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NORTH DAKOTA ACADEMY OF SCIENCE
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81st ANNUAL MEETING

April 27-28, 1989

Grand Forks, North Dakota

Editor's Notes

The Proceedings of the North Dakota Academy of Science was first published in 1948, with Volume I reporting the business and scientific papers presented to the fortieth annual meeting, May 2 and 3, 1947. Through Volume XXI, the single yearly issue of the Proceedings included both Abstracts and Full Papers. Commencing with Volume XXII the Proceedings were published in two Parts. Part I, published before the 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 all 31 papers presented in the four symposia at the 1989 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 53 communications presented in the Professional section of the 1989 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. This section is arranged alphabetically, by first author's last name.

The third section of this volume contains 21 collegiate communications, representing all those papers presented in the A. Rodger Denison Student Research Paper Competition. Undergraduate and graduate students reported on the results of their own research activities, usually carried on under the guidance of a faculty advisor. While the student competitors were required to prepare a communication similar to those prepared by their professional counterparts, these communications were not 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 place and runner-up 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.

This issue of the Proceedings includes copies of the constitution and bylaws of the Academy, and lists of the officers, committee membership, and the entire membership.

John R. Reid
Editor

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NORTH DAKOTA ACADEMY OF SCIENCE

I. Rules for Preparation of Proceedings Communication

1. Each paper presented at the annual meeting of the Academy must be represented by a communication in the Proceedings, including A. Rodger Denison student research competition papers.
2. Only communications intended for presentation at the annual meeting will be considered for publication. They must present original research in as concise a form as possible. Quantitative data should be presented with statistical analysis (i.e., means with standard errors). Papers which merely summarize conclusions or ideas without supporting data are discouraged and will not normally be accepted. The communication should include the purpose of the research, the methodology, results, and conclusions.
3. **Authors are encouraged to utilize the full space available in order to provide sufficient information to fully describe the research reported.**
4. Communications must be prepared on the special blue-line form and sent, with three legible xerox copies, by first class mail to the Secretary, North Dakota Academy of Science, Box 8123 University Station, Grand Forks, ND 58202. The form must not be folded; a cardboard backing should be used to avoid damage. The Proceedings will be published by direct photo-offset of the submitted communication. No proofs will be prepared.
5. All typing, drawing and secured art or photographic materials must be within the boundaries of the blue-line form. Consult the example on the reverse side of the special form for proper style (i.e., titles, authors, address, tables, figures, references, indentations, headings, and punctuation). *Indicate the author to present the communication by an asterisk (*) after that person's name.*
6. Tables, diagrams, and photographs are acceptable provided they are secured to the special form and do not occupy a total area of more than 100 square centimeters.
7. Only essential references should be cited, and should be indicated in the text by numerals and quoted at the end of the communication. Up to three authors' names may be cited in full; with four or more authors only the first should be cited. The following form of citation should be used:

   Journals: Neary, D., Thurston, H. and Pohl, J.E.F. (1973) Proc. N.D. Acad. Sci. 40, 83. (Abbreviate titles.)

   Books: Batsone, G.F., Blair, A.W. and Slater, J.M. (1971) A Handbook of Pre-natal Paediatrics, pp. 83-90. Medical and Technical Publishing, Lancaster.

   Individual chapters in books: Farah, A.E. and Moe, G.K. (1970) in 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.

8. Use a typewriter with elite type and with a carbon or good quality black silk ribbon. Single space and begin paragraphs with a 3 space indentation. Special symbols, not on the typewriter, must be hand lettered in black ink. Dot matrix type is not acceptable.
9. Abbreviations: Only standard abbreviations should be used, and should be written out the first time used with the abbreviation following in parentheses.
10. Titles: It is suggested that authors select a sufficient number of keywords to describe the full content of their paper, and then construct a title using as many as these as practicable. Titles normally should not exceed 140 characters in length. In particular, they should be free from unnecessary phrases such as "a preliminary investigation of" or "some notes on" which add little or nothing to their meaning.
11. Session Assignment: In order to assist the program committee in organizing the presentations, please indicate on the reverse side of the blue-line form your 1st, 2nd, and 3rd preferences for the topical classification of your paper.
12. The authors' permission for the North Dakota Academy of Science to publish is implied by a submission. The Academy does not restrict the right of authors to include data presented in a communication in full papers submitted at a later date to other publishers.

II. Rules for Oral Presentation of Paper

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

SYMPOSIUM

ON

A CENTURY OF EXCELLENCE

Presiding: Forrest H. Nielsen, President, ND Academy of Science, Director, USDA, ARS, Human Nutrition Research Center, Grand Forks, ND

1. A Geologist looks at the Past.
   Wilson Laird, Professor of Geology and State Geologist Emeritus, University of North Dakota, Kerrville, Texas

2. A Century of Excellent Progress in Agriculture in North Dakota.
   J. F. Carter*, Professor Emeritus, North Dakota State University, Fargo, ND

3. Mapping North Dakota's Natural Vegetation.
   W. C. Whitman and W. T. Barker, Departments of Botany and Animal and Range Sciences, North Dakota State University, Fargo, ND

4. Chemistry in North Dakota - A Century of Progress.
   H. J. Klosterman*, Professor Emeritus, North Dakota State University, Fargo, ND

5. A Century of Excellence: Computing and Its Influence.
   Gene A. Kemper*, Associate Vice President For Academic Affairs, University of North Dakota, Grand Forks, ND

(1)

A GEOLOGIST LOOKS AT THE PAST

Wilson M. Laird*
Professor of Geology and State Geologist Emeritus
University of North Dakota
Grand Forks, N.D. 58202

This brief review gives the work of the geologists who participated in the development of the mineral industries in the early days of the State. Serious work on the geology of North Dakota started about 1890 and has continued at an ever increasing pace up to the present. This interest in the State's geology was stimulated in great part by the development of the oil industry which began in 1951.

