489 2422 1442 1468 1090 1121 658 635 1200 40 63 52 52 19 41 31 11 78 97 29 151 173 41 99 122 48 19

l SD **–** Short duration

SL - Seasonlong

CCCURRENCE OF MULTIPLE ANTIBIOTIC RESISTANT ENTERIC BACTERIA IN DOMESTIC SEWAGE AND OXIDATION LAGOONS

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Several genera of enteric bacteria were isolated from the Grand Forks, North Dakota sewage collection and treatment systems which demonstrated multiple antibiotic resistance (MAR) to three currently utilized antibiotics: Ampicillin, Tetracycline, and Kanamycin. These plasmid-containing enteric microorganisms made up approximately 5% of the total population of organisms isolated on MacConkey medium during the Summer (June, 1983) and about 0.4% of the total population of organisms isolated on MacConkey medium during the Winter (January, 1984).

Experimental transfer of the MAR-plasmids to an $E.\ coli$ strain K-12 (nalidixic acid resistant and lactose negative) occurred at rates as high as 90% with some of the isolates and as low as 0.01% with other isolates. Some of the MAR isolates were not capable of transferring the MAR-plasmid to the K-12 receiver strain.

Transfer of the MAR-plasmid was best achieved when the donor organisms were in the stationary phase of growth; the growth phase of the recipient seemed less important.

Table 1

Seasonal Incidence of Multiple Antibiotic Resistant Enteric Bacteria in the Grand Forks Sewage Treatment System

Sample/	Colony Forming	Units/ml
Nato	Total Coliforms	MAR*
Composite Raw 6/83 8/83 11/83 1/84	2 x 10 ⁴ 3 x 10 ⁵ 7 x 10 ⁵ 2 x 10 ⁵	1 x 10 ³ 4 x 10 ³ 2 x 10 ² 7 x 10 ²
Primary Lagoon 6/83 11/83	3 x 10 ³ 1 x 10 ³	8 x 10 ¹ 4 x 10 ¹
Secondary Lagoo 6/83 8/83 11/83	3 x 10 ³ 4 x 10 ² 3 x 10 ¹	2 x 10 ³ 3 x 10 ¹ <1

^{*}MAR - multiple antibiotic resistant organisms.

Table 2

Experimental Transfer of Multiple Antibiotic Resistant Plasmids from Wild Type Biotypes Isolated from Grand Forks Sewage Treatment System to a Receiver Strain

Wild Type	% Transfer to
Donor	Receiver Strain
E. coli (0144473*) E. coli (4144572) E. cloacae (3005573) E. coli (5044552) C. freundii (1004572) E. agglomerans (0005553) E. coli (5144562) K. pneumoniae (5205773) E. coli (414556572) E. coli (1144572) E. coli (0144572)	90 50 43 41 35 20 10 5 1 0.1 0.01

^{*}API biotypes

THE EFFECT OF BARLEY STRIPE MOSAIC VIRUS ON ISOZYME PATTERNS IN BARLEY SEEDLINGS

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Isozymes are multiple molecular forms of an enzyme having similar or identical catalytic activities and occuring within the same organism. Isozyme techniques have been extensively used in many of the biological sciences since they have advantages of direct visulization of gene products, specificity and simplicity (1). Little work has been reported on the interaction of a plant virus and its host in relation to isozyme variations. This paper reports some of the results in our preliminary work on barley isozymes as affected by barley stripe mosaic virus (BSMV). Two of the isozyme systems, acid phosphatase and acetylesterase, involved in respiration were detected.

MATERIALS AND METHODS Specific isozymes were detected by utilizing gel electrophoresis and selectively staining methods(1,3). BSMV susceptible (Black Hulless, C.I. 666) and BSMV resistant (C.I. 4197) barleys (Hordeum vulgare L.) were used. Seedlings were grown 7 days at 25+10C with a 16 hr light period and then inoculated with CV52, a virulent, BSMV strain, and CV42, a mild strain, respectively. Seven days after inoculation the seedlings were harvested and homogenized with 1:2 (W/V) extracting buffer (0.1 M Tris-HCl, pH 8.0, containing 0.5 M sucrose, 0.1% ascorbic acid, and 0.1% cysteine-HCl). The homogenate was filtered, and centrifuged at 30,000 g for 30 min and then at 110,000 g for 30 min to obtain a supernatant of barley soluble proteins. Sucrose and bromophenol blue, the tracking dye, were added to a concentration of 10% and 0.002%, respectively. Electrophoresis was based on Davis (2) procedure. A non-denaturing gel system of 7% polyacrylamide was used for isozyme separation. Electrophoresis of the soluble proteins was carried out at 2mA/gel for 30 min and then at 3 mA/gel until the tracking dye was near the bottom. Specific enzyme staining: Acid phoasphatase - Gels were preincubated with 0.1 M acetate buffer, pH5.0, and incubated at 37° C with a staining solution containing α -naphthyl acid phosphate (lmg/ml), fast blue RR salt (lmg/ml), NaCl (20mg/ml), polyvinylpyrollidone (5mg/ml) and a few drops of 10% MgCl₂, in 0.1 M acetate buffer, pH 5.0, until the isozyme bands were developed (5). Acetylesterase - Gels were preincubated with 0.2M Tris-HCl, pH7.0, and incubated at room temperature with a staining solution containing α -naphthyl acetate (0.3 mg/ml), fast blue RR salt (lmg/ml), in 0.2 M Tris-HCl buffer, pH

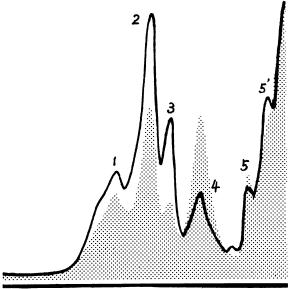


Fig. 1. Acid phosphatase patterns in Black Hulles barley infected with CV52 strain of BSMV (shaded area refers to the healthy check.)

7.0, for about 3 hrs. After staining the gels were washed and stored in 7% acetic acid. The isozyme patterns were examined and scanned at 580 nm.

RESULTS AND DISCUSSION Significant changes of acid phosphatase activity in both barley cultivars were found after BSMV inoculation. Activities of three of the major isozyme bands (1,2 and 3, Fig. 1) in C.I. 666, were greatly increased, the activities of two other bands (4 and 5) were decreased, and as a whole the enzyme activity was increased. The change of the isozyme activities was correlated with the virulence of the virus strains. In C.I. 4197 which showed no symptoms after inoculation, the activity of all acid phosphatase components was decreased. No significant changes on the acetylesterase patterns were found in either cultivar after inoculation. The increase in acid phosophatase activity may be associated with the change in respiration of the susceptible barley cultivar after BSMV infection. It is interesting that in the resistant cultivar C.I. 4197, the isozyme pattern was changed after inoculation but the plants showed no visible symptoms. The alterations of the isozyme patterns could serve as markers or "biochemical symptoms" in the study of resistance and susceptibility in future work.

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FOSSIL FISH SCALES FROM THE ALMONT GINKGO SITE

47

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The purposes of this research were to: 1) determine the life history and type of fish by examining the fish scales, 2) determine the environment of deposition at the site, and 3) project the paleowater-ecology of the site.

The Almont Ginkgo Site is a fossil site principally containing flora of a Paleocene age. It is located on the Naegeli Ranch north of Interstate Highway 94 north of Almont in Morton County, North Dakota. The small hill which comprises this site is at an elevation of 2200 feet above sea level. In the Fall of 1983, fossil fish scales were collected from surface middens of already disturbed stone and from the digging of three random sample units. Near perfect preservation of the scales has allowed for analysis of the types and growth rings and structure. Each scale seems to be a combination of the cycloidal and ctenoidal types common to fish today. Each is one unit wide compared to 3/4 unit from front to back. Most measure in the range of lcm x 3/4cm. They have radiations from the nucleous to the back which number from 5 to 7. There are about 50 identifiable scales and perhaps some regenerated ones without the growth rings. Most of the scales seem to be from fish about three to four years old.

Hand specimens of the rock showed it to be a highly siliceous cherty shale with brittle, slabby properties. It appears to have an iron-stain throughout and ranges in color from yellowish to black. It is well layered and many times breaks along the bedding planes. This lamellar bedding can develop only in quiet waters where it cannot be destroyed. In quiet deeper waters, delicate organisms can be preserved. Presence of laminations indicates a lack of burrowing bottom scavengers, since they tend to homogenize the sediment. Agitation which is too vigorous prevents fine laminations because siltand clay-sized particles are not likely to be deposited if they remain in suspension. When current flow essentially ceases, the fine-grained uniform suspension settles out to form a poorly sorted mixture of fine sand, silt, and some clay.

Thin section analysis of the shale shows some microscopic varves. The time factor in sedimentation can be seen in the varve type of bedding, where paired layers of sediment of contrasting composition or texture are developed for each seasonal cycle. The microscopic varves of these specimens seem to be based mostly on coloration change rather than on a change in particle size. Alterations of light and dark layers can be correlated with differences of settling velocity of the introduced materials or with seasonal variations in the characteristics of the introduced materials. In some siltstones, laminations are seen to be due to variations in mineral composition of the silt particles. Heavy—mineral accumulations in certain lamellae, particularly where the minerals are iron-bearing and are subject to oxidation and rust-staining, produce noticeable color contrasts.

Many cherts are associated with contemporaneous volcanoes. Inorganic silica may be derived by prolonged submarine leaching of slowly accumulating volcanic ash. Iron-silicate minerals may be finely laminated materials. They generally occur as clay-sized to fine-sand-sized flakes with abundant admixed chert and iron oxides. Iron staining is common on the weathered surfaces. Shale pigments may be of ferric iron which is disseminated hematite or limonite and imparts a red or purple color; of ferrous iron which is a greenish color but which turns to light yellow, tan, or brown on exposure; or of carbon giving a black color. While some sooty black appearance of the shales may be due to the presence of carbon, such shales also may have a high iron-sulphide content and may have accumulated under anaerobic conditions. The essential condition is complete lack of bottom circulation so that dissolved oxygen, soon exhausted, cannot be replenished, while a high proportion of organic matter may be contributed by animal and plant remains falling from the superficial aerated layers. Where there is a paucity of oxygen the normal bottom-scavenging animals will not be able to live because of toxic conditions resulting from the accumulation of organic wastes.