In the early days of the State, the geologists working with the North Dakota Geological Survey were the most prominent geologists. Included in this group was Earle Babcock, first State Geologist, who was really trained as a chemist, and Dr. Frank Wilder who set the N.D. Survey on its future scientific path. The work of Dr. Arthur Leonard, especially his work on the lignite resources of the State, was noteworthy. Dr. Leonard, incidentally, was the State Geologist with the longest tenure.

Work on the water resources of the State was initiated by Professor E.F. Chandler, later Dean of Engineering at the University of North Dakota. The state's underground water was explored by Dr. Howard E. Simpson who studied the ground water resources of surrounding states as well. One of the pioneer studies on the fluoride in ground water was done by Dr. George Abbott, chemist at the University of North Dakota, who was also secretary of the North Dakota Academy of Sciences for many years.

Geologic studies in connection with the search for oil in North Dakota were done both by state and federal government geologists. In the final decision to drill, the geologic work of the geologists of the oil companies were predominant. Among these was A. Rodger Denison of the Amerada Peroleum Corporation which drilled the discovery well on the Nesson anticline.

The North Dakota Geological Survey was active in oil and gas conservation matters and encouraged the legislature to pass adequate oil and gas conservation laws which are still on the books today.

The lignite industry has been important in the industrial development of the State from the very first. Today, with the numerous electrical generating plants, North Dakota is in the forefront of the energy producing states. Many other products can be made from this valuable resource and much work remains to be done on it although research on lignite has been important from the very early days.

There is still much to be done on the research and development of the clays of the State which was one of the first natural resources studied. Other resources, such as the deeply-buried potash, need further geologic and economic study.

While much work has been done there is still much to do, and both academic and industry geologists are still actively pursuing still unresolved problems such as pollution control, contamination of underground water, and other waste disposal problems. The future, while different from the past, is still geologically exciting.

(2)

A CENTURY OF EXCELLENT PROGRESS IN AGRICULTURE IN NORTH DAKOTA

J. F. Carter, Professor Emeritus
North Dakota State University

Prior to 1890, most of North Dakota east and northeast of the Missouri River was "settled" via homesteading and/or purchase of land grants from railroads. The farms were highly diversified or nearly self-sufficient by producing several crops, using horse power, and producing beef, dairy animals, swine and poultry. By 1989, farm numbers have decreased greatly, farms have become specialized to crops primarily, except where native grassland composes enough of the farm or ranch to justify livestock, primarily cattle.

The "land and its people" and the institutions which the people support are responsible for a century of "excellent progress" in agriculture. The people have accepted, promoted and/or initiated changes in agriculture from advances in scientific agriculture and technology. They have supported the educational institutions for general education and specifically the North Dakota State University and North Dakota Agricultural Experiment Station (NDAES), including Branch Stations, which have developed continuing new superior products and practices for North Dakota agriculture, and an Extension services that has communicated results of research. The system also has evolved a greatly improved agribusiness, including marketing systems, with favorable interactions of producer and agribusiness. Thus, the producers have used their own initiatives and the products of agricultural research and the service of agribusiness to develop the highly sophisticated agriculture of 1989.

Since 1910, farm size has increased 3-fold and farm numbers decreased from a peak of 84,000 to 32,500 in 1987, with less than 15% of farms having livestock. Modern machines and tools have replaced much farm labor on farms of 1989.

Some specific examples of excellent progress in agriculture are research products of the NDAES utilized by farmers to produce 75% of new wealth in ND in past years, now just over 50%. Examples are:

1. Wheat production in ND was 26,000,000 bushels in 1889 but increased to 328,000,000 bushels in 1981 with ND the leading wheat producer twice in the last decade. Scientists at NDSU have produced agronomically superior new varieties, especially resistant to stem rust, while maintaining excellent end use quality for domestic and export markets of our wheat. New varieties have returned many millions of dollars above potential income from older varieties, often a 200:1 return on research investment. Improvement of other major crops, e.g., barley, oats, corn, flax, dry beans, etc. has greatly improved production and return to farmers.
2. Two highly valuable minor crops, potato and sugarbeet, have been brought to successful production by cooperation of producers, commodity organizations, and NDSU. NDSU has been a regional and national leader to improve potato by breeding, and improved sugarbeet production practices based on research.
3. Cooperation of NDSU and ARS, USDA, led to development of basic hybrid production techniques and germplasm in sunflower on which this nation's, indeed the world's, sunflower hybrid crops are based.
4. Farmers and ranchers have benefited greatly by research at NDSU on breeding, management, production testing, health protection, etc. of livestock, and protection of crops from the pests, weeds, diseases and insects.
5. Producers, NDSU and the Soil Conservation Service have cooperated on soil survey, soil testing and fertilizer technology to produce the most from the basic soil resource while maintaining soil productivity.
6. Agricultural engineers have assisted to bring new machine technology and electrification to ND farms for "better living" and much improved convenience and efficiency.
7. Research from economics has aided greatly in decision making on farms regarding input/output, marketing strategies, crop alternatives, etc.