Lack of any evidence of faunal remains other than the fossil fish scales which could have been transported into the area, leads me to conclude that the waters in which the flora and the fish scales were deposited must have been toxic to fauna possibly from lack of oxygen and from acidic conditions which may have been present from the ash of volcanoes to the west of the area in the Paloecene times. The almost perfect preservation of the fossil specimens could also be due to anaerobic conditions and to the absence of water currents or burrowing animal disturbances. I believe that the water was a low energy, quiet water possibly a narrow landlocked lake of the ox-bow type which was gradually drying up. This could account for the lack of many structures in the shales and the accumulation of the 10 to 15 inches of shale would have taken many years. The shales are what is left of a lake pavement while the surrounding area has been eroded away.

INFRA-RED ASSAY OF D20 FOR THE DETERMINATION OF TOTAL BODY WATER

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Deuterium oxide (D₂0) dilution has been widely used to measure total body water (TBW) in humans. Assay of D₂0 concentrations in plasma has been done by mass spectrometry (1), gas chromatography (2), infra-red spectrometry (3,4), and other techniques, typically using doses of D₂0 in the 1-2 g/kg body weight range and yielding plasma D₂0 concentrations of 0.5 to 3 mg D₂0/g H₂0. We report an inexpensive infra-red method with good precision using doses of 10 g D₂0.

Human volunteers were recruited from college athletic teams. Their participation was approved by the USDA and UND Human Studies Committees and all procedures were consistent with the Declaration of Helsinki. Subjects gave written consent to participation in the study.

Subjects consumed weighed 10 g doses of 99.7% D_20 with 300 ml of apple or orange juice after an overnight fast. Blood was collected at 30, 60, 90, 120 and 180 minutes after the dose. All urine passed during the 180 minute interval was also collected. Plasma and urine were stored frozen under N_2 until analysis.

Precipitation of plasma protein by CuSO₄ (3), FeCl₃ or HgCl₂ was unsatisfactory as each yielded little supernatant. CdCl₂ gave satisfactory yields of supernatant but recovery of D₂O from spiked plasma was only $76\pm7\%$ (mean \pm SD). Distillation (3) of untreated plasma gave a recovery of ($56\pm9\%$) D₂O. Lyophilization of untreated plasma gave D₂O recovery of 99.6 $\pm5.6\%$.

DoO concentration in purified water samples was measured using a Foxboro Analytical Miran-1FF (5), (South, Norwalk, CT) fixed filter single beam infra-red analyzer and a sealed CaF2 cell with 0.2 mm path length. Within the range of $10-25^{\circ}\text{C}$, absorbance increased linearly with decreasing temperature. Temperature was controlled at $15\pm0.5^{\circ}\text{C}$ by water circulating through a cell jacket. Standard curves were obtained each day. Precision of duplicate measurements was 3% or better. Day-to-day variability in the slope of the calibration curve was less than 10%.

Gravimetric standards were prepared using weighed amounts of D_2O and deionized water. Concentrations ranged from 0.01 to 0.3 mg D_2O/g H_2O .

Equilibration of D₂O concentration in plasma was reached in 2 hours or less in all 10 subjects. Values for TBW ranged from 33.4 to 56.8 L. An indirect validation of this method showed a good correlation (r=0.93, p<0.001) between individual values of TBW and fat-free mass determined by hydrostatic densitometry.

Previously published methods have employed doses of D₂O five to ten times greater than those employed here. The lower, 10 g dose allows repetitive measurements in the same subject without undue elevation of fasting plasma D₂O concentrations. Furthermore, the availability of this method should reduce the use of tritiated water, a radioactive marker for determination of TBW, which will facilitate the measurement of TBW in the study of human body composition. The instrument employed is simple and inexpensive.

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THE UNEXPECTEDLY FACILE OXIDATION OF NATURALLY OCCURRING SULFUR IN OVERBURDEN MATERIALS OF WEST-CENTRAL NORTH DAKOTA

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The generally accepted hydrogeochemical model for the evolution of ground water in western North Dakota and similar semi-arid areas (1) considers the oxidation of naturally occurring reduced sulfur (pyrite) to be the ultimate source of these region's high sulfate levels. This process is expressed by the following reaction:

4 FeS₂ + 15 O₂ + 14 H₂O
$$\longrightarrow$$
 4 Fe(OH)₃ + 16 H⁺ + $8SO_4^{2-}$

Since the products of this oxidation seriously threaten the ecological and geochemical stability of overburdens disturbed by surface mining, an accurate assessment of the rate at which it occurs is essential for the design of successful reclamation strategy. Although conventional wisdom dictates that the rate at which this reaction proceeds is quite slow, this assumption has not been confirmed by scientific studies. Thus, research was carried out to determine both the rate and extent of this oxidation.

Two types of tests were performed on core samples of undisturbed overburden: exhaustive leaching and accelerated weathering. The leaching tests, in which samples were ground, put in excess excess amounts of distilled water, and then continuously shaken in constant temperature baths for periods ranging from two to seventy-two hours, were designed to remove all of the oxidized sulfur present in the soil. The accelerated weathering experiments, which subjected the residual materials recovered from leaching to extremes in temperature, moisture, and pH and to excess oxygen for a period of twenty-eight days, were intended to determine the effects that severe conditions would have on the oxidation of pyrite.

water recovered from the leaching process was analyzed for dissolved sulfates. It was found that the amount of sulfates leached from a sample varied proportionally with the amount of water used to leach it and that this amount diminished considerably with successive leachings. Interestingly, more sulfates were present than could be accounted for by the amount of oxidized sulfur known to exist in the soil. The most direct conclusion that can be drawn from this data is that sulfates either exist in a partially soluble form (CaSO₄) or are readily converted to such a form. Thus, the major portion of the total amount present was removed in the initial leaching, with the only limitation on this amount being the equilibrium constant of gypsum (CaSO₄). Also evident is the fact that at least a portion of the reduced sulfur had become oxidized and was removed as sulfate.

Weathered residual material was releached and the resulting water was compared to that produced by releached, unweathered residuals. No perceptible increase in sulfate production over the unweathered soils was found to exist. Two possible conclusions could be drawn from this fact: either the conditions required to oxidize pyrite were more severe than any that it had been subjected to or the comparatively mild grinding and leaching of the soil had been sufficiently harsh to oxidize all of the oxidizable reduced sulfur present. The conclusion previously drawn from the leaching experiments led to the adoption of the latter of these alternatives.

Work that has since been done by the Alberta Research Council (2) to test the facility of pyrite oxidation lends almost conclusive support to this hypothesis. In their experiment, soil was leached in an inert atmosphere. When it was determined that all available sulfates had been leached, oxygen was introduced into the system. A sharp increase in the amount of sulfate produced was seen almost immediately (less than 24 hours).

The implication of these findings is that the very act of disturbing overburden material is sufficiently severe to oxidize the pyrite present. In the light of this fact, it is obvious that the selective handling and restoration of spoil material, a measure proposed by many, would be nonproductive. Instead, reclamation techniques should be designed with the intention of minimizing the harmful effects of the inevitably present salts on both the water and ecology of the system.

This research was a portion of study done by the North Dakota Geological Survey. Funding was provided by the United States Bureau of Mines.

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A FOSSIL HORSE FROM THE BRULE FORMATION, STARK COUNTY, NORTH DAKOTA

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During the summer of 1982, the fossilized remains of a small horse similar to the genus Miohippus were found in an exposure of the Brule Formation in the Little Badlands south of South Heart, in the SE &, Sec. 7, T. 137 N., R. 97 W., Stark County, North Dakota. The site is on the ranch of Robert Fitterer and is near the type section of the Dickinson Member of the Brule as defined by Stone (1). The bones were found weathered out on the surface of a small drainage divide. Further digging revealed many more bones still enclosed in the matrix of calcareous silty claystone. Because of the hardness of the matrix, it was possible to transport chunks of this material intact back to the laboratory for further preparation. Among the elements found are a pelvic girdle, about 80% complete, three distal phalanges (hoof bones), apparently from the same foot, a number of other phalanges, two proximal condyles from the femurs and a part of one femur shaft, about 60% of a tibia, parts of at least six vertebrae including the complete axis (2nd cervical), and numerous rib fragments. A tooth (left upper molar) that was found weathered out on the surface nearby is believed to be associated with the postcranials. All of the bones were removed from an area of about 1.5 m and it appears that they represent the remains of a single individual.

The evolutionary line of the horses from the lower Eocene through the middle and upper Oligocene is straight and unbranching. Morphological changes between related genera are gradational and often indistinct. This gradational relationship is found between the Oligocene genus Mesohippus and the slightly more advanced Miohippus. Thus definite assignment to one or the other of these genera must be based on all available criteria, especially in the case of incomplete skeletons, and in fact may not be possible. The general increase in the size of the horses has been the most notable and constant aspect of their evolution during the Tertiary. It should therefore be reasonable to use the size of adult specimens as a criterion for evaluating their similarity. With this goal in mind, measurements of the tooth and pelvis were made. Measurements of the single molar found with this specimen give dimensions of from 2.7% to 9.8% larger than published values for Mesohippus (2, 3). Additionally, rough measurements on the pelvic girdle suggest that this individual was somewhat larger than the average Mesohippus. Due to the relative rarity of Miohippus specimens, direct size comparisons have not been made with specimens of that genus. The upper cheek teeth of both genera are marked with a distinctive W-shaped ectoloph. In Miohippus there is the beginning of a small spur on the metaloph which appears to be incipient in this specimen. Another important distinguishing character is the strength of the cannon bone-ankle joint (4). In Mesohippus the middle cannon bone articulates with the ectocuneiform alone while in Miohippus this joint has strengthened and the cannon bone contacts both the ectocuneiform and cuboid. Unfortunately, none of these bones were available and could not be used in the determination.