Finally, the College of Agriculture and NDSU have educated thousands of students in modern agriculture, from one or two students in 1889 to 182 B.S., 57 M.S., and 15 Ph.D. students graduated in 1988, who return to the farm or take professional careers in agriculture.

A century of excellence in agriculture has led our producers, research institutions, extension system, and agribusiness to the "cutting edge" of modern agriculture.

(3) MAPPING NORTH DAKOTA'S NATURAL VEGETATION 1/

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Depts. of Botany and Animal and Range Sciences
North Dakota State University, Fargo, N.D. 58105

An earlier N.D. Academy of Science symposium (1) dealt with defining information needed to revise existing maps of North Dakota's natural vegetation. The intention of the present effort is to provide an historical perspective of the mapping efforts that have been made to reconstruct a picture of the natural vegetation existing in the area prior to settlement. Such maps are considered to represent ecological climax vegetation for the naturally occurring soil-plant landscape units of a region.

In the mid-1800's the legend persisted that our region was part of the Great American Desert. This desert region was thought to stretch eastward from the base of the Rocky Mountains for a distance of 500 to 600 miles. This concept was fostered by prominent national figures and was prevalent in the period 1850-1860 (2). By 1890, however, vegetation maps were in existence which showed North Dakota as having a grassland vegetation of two types. In the east was a tall grassland, while the western region supported a short grassland (3).

The first significant map showing the natural vegetation of the United States as a whole was that of Shantz and Zon printed in 1923 (4). This map shows for North Dakota an eastern tall grass vegetation, and a western short grass type. It also delineates wooded areas in the Turtle Mountains, Devils Lake area, and in the stream valleys of the state. The vegetation in these latter areas was designated as the Oak-Hickory type. A more detailed black and white map of the grasslands accompanied the colored map of overall vegetation. On this map five grassland types were shown in North Dakota. From east to west across the state these types were the Bluestem Sod in the Red River Valley, Needlegrass-Slender Wheatgrass across the drift prairie to the Missouri Coteau, then extending across most of the rest of the state was the Grama-Western Needlegrass type. Most of the Badlands area was shown as supporting a Western Wheatgrass-Sagebrush type with small intrusions in the southwest and west of a Grama grass type.

The Kuchler map of 1965 (5) has become the basic map of potential natural vegetation of the United States and the basis for most maps of North Dakota's natural vegetation. For North Dakota grasslands it distinguishes four types: the Bluestem Prairie, Wheatgrass-Bluestem-Needlegrass, Wheatgrass-Needlegrass, and a small fragment of Nebraska Sandhills Prairie. For woodlands it shows the Northern Floodplain Forest along the major streams, an Oak Savanna type in the Turtle Mountains, parts of the Souris River loop, the Devils Lake Area, Pembina Mountains, and in the Sheyenne Delta. This map is the first to show the pine forest area in Slope County where it is designated as Eastern Ponderosa Forest.

In 1975 Whitman and Wali (6) used the ERTS Image Mosaic map with designations of vegetation types superimposed on the satellite image. This was not particularly effective, although it did recognize the wooded Killdeer Mountain area not previously included on state vegetation maps. The Soil Conservation Service, USDA, adaptation of the Kuchler map was used by Shaver in his 1977 discussion of the state's rangeland resources (7). The 1974 and 1977 Forest and Range Ecosystems of the United States maps (8, 9) prepared by the U.S. Forest Service are based primarily on the Kuchler map, but they do have some different details. The cover-type maps of North Dakota developed by the Regional Environmental Assessment Program in 1976 (10), from computer processed Landsat imagery, covering individual counties as well as the state as a whole, could be very useful in the development of more detailed future maps.

The 1986 Karch and Roloff map (11) of major North Dakota wooded areas does provide some additional detail. The Barker-Whitman map (1988) of Northern Great Plains vegetation (12) adds some detail and introduces the concept of a Rough Fescue mixture along a portion of the northern border of North Dakota.

A comparison of natural vegetation maps of such states as Kansas, Nebraska, South Dakota, Minnesota, and Montana with North Dakota's existing maps indicates that the preparation of a more detailed map would be in order. The map of Montana climax vegetation prepared by Ross and Hunter (13) could well serve as a general model. Reference to this map, the General Soil map of North Dakota (14), the REAP cover-type maps, and presently existing knowledge of plant-soil relations could result in the delineation of 12 to 15 distinguishable vegetation types as existing in North Dakota prior to settlement.

1/ A list of pertinent literature will be provided at the symposium by the authors.

(4)

CHEMISTRY IN NORTH DAKOTA - A CENTURY OF PROGRESS

H. J. Klosterman
Professor Of Biochemistry (Emeritus)
North Dakota State University

The foundations of the chemical sciences were well in place in the late nineteenth century, when statehood was established for North Dakota. Discoveries by the last half of that century had resulted in the determination of the atomic weights of most of the common chemical elements as well as their respective chemical properties. The pioneering work of LeBel and van't Hoff permitted a rational description of the spatial arrangement of atoms within molecules, especially carbon-containing molecules, and led to a more thorough understanding of the constituents of living sytems. These same insights provided a great stimulus to the development of synthetic organic chemistry.

Contemporaneously with the advances in the developments in the chemistry of the compounds of carbon, new and deeper insights into the factors that govern chemical reactivities led to a comprehensive treatment of the thermodynamics of chemical equilibrium, especially in the writings of J. Willard Gibbs.

The last decades of the nineteenth century were also marked by an intense interest in the quantitative measurement of the material world. Knowledge of these quantitative chemical skills was brought to the newly organized state by the first faculty appointments at the Agricultural College (NDSU) and the University (UND). Earl Babcock became the first faculty member at UND with an assignment in chemistry and Edwin F. Ladd was appointed professor of chemistry at the Agricultural College in 1890. Both Babcock and Ladd introduced analytical chemical procedures and applied these to problems of health, scientific agriculture and consumer protection.

From these small beginnings the chemical science diversified as newer knowledge and methodologies became available, and became destined to play a major supporting role in the development and application of scientific agriculture, mining, public health and animal disease control, nutrition and consumer protection.

The decades from 1950 to the present have been marked by very large increases in both the number and expertise of professional chemists at the colleges and universities, the energy spector and in state agencies related to public health and consumer protection. New techniques in spectrometry of all types, chromatography and use of radiolabeled isotopes have greatly expanded the role of chemistry in a wide range of physical, biological and health-related sciences. Along with this expanding supporting role, recent decades have also witnessed a new role for chemistry--a substantial effort in basic science reflecting the maturing of chemical sciences in North Dakota and of the contributing scientists.

(5)

A CENTURY OF EXCELLENCE: COMPUTING AND ITS INFLUENCE

Gene A. Kemper
Associate Vice President For Academic Affairs
University Of North Dakota

There have been enormous developments in computer system technology during North Dakota's century of statehood. Historically, some of these developments occurred at the time of our admittance as a state, the depression of the 1930s, the global conflict of the 1940s, and the ensuing years. A brief review of these developments will establish a perspective of the status of the computing technology in the early 1960s when the University of North Dakota and North Dakota State University both installed their first computer systems.

The development of computer services in higher education is a valuable asset to education, business, industry and government use of computers in North Dakota. From the establishment of the two university computer centers, to the formation of the statewide Higher Education Computer Network, and beyond the state with connection to national supercomputer networks and world-wide message networks, North Dakota is indeed participating in the computer revolution and the information age. The primary focus of the presentation is the development of higher education computer services, applications, and influence on the state since the early 1960s.

A bit of prophecy will identify an exciting research activity that has the potential to sustain the technological computing revolution.

SYMPOSIUM

ON

METHODS AND APPLICATIONS OF BODY COMPOSITION ASSESSMENT IN HUMANS AND ANIMALS

Presiding: Henry C. Lukaski, USDA, ARS, Human Nutrition Research Center, Grand Forks.

43. Methods of Human Body Composition Assessment.
Henry C. Lukaski, USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND

44. Anthropometry and Auxology: An Historical Perspective.
John A. Williams, Department of Anthropology, University of North Dakota, Grand Forks, ND

45. Assessment of Physique and Its Relationship to Physiological Function.
William W. Bolonchuk, Department of Health, Physical Education, and Recreation, University of North Dakota, Grand Forks, ND

46. Effect of Bone Mineral Content on the Estimation of Body Fatness by Hydrodensitometry.
D. T. Drinkwater, and A. D. Martin, Sport and Exercise Sciences Research Institute, University of Manitoba, Winnipeg, Manitoba

47. Compositional Changes After Weight Loss in Obese Humans.
Donna J. Terbizan, Department of Physical Education and Athletics, North Dakota State University, Fargo, ND

48. Changes in Human Body Composition in Disease.
William W. Goodall, Department of Clinical Affairs, School of Medicine, University of North Dakota, Grand Forks, ND

49. Assessment Body Composition of Livestock.
Martin J. Marchello, Animal and Range Sciences Department, North Dakota State University, Fargo, ND

(43) METHODS FOR THE ASSESSMENT OF HUMAN BODY COMPOSITION

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The application of methods for the assessment of body composition extends across many scientific disciplines. The physical anthropologist or human biologist uses estimates of body composition to compare populations, and to describe structural changes associated with growth, development, and aging. Human nutritionists assess fat free mass and fat mass in evaluation of energy status in malnutrition, pregnancy, and obesity. Animal scientists seek to estimate live animal carcass composition in meat-producing livestock. Physiologists rely upon methods of body composition assessment in physical fitness appraisal, athletic counseling, and the establishment of references for physiological variables, particularly in energy metabolism. In clinical medicine, there is an increased interest in assessing compositional changes associated with metabolic diseases and in developing new bases for drug therapies and anesthesia. Thus, body composition methods are multidisciplinary research tools.