On the basis of the evidence gathered thus far, it seems that the specimen represents an individual evolutionarily midway between a "typical" Mesohippus and "typical" Miohippus. However, the large size and presence of the metaloph spur weigh toward tentative assignment to the latter genus. Further study will be necessary to make a more definite determination that may be helpful in stratigraphic correlation.

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SEDIMENTOLOGY OF RYE BEACH AND WALLIS SANDS, NEW HAMPSHIRE

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Criteria for the recognition of the depositional environment of sandstones are important in the reconstruction of paleoenvironments and are of considerable importance in connection with the search for stratigraphic oil traps. Textural or mineralogical characteristics of different sand types have been studied to determine depositional environments. Presently, there are no satisfactory petrographic criteria to evaluate deposition. Due to the fact that near shore sands are shifted from one environment to another it is necessary to relate the petrographic characteristics to the terminal environment (1).

Samples were collected from two sites along the New Hampshire coastline which provides unique sedimentary characteristics; Wallis Sands State Park and Rye Beach which are approximately 3 miles apart. The sediments were analyzed for mineralogical composition, grain size based on the phi scale, and values were used to determine certain statistical functions.

The uniqueness of these depositional environments becomes apparent when one begins to analyze possible source areas. There are at least three source areas in the local area, 1) the Nevington Moraine, stretching from York County, Maine, through New Hampshire and into Essex County, Massachussetts, 2) the Piscataqua River, with its mouth at Portsmouth Harbor, north of the sample sites, and 3) shallow water sediments of the Atlantic Ocean, which fluctuate between coarse sediments reworked from sea cliffs or glacial till and fine grained sediments in protected waters of bays or at mouths of low gradients streams or rivers (2).

The distribution of sizes in sediments is related directly to the availability of different sizes of particles and the processes operating where sediments are deposited (3). Sample site 1 yielded a more uniform distribution, the mean at 1.330° sample 2 had a very sharp, peaked curve with a mean value of 2.350°.

The mineralogy of clastic sediments reflects the nature of the source rock, where as the textural parameters are related to the mode of transportation and the energy conditions of the transportation medium. Minerology of the sediments was similar to the local bedrock, the highest concentrations being quartz, amphibole, pyroxene and igneous rock varieties such as diorites and granites.

Water or tide motion is so vigorous that clay and silt particles are always kept moving and do not come to rest with the sands. For these sand types the positions of breaks between the straight line segments on the probability plots are predictable and are functions of the processes that deposited the sediment (4). Sample 2 exhibits this trait; segments can be broken from the largest particle sizes into a rolling and sliding population, two saltation populations and a suspension population. The probability plots of each sample indicate the segments representing suspended particles constitute less than 2% of the samples. Frequency distributions of the samples are slightly, negatively skewed, typical of beach sands. Values of the standard deviation (S. D.) are important in this study as river sands generally have a higher value than beach sands. Sample 1 had a S. D. of .96, whereas sample 2 had a S. D. of only .37 indicating the Piscataqua River may contribute sediment to site 1 rather than site 2. Site 1 also exhibits traits of a higher energy beach than site 2, as the sediments are slightly more well rounded. Sands from both sites are a product of the local bedrock and landforms, however, sediments are influenced by the Piscataqua River and deposited along the southern New Hampshire coast.

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COLD TOLERANT BIRD FLEAS: SURVIVAL AND THEIR ALTERED GLUCOSE AND GLYCOGEN RESERVES

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Insects in temperate and subarctic regions have evolved several methods of overwintering, including the production of antifreeze. Many free-living species have been studied, but little is known about cold hardiness of fleas. Ceratophyllus idius is an ectoparasite of purple martins (Progne subis) and tree swallows (Iridoprocne bicolor) across much of temperate North America, and commonly occurs in neground material. In eastern North Dakota, migration of martins (late August, late April) separates the parasites from their hosts for about 8 months. Despite host absence and stressful winters, some portion of the flea population survives to reinfest returning birds in April (1). In the late autumn, this flea produces glycerol, a cryoprotectant (2). This paper re-examines some patterns of survival and the changing glucose and glycogen levels of purple martin fleas from early fall to mid-winter.

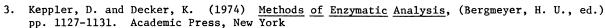
Nest material was collected from 3 martin houses in Grand Forks, North Dakota during August 30-September 3, 1983. This material was held at room temperature until 1550 adult C. idius could be placed in lots of 50/gauze-covered glass vial. At mid-September the first lot was killed in a dry ice-acetone bath and held frozen for future analysis. The remaining 30 lots were cooled to 1°C by lowering the temperature 3° every fourth day. In the final step, 15 vials were placed on the roof of the biology building (double boxed and shaded), while the other 15 were placed in a -6°C freezer. At monthly intervals (mid-October to mid-January) flea samples from each location were allowed to warm to room temperature. Survivors were freeze-killed, sorted and weighed by sex, and homogenized in 80% methanol. Liberated glucose was analyzed by colorimetric methods (Sigma; technical bulletin No. 510). Glycogen was hydrolyzed by amyloglucosidase (3) and also read as glucose units. Determinations were made with a Beckman DU-7 spectrophotometer, and concentrations calculated as µmoles/g wet wt.