Assessment of body composition can be performed safely and non-disruptively using methods developed to quantitate a specific component of the body (1). Based upon direct chemical analyses of mammals, including some human cadavers, the body contains four distinct chemical groups: water, protein, fat, and ash or bone mineral (2). In functional terms, the body consists of two chemically distinct compartments, fat and fat free (2). The composition of the fat free body is assumed to be relatively constant with a density of 1.10 g/cc at 37°C, a water content of 72-74%, and a potassium concentration of 50-60 and 60-70 mmole/kg for women and men, respectively. In contrast, fat or stored triglycerides, which is anhydrous and contains neither nitrogen nor potassium, has a density of 0.90 g/cc at 37°C.

Based upon these distinct physical and chemical differences between the two compartments, methods have been developed for estimating either fat free mass or fat mass. Body fat can be measured using hydrodensitometry, anthropometry, and ultrasound. Hydrodensitometry, or underwater weighing, measures body volume by water displacement. Using measurements of body mass in air and mass in water and determination of residual lung volume, body density is calculated and is used to estimate body fatness, expressed as a percent of body weight.

Measurements of skinfold thickness, including both adipose tissue and skin, are used either independently or in conjunction with body circumferences to predict body density which is used to estimate body fatness. Determinations of adipose tissue thickness can also be determined using ultrasonic sound waves. Whereas anthropometry and ultrasound are easy to perform, they generally yield smaller predictions of body fat than does hydrodensitometry because they rely upon regional measurements of the body to predict total body fat.

Because water, protein, and potassium are present only in the fat free body, methods have been developed to measure them. Water is measured using dilution of hydrogen isotopes. Body potassium is determined by external counting of the gamma ray emissions of the naturally occurring isotope potassium-40 using a whole body counter. Total body nitrogen is measured using prompt gamma neutron activation analysis in which an individual is irradiated with neutrons from a uniform isotope source. The nitrogen in the body becomes transiently radioactive and emits a specific energy gamma ray whose total activity is quantitated using external detectors. Measurements of total body water, potassium, and nitrogen are used to predict fat free mass based upon their known concentration in the fat free body.

Electronic methods are also available that estimate body water. Bioelectrical impedance analysis, which uses foil surface electrodes and introduces a radio frequency current into the body, depends upon electrical conduction to predict body water. Similarly, total body electrical conductivity, which uses a high frequency electronic field through which the body is moved, measures the disruption of the field as the body passes through it and relates a change in field strength to body water volume. Both methods have also been used to estimate fat free mass.

An alternate approach is to partition the fat free body into a metabolically active, intracellular component and a relatively inactive extracellular fraction. The body cell mass includes viscera, muscle, and brain; it represents the energy expending portion of the body. The extracellular mass consists of the skeleton, connective tissue, transcellular and extracellular fluids. The extra- and intracellular masses are clinically important compositional variables that are estimated using radioisotopes.

1. Lukaski, H.C. (1987) Am. J. Clin. Nutr. 46, 537-556.
2. Keys, A. and Brozek, J. (1953) Physiol. Rev. 33, 245-325.

(44) ANTHROPOMETRY AND AUXOLOGY: AN HISTORICAL PERSPECTIVE

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In simple terms, anthropometry is the scientific technique of measuring the human body. The term anthropometry was first used in the seventeenth century by the German physician Johann Elsholtz (1,2, 3). Today anthropometric measurement takes many forms, from the measurement of body dimensions in living peoples to measurement of bone diameters in prehistoric skeletons. While anthropometric data are employed by many diverse disciplines anthropometry is primarily a methodology of physical anthropology.

Modern anthropometry has its roots in the beginning years of anthropology. In the early 19th century anthropologists began an interest in the relationships between living human populations (4,5). These individuals were the first to quantify the differences in human races. It was not until the late 1800's, however, that anthropometry truly became the medium of anthropology. It was during the latter half of that century that the scientific community grappled with Charles Darwin's concept of evolution and the evolution of life.

It is in the field of human growth and development, auxology, that anthropometric analysis has perhaps maintained the highest profile. Like anthropometrics in general, auxological anthropometry has very old roots. Although anthropometry was first used in the 17th century it was not until the latter half of the 18th century that it was used in a longitudinal growth study. Count Philibert de Montbeillard published measurements taken on his son during the years 1759 to 1777 (1,2). With the advent of the 20th century longitudinal growth studies became increasingly more common. American studies dominated the early pre-world war II years of modern auxological anthropometry (1,2,3). Between the years 1904 and 1948 17 studies were initiated with 11 reaching completion and two still in progress today. In the years following the second world war modern growth studies came into their own. The Fels Institute for the Study of Human Development, the Harvard Growth Study, the Denver Child Research Council, the Brush Foundation for the Study of Human Growth and Development, and in Great Britain the Institute of Child Health (also known as the Harpenden Study) became synonymous for longitudinal growth and development studies (1,6). It is through these studies that auxological anthropometry has reached its current level of sophistication.

From very modest beginnings auxological anthropometry has expanded beyond the scope of normative growth studies. Today studies now often focus on specific research problems (7). Roberto Frisancho, for example, has shown the applicability of auxological techniques to the growth dynamics of high altitude populations. Others, including Robert Malina and Francis Johnston, have concentrated on the specific circumstances surrounding adolescent growth. With a look to the future studies are now focussing on environmental factors such as environmental pollution.

As we move into the 1990's computers have become common fixtures in growth study centers. At the same time, some researchers have been experimenting with automated anthropometry. Both events are moving us farther and farther away from the days of time consuming manual data extraction. While anthropometric auxology will always suffer from inherent drawbacks involved with human observers and subjects it still remains the principal technique for the analysis of human growth and development.

1. Cameron, N.T. (1984) The Measurement of Human Growth. Croom Helm, London.
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(45) ASSESSMENT OF PHYSIQUE AND ITS RELATIONSHIP TO PHYSIOLOGICAL FUNCTION

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The assessment of physique has revealed a relationship between the dominant somatotype and body composition (1,2). Fat weight was positively related to endomorphic dominance, fat free weight was positively related to the mesomorphic dominance in the male but unrelated to mesomorphic dominance in the female, and fat weight and fat free weight were negatively related to ectomorphic dominance.

Modification of the dominant component of somatotype was also related to body composition (3). Endomorphic modification of the physique was associated with greater fat weight in both the ectomorphic and mesomorphic dominant physiques. Mesomorphic modification was associated with greater fat free weight in the ectomorphic and endomorphic physique while the ectomorphic modification was associated with less fat weight and less fat free weight in the endomorphic and mesomorphic dominant physique.

The relationship between physique and physiological function has developed from the established hypothesis of the relationship between structure and function. Tanner (4) reported the relationship between skinfolds and serum lipids which was followed by several investigations including Gordon's (5) recent report of the relationship between somatotype and serum lipids. Sheldon (6) observed that certain body types reoccur among participants of given athletic activities. Novak's (7) results agree with Sheldon's observation and identify the performance differences between athletes and nonathletes. Sills (8) and Cureton (9) demonstrated the relationship between somatotype (both athletic and nonathletic groups) and exercise performance. Schreiber (10) has supplied the only physiological function data by somatotype that appears in the literature. This study reported higher concentrations of blood lactate in ectomorphs than in mesomorphs or endomorphs over an eight week training period.

Table 1
Physiological Function Variables by Somatotype

	N	Power kgm/min	VE ml/min	A ml/kg/min	VO_2 ml/min	VCO_2 ml/min	Heart Rate BPM	Lactate mM
Endomorph	8	1106	81	30.7^a	2586	2871	171^a	4.9^a
Mesomorph	28	1307	96	38.4^b	2983	3281	186^b	6.6^b
Ectomorph	15	1260	100	40.7^b	2710	3247	184^b	7.4^b

^{a,b}Different superscripts indicate significance, $p < 0.05$.

The response of physiological function was studied in 51 male subjects at the Grand Forks Human Nutrition Research Center. A test of physical work capacity (PWC) to voluntary maximum revealed greater ($p < 0.05$) oxygen consumption, heart rate and lactate production by mesomorphs and ectomorphs than endomorphs (Table 1). These findings suggest that physiological function is positively related to physique as determined by dominant somatotype.

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(46) EFFECT OF BONE MINERAL CONTENT ON THE ESTIMATION OF BODY FATNESS BY HYDRODENSITOMETRY

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Despite the expanding application of body composition analysis in many different disciplines, none of the existing techniques for estimation of percent body weight as fat (%fat) has ever been validated in humans. Since the amount of fat in the body cannot be measured directly in vivo, all current techniques must rely on the measurement of various indicators and make assumptions concerning the relationship of those indicators to body fat. For example, hydrodensitometry, long accepted as the "gold standard" for fat estimation, utilizes the measurement of whole body density. This method assumes the body comprises two components: a fat mass and fat-free mass, the densities of which are known and constant (1). Since anthropometric methods, bioelectrical impedance, total body conductivity and other techniques for estimating %fat are frequently "calibrated" against hydrodensitometry, it is essential to assess the validity of these assumptions. The density of fat is approximately 0.900 g/ml for all individuals, regardless of sex or age (2), but because the density of the fat-free mass in any individual is a function of the densities and proportions of the various tissues which comprise it, the density of the fat-free mass may vary widely from an assumed value of 1.100 g/ml.

Our recent cadaver dissection data (3) showed the proportions (42-59% muscle; 16-26% bone) and densities of the tissues comprising the adipose tissue-free mass (analogous to the fat-free mass), to vary considerably among subjects. The tissue which appears to most influence the density of the fat-free mass is bone. Schutte et al. (4) using size- and shape-matched white and black college age males observed that the body density of the black males was invariable greater than that of the white males. He concluded that this difference was due primarily to a greater bone mineral content in the black males and tentatively reported a new value of 1.113 g/ml for the density of the fat-free mass of this racial group. Lohman et al.(5), studied prepubescent children, who, due to their "chemical immaturity", have lower bone mineral density than adults, and derived a density value of 1.084 g/ml for their fat-free mass.

Since variation in bone mineral content leads to variation in fat-free density, the estimation of %fat by hydrodensitometry can result in large errors. In people with very dense bone, hydrodensitometry can yield an underestimate of true %fat to the extent that in very lean, muscular, young men negative values of %fat may be estimated. An example of this is the report of whole-body densities as high as 1.130 g/ml in Canadian football players (6), resulting in anomalous negative body fat predictions. In such subjects the density of the fat-free mass was speculated to be as high as 1.150 g/ml. Conversely, low bone mineral content, prevalent in the elderly but also seen in women with anorexia nervosa, athletic amenorrhea and premature menopause, will lead to over estimation of percent fat, an error not readily detected.