Large decreases in the glycogen levels during the first two months of the experiment occurred in both ambient and freezer conditions (Fig. 1). Concomitant with this decline was a 3 to 4-fold rise in glucose levels. This increase was not noted in a previous experiment (2), although such changes do occur in cold-stressed larvae of goldenrod gall flies (4). The elevation of glucose may reflect the flea's inability to synthesize antifreeze as quickly as glycogen is broken down. Beyond mid-November, freezer-held fleas showed a consistent decline in glucose and glycogen levels, while those in ambient-held specimens displayed temporary stability prior to declining in January. This pattern confirms an earlier observation for ambient treated fleas (2).

Pooled counts of monthly samples (Fig. 2) showed that more freezer-held fleas (100/255 = 39.2%) survived than those subjected to roof top conditions (83/326 = 25.5%). Decreased survival at ambient parallels a previous study (2), as does the tendency of females to outsurvive males (1). Reasons for differential survival are unknown, but it seems likely that wide ranging temperature extremes and repeated freeze-thaw oscillations are more stressful than constant cold. Results thus far do not suggest an obvious relationship between glucose/glycogen levels and flea survival. Cold hardiness may be more directly tied to glycerol levels and/or other undetermined cryoprotectants.



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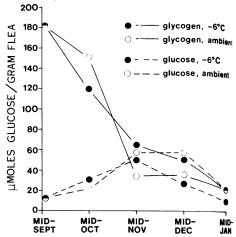


Figure 1. Glucose and glycogen levels as a function of time and regime. Pooled data for both sexes: results as means of 3-9 replicates. Glycogen expressed as µmol glucose after enzymatic hydrolysis.

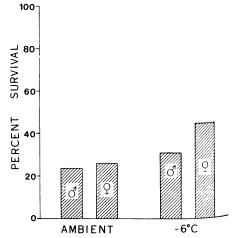


Figure 2. Flea survival.

Pooled data by sex through
mid-January 1984.

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Recent research at the Almont Fossil Site in the northwest corner of Morton County (SW\sE\sec.26T. 140N.R.86W.) has provided evidence for a lowland forest community. Fossil flora evidence is as follows:

Ampelopsis acerifolia (Newberry) Brown Carya antiquorum Newberry

Circidiphyllum arcticum (Heer) Brown

Cinnamomum sezannense Watelet

Cyclocara brownii

Ginkgo adiantoides (Unger) Heer

Juglandicarya species?

Metasequoia occidentalis (Newberry) Chaney

Viburnum species?

Lithology at the locality is brown to dark brown siliceous fine-grained shales that break in concoidal factures. These shales are part of the Paleocene Sentinel Butte Formation. Taking this into account with the fossil evidence suggests a lake or oxbow flanked by the various trees. The high silica content would suggest volcanic ash (1) carried by wind currents, settled out in the water. The silica leached from the ash would act as a cement to provide a good matrix for fossil preservation.

Based on what we know of living species of $\underline{\text{Ginkgo}}$, $\underline{\text{Metasequoia}}$, and $\underline{\text{Cercidiphyllum}}$ found in China the environment would have been a temperate, 100 wland forest. The temperatures probably ranged from -1° to 29°C and rainfall ranged between 102-203 cm a year.

The Shui-hsa Valley, in the mountainous tableland southeast of the Yangtze River in China, is the site of the discovery in 1944 of Metasequoia glyptostroboides, the living counterpart of Metasequoia occidentalis. These trees thrive along the banks of streams at the lower ends of ravines. The winters here are warm with little or no snow. Precipitation is evely distributed throughout the year and the humidity is high. Modern species of Cercidiphyllum and Ginkgo are also found in this valley.

(2) The flora assemblage found at the Almont site leads to the conclusions that the paleoenvironment was similar to the present day environment of the Shui-hsa Valley.

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USE OF ROTATING BIOLOGICAL CONTACTORS FOR TREATING COAL GASIFICATION WASTEWATER

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The two objectives of this study were to determine the feasibility of using Rotating Biological Contractors (RBC) for the treatment of coal gasification wastewater and to define a range of hydraulic and organic loadings for the reactors, within which process stability can be maintained.

A bench scale RBC was assembled using plexiglass and PVC pipe. The reactor contained 39-5 $_{inch}$ diameter equally spaced discs on a 14 inch long shaft that rotated in a rectangular tank with a concave bottom. The total disc surface area was 10 ft². The system operated at 40 percent disc submergence and a tank wastewater volume of 0.32 gallons. The discs were rotated by a Cole-Palmer motor drive at an average peripheral velocity of 0.19 ft/s. The unit was fed continuously using a Masterflex peristaltic pump while operating at a mean temperature of 70°F.