With the increasing availability of dual photon absorptiometry there has been a move towards evaluating the effect of bone mineral content on the hydrodensitometric estimation of body fatness (7-10). Drinkwater et al. (10) presented data on a homogeneous group of active men over 60 years of age showing the relationship of bone mineral density, as determined by photon absorptiometry at the distal radius and ulna, lumbar spine, and proximal femur, to body density determined by hydrodensitometry. The effect of bone mineral density and fatness indicators (skinfolds, girths) on body density was determined by multiple regression. Chest skinfold and waist girth accounted for 44% of the explained variance; trochanter mineral density an additional 30%. When whole-body density was normalized for bone mineral density, correction factors ranged from -0.0274 to 0.0175 density units corresponding to corrections of +11.9% and -7.8% fat, respectively, to the originally determined %fat values (mean 16%).

Thus, when estimating %fat by hydrodensitometry, bone mineral content and its relationship to fat-free density must be considered and accounted for, especially when evaluating lean subjects.

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(47) COMPOSITIONAL CHANGES AFTER WEIGHT LOSS IN OBESE HUMANS

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Obesity is characterized by an excess of fat patterned over the body. It has been associated with such diseases as diabetes, coronary heart disease (CAD), and hypertension, as well as others. To lower the risks of these individuals in relation to these diseases, a decrease of the excess fat is required. Measurement of body composition can reveal the amount of fat loss necessary to return these individuals to a level of acceptable health.

Very low calorie diets have been used to help obese individuals reduce the level of excess fat on their body. Barrows and Snook (1), studied changes that occurred in 15 obese patients on such a diet for 12.9 to 22.4 weeks. They measured body composition changes through anthropometrics and hydrostatic weighing. Total weight loss was 11.8 to 32.5 kg, with average percent fat decreasing from 46.8% to 35.5%. Of the total mean body weight loss of 19.9 kg, 83% was lost from body fat and 17% from lean body mass (LBM). Foster et al. (2), studied 80 moderately obese women also involved in a very low calorie diet. The purpose of this study was to determine the composition of the subjects excess weight as well as to measure resting energy expenditure. The body composition of these women was assessed by anthropometry, total body potassium, and total body water. Averaging these measurements, the authors concluded that the subjects had 48.3% fat and 51.7% fat free mass (FFM). Calculating excess body weight, they stated that of the excess weight, 68% was fat and 32% was FFM. They also were concerned with the fact that obese individuals have an excess of FFM before dieting, and that a reduction in FFM may be deterimental to the individual. Very low calories diets change body composition, but there are some drawbacks to the procedures.

Exercise training has been added to the very low calorie diet treatments in an effort to decrease the amount of weight lost in lean body mass. Henson (3), studied 14 obese women for 9 weeks, measuring body composition through skinfolds and electrical impedance. Exercise was performed on a cycle ergometer at 70% maximal oxygen consumption for 5 days/week, during weeks 4-6. In this time, lean body mass did not change, while weight and body fat percentage decreased. Lampman (4), exercised obese patients 4 days/week for 60 minutes in adition to a very low calorie diet. Weight loss reductions were primarily due to body fat loss, although slight reductions were observed in lean body mass. Hill (5), measured body composition changes of 8 obese women who consumed 800 kcal/day for 5 weeks. Five of these subjects also participated in daily aerobic exercise. All subjects lost an average of 8.1 kg during the study, 67% of the loss from fat and 33% from FFM. Exercising subjects lost 8.2 kg with 74% of the weight loss from fat and 26% from FFM, while sedentary subjects lost 8.0 kg, 57% from fat and 43% from FFM. Thus, inclusion of an exericse regimen with a hypocaloric diet results in increased body fat loss and a sparing of FFM.

Fat patterning has recently become an area of interest. Upper body obesity has been connected with CAD, hypertension, and diabetes. Recent research has demonstrated that most exercise and diet regimes help to reduce upper body obesity while having little effect on lower body stores. Wadden et al. (6), studied 68 women who lost an average of 12.3 kg, with the heavier and fatter subjects losing more weight and fat over the period of the study. Body fat distribution was estimated by circumference measures. It was demonstrated that most fat was lost from upper body deposits.

Treatment of obesity requires a decrease in the levels of excess fat with a retention of FFM or LBM. Very low calorie diets and exercise seem to be useful in achieving this goal. Fat patterning research has begun to demonstrate that very low calorie diets and exercise helped to reduce upper body obesity, which may help to reduce CAD, hypertension, and diabetes in these individuals.

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(48) CHANGES IN HUMAN BODY COMPOSITION IN DISEASE

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A number of approaches have been used to assess body composition in clinical practice. The most commonly measured variable is body weight. Unfortunately, changes in body weight among individuals with chronic diseases does not indicate compositional changes. For example, patients with congestive heart failure may demonstrate a weight gain that is not due to increases in body fat or fat free mass, but is attributable to increased body water due to fluid accumulation in the thorax.

Because weight loss occurs during the progression of many degenerative diseases, clinicians have attempted to estimate its effects on body protein stores using anthropometric measurements. The advantage of using standardized measurements of the body is that they are simple, inexpensive, and noninvasive. Using measurements of the triceps skinfold thickness and midarm circumference, Heymsfield et al (1) developed an equation for the estimation of bone-free arm muscle area. This equation was validated using computed axial tomography. It was determined in a clinical trial that the minimum range of arm muscle area compatible with survival was 9-11 cm^2.

Whereas the body can be classified into two chemically distinct compartments, fat and fat free, the use of traditional methods of body composition assessment, such as densitometry, in clinical patients was not feasable. Thus, there was a need to conceptualize functional compositional entities that would be useful in clinical practice. F.D. Moore et al (2) developed the concept of the mass of body cells as a measurable entity. The body cell mass includes muscle, viscera, glands, organs, and cellular components of the brain. It represents the metabolically active portion of the body. In contrast, the extracellular mass includes the tissues and fluids outside of the cell, and whose function it is to support and to transport. The extracellular tissues include the skeleton, connective tissue, and fluids bathing the cells.

Body weight is composed of two major components, fat and fat free. The fat free mass can be divided into cellular constituents: body cell mass and extracellular mass. Body cell mass is determined by a multiple isotope dilution technique that involves use of radioactive sodium and tritiated water. Determinations of total exchangeable sodium and total body water are used to calculate exchangeable potassium which is a measure of body cell mass. Similarily, exchangeable sodium is an index of extracellular mass.

These variable were used to describe compositional changes in malnourished and in post-surgical patients (3). Body weight (-16%) and body fat (-37%) were less in the malnourished patients, but fat free mass was similar between the groups. Body cell mass decreased 40% and extracellular mass increased 25% in malnutrition. After surgery, patients weighed less (-4%), had less fat (-3%), but similar fat free mass. Extracellular mass increased 10% and body cell mass decreased 14%.

A recent approach is to determine the elemental composition of the body using neutron activation analysis. An individual is exposed to a moderated beam of neutrons which interact with the elements of the body (4). Of interest are nitrogen and calcium which can be used to estimate body protein and bone mineral, respectively. Estimates of body protein and bone mineral together with body water (determined by hydrogen isotope dilution) allow for the calculation of body fat by difference from body weight.

This sophisticated approach has been used by S.H. Cohn and his colleagues (5) to describe the effects of cancer on body fat and muscle mass. Relative to age and sex matched healthy subjects, patients with lung, gastrointestinal, and head-neck cancers had reduced body fat and receiving combined nutritional (parenteral, enteral, and oral) support over a six month period, those gaining weight increased body water and fat equally, whereas those losing weight experienced a loss of protein.

These examples of the use of body composition methods to assess fat, protein, water, and body cell mass indicate their potential usefulness in understanding how disease affects body energy stores.

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(49) ASSESSMENT OF BODY COMPOSITION OF LIVESTOCK

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Efforts to predict body composition of live animals have had limited success due to body dynamics and variability in genetics, feeding practices, body types and methodologies. Even so, the meat industry has made dramatic progress in the past 20 years in reducing the fat content of domestic animals in response to consumer demand for leaner meat. A major goal of the meat industry is to develop a tool that is simple, noninvasive, practical, accurate and inexpensive. Methodologies that will reliably predict the body composition of the live animal has potential to produce the ideal meat animal as well as production and physiology aspects. Table 1 lists the various techniques used to determine body composition and some new techniques that have potential application.

Table 1

Techniques Used to Determine Body Composition

A. TRADITIONAL
- Weight
- Linear measurements of live animals
- Linear measurements of carcasses
- Visual assessment and subjective evaluation
- The back fat probe
- Reflection probe
- Ultrasonics

B. ANALYTICAL
- Whole-body ^{40}K counting
- Body density
- Anyl-Ray
- Tissue sawdust technique
- Electronic
  - a) EMME
  - b) TOBEC
  - c) Bioelectrical impedance
- Dilution technique
  - a) Urea
  - b) Deuterium oxide

C. INNOVATIVE
- Video image analysis
- Near-infrared reflectance
- Soluble short-lived radioactive gas tracers
- Computerized tomography
- Nuclear magnetic resonance imaging

Topel and Kauffman (1988) reviewed most of these techniques. Some of the equipment used to determine body composition cost less than a dollar while other equipment will run into the millions. Traditionally, the United States livestock industry has preferred live weight and visual assessment for estimating body composition. Even the use of the back fat probe has had limited use but yet its accuracy is as high as the best ultrasonic techniques. With the advent of new methods such as bioelectrical impedance, computerized tomography, and nuclear magnetic resonance imaging, progress will be made. It may require a combination of the new with the old and maybe incorporating dilution techniques to obtain body water. Based on recent literature and the utilization of current equipment in combination with new methods we may be able to develop the ultimate technique to ascertain optimum market weight, improve the animal by management changes, make payments to producers according to the value of the carcass and determine treatment effects without sacrificing the experimental animal.

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SYMPOSIUM

ON

AVOIDING PITFALLS IN STATISTICAL ANALYSIS

Presiding: Douglas H. Johnson, United States Department of the Interior, Fish and Wildlife Service, Northern Prairie Wildlife Research Center, Jamestown, ND

50. Scientific Versus Statistical Significance: Significance Testing With Large Samples.
J. M. Legler, and C. E. McLaren, Department