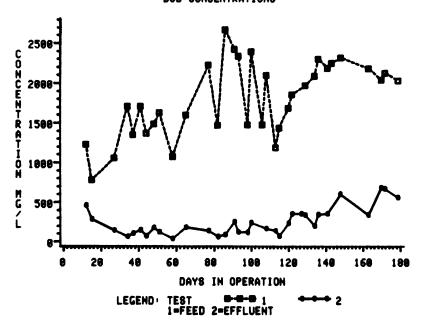
The wastewater was generated at the University of North Dakota Energy Research Centers (UNDERC) slagging, fixed-bed gasifier and underwent pretreatment prior to the biological treatment. The wastewater was solvent extracted and steam stripped to produce stripped gas liquor (SGL) with final feed concentrations of 150 mg/l phenol and 500 mg/l ammonia. Toxic substances in the SGL also included 210 mg/l thiocynate and 39 mg/l cyanide (1). The mean BOD $_5$ of the SGL was 1891 mg/l. The unit operated for 190 days.

At hydraulic loadings of 0.1 $\rm gpd/ft^2$ (1.4 x $\rm 10^{-3}$ lb. $\rm BOD/ft^2$ day) the unit achieved a 93 percent removal of BOD₅, 91 percent removal of alcohol and an 83 percent removal of phenols at steady state. The RBC removal rates decreased when the hydraulic load was increased to 0.15 $\rm gpd/ft^2$ (2.53 x $\rm 10^{-5}$ lb. $\rm BOD/day$). $\rm BOD_5$ removal was reduced to 73 percent, alcohol removal was reduced to 78 percent and the removal of phenol was reduced to 80 percent.

Despite a 50 percent increase in the hydraulic loading to the RBC the unit remained stable with only a 20 percent reduction in BOD_5 removal. The study showed that a RBC can achieve high biodegradable organic removal under the range of hydraulic and organic loadings tested. The RBC also proved to be stable during rapid fluctuations in thiocynate and cyanide loadings that upset activated sludge reactors being operated simultaneously with the same SGL.

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ROTATING BIOLOGICAL CONTACTORS BOD CONCENTRATIONS



55 X-RAY DIFFRACTION CHARACTERIZATION OF SOLID WASTES FROM TWO NORTH DAKOTA COAL-FIRED POWER PLANTS

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Solid wastes from the abundant coal-fired power plants in west-central North Dakota constitute a disposal problem for these plants and the adjacent mines which are often used as disposal sites for the wastes. Utilization of these solid wastes in applications such as a replacement for cement is an alternative to disposal. The "mineralogy," crystalline (and non-crystalline) phases that comprise the solid wastes, affects the behavior of the wastes in contact with groundwater or in some commercial applications. Solid wastes from two N.D. power plants were studied by X-ray diffraction as part of research conducted jointly by the University of North Dakota and North Dakota State University on the characterization, utilization and disposal of these wastes.

The coal combustion wastes studied were fly ash, bottom ash and flue gas desulfurization (FGD) waste. The two plants (Milton R. Young station at Center N.D. and Coal Creek station at Underwood

N.D.) utilize different lignite coals and employ different combustion systems.

An energy dispersive X-ray fluorescence (XRF) spectrometer was used to qualitatively determine elements (above Z = 11) present in each specimen studied by X-ray diffraction (XRD). Diffraction data were obtained with a Philips diffractometer and phases were identified by reference to the JCPDS Mineral and Inorganic Powder Diffraction Files. Since non-crystalline glass was predominant in the fly ashses and one of the bottom ashes these samples were subjected to devitrification treatments at 300, 700, 900 and 1100 C and the devitrification products were analyzed by XRD. Quantitative XRD analysis (standard additions method) for lime (CaO) and periclase (MgO) was also performed on the fly ash samples.

The crystalline phases identified in several fly ashes from the Milton R. Young (MRY) station were: anhydrite (CaSO₄), periclase (MgO), thenardite (Na₂SO₄), melilite (Ca,Na)₂(Mg,Fe,Al)(Si,Al)₂O₇, quartz (SiO₂), ferrite spinel (Fe,Mg,Mn,Ti)(Fe,Al)₂O₄, aphthitalite (Na_{2-x}K_x)₂SO₄ and lime (CaO). Fly ash specimens from Coal Creek (CC) station contained quartz, ferrite spinel, anhydrite, periclase and lime. C_3A (Ca₃Al₂O₆) may also have been present but unambiguous identification was not possible. The anhydrite and lime reacted with the glassy phase (and/or C_3A if present) to form the cementitious phase ettringite (Ca₆Al₂(SO₄)₃(OH)₁₂.25H₂O) in a CC fly ash that had been wetted for use in constructing an ash holding pond. Quantitative analysis showed the MRY fly ash to contain about 0.5 wt% crystalline lime and 2% periclase, while CC fly ash contained about 1% crystalline lime and 2% periclase. Because the amounts were small the estimated standard error is + 20% of the amount present.

Partial devitrification of the MRY fly ash had taken taken place after the 700 C treatment. After the 900 and 1100 C firings the samples had completely devitrified into a phase assemblage consisting of the following solid solutions (s.s.): melilite (Ca,Na)₂(Mg,Fe,Al)(Si,Al)₂0₇, sodalite (Ca,Na)₂(NaAlSi0₄)₆(S0₄)₂, ferrite spinel (Fe,Mg,Mn,Ti)(Fe,Al)₂0₄, pyroxene (Ca,Na)(Mg,Fe,Al)(Si,Al,Ti)₂0₆, nepheline (Na,Ca)(Al,Fe)(Si,Al)0₄. In the CC fly ashes no apparent devitrification had taken place after the 700 heating. After the 900 C firing the samples exhibited approximately 50% crystallization into melilite and pyroxene s.s. (quartz, ferrite spinel and periclase were still present from the original ash). After the 1100 C treatment the pyroxene phase dominated over melilite.

Due to the differences in combustion methods and conditions there was a vast difference between the bottom ashes from the two plants. The MRY bottom ash was a non-crystalline black glass. However, the CC station produces a gray-brown clinker composed of large chunks of almost pure pyroxene s.s. and finer particles. These particles proved to be predominantly crystalline solids composed of pyroxene s.s., melilite s.s., quartz, ferrite spinel s.s. and minor hematite (Fe_2O_3). The MRY bottom ash devitrified into pyroxene, melilite, nepheline and ferrite spinel solid solutions.

FGD waste from the MRY plant consisted of gypsum ($CaSO_4.2H_2O$) and fly ash which is used in the sulfur scrubbing process. The FGD waste from the CC plant had major $CaSO_3.1/2H_2O$ in addition to gypsum

and_fly ash.

Because of the presence of soluble alkali, Ca and sulfate salts the potential for ground water mineralization from buried ash is greater in the MRY fly ashes. The soluble salts also produce salt effluorescence on the surface of set concrete which makes its use as a commercial cement replacement in concrete unattractive. The CC fly ash is self-pozzolonic (presence of ettringite in wetted ash was noted above) and is used commercially as a replacement for concrete. The differences in the behavior of the fly ashes may be due to their differences in glass compositions and properties as evidenced by their different devitrification behaviors. The bottom ashes from the two plants are generally low leachability solids and can be used as feed materials for ceramics and mineral wool and as aggregate in concrete.

BORON DEPRIVATION IN RATS

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Between 1939 and 1944, attempts to induce a boron deficiency in rats were unsuccessful although the diets apparently contained only 155-163 ng boron/g (1,2). After those reports, boron was generally accepted as being essential only for plants, not for animals. Recently however, evidence was reported that indicated boron might be an essential nutrient that interacts with magnesium in the growing chick (3). Therefore, we decided to reexamine the possibility that boron is an essential nutrient for the rat.

Male weanling Sprague-Dawley rats were assigned to groups of nine in a fully-crossed, two way two-by-two design. The treatments were the supplementation of the basal diet (containing 0.3 to 0.4 μg boron/g) with boron (as boric acid) at 0 and 3 $\mu g/g$, and with magnesium at 200 and 400 $\mu g/g$. Environmental conditions have been described (3). The rats were fed their respective diets for five weeks, weighed and decapitated subsequent to cardiac exsanguination with a heparin-coated needle and syringe. The liver, spleen, kidney, heart and brain were removed and weighed immediately. Selected parameters listed in the table, or mentioned elsewhere, were determined by our usual methods (3).

Treatment, μg/g diet Β Mg		Weight, g	Hemoglobin, g/100 ml	Kidney wt, g Body wt, 100 g	Spleen wt, g Body wt, 100 g	Dried Kidney Calcium, μg/g
	200	200	12.52	0.227	0.251	150
0	200	208	13.53	0.327	0.251	152
3	200	226	14.51	0.337	0.225	143
0	400	216	13.79	0.327	0.239	152
3	400	233	14.15	0.348	0.227	162
		<u>A</u>	nalysis of Var	iance - P Values		
Boron effect		0.003	0.0001	0.007	0.009	NS
Magnesium effect		NS	NS	NS	NS	0.05
Boron x Magnesium		NS	0.05	NS	NS	0.06

Boron deprivation depressed growth, hematocrit, hemoglobin, and kidney wt/body wt ratio, and elevated spleen wt/body wt ratio. Boron deprivation had little affect on liver wt/body wt ratio, heart wt/body wt ratio, kidney magnesium or plasma urea. The rats fed 200 µg magnesium/g diet apparently were not magnesium-deficient because they did not show magnesium deficiency signs of depressed growth and hematopoiesis and elevated kidney calcium. Apparently because some rats were not magnesium-deficient, there was no highly significant interaction between boron and magnesium. In the studies in which an interaction between boron and magnesium was found, some of the chicks were magnesium deficient (3). Nonetheless, there were some findings in the present study that suggested a relation between boron and magnesium. Boron deprivation apparently depressed hemoglobin more in magnesium-low than in magnesium-adequate rats. On the other hand, decreasing dietary magnesium apparently affected kidney calcium more in boron-supplemented than in borondeprived rats. In support of a relationship between boron and magnesium are the findings of Elsair et al. (4). They found boron alleviated secondary hyperparathyroidism induced by fluoride toxicity in rats. Perhaps the hyperparathyroidism was caused by an induced magnesium deficiency because fluoride is an antagonist of magnesium (5). Signs of hyperparathyroidism are characteristic of magnesium deficiency in rats (6).

In summary, the findings are consistent with boron being an essential nutrient for the rat. Furthermore, boron might have a physiologic role that influences magnesium metabolism.

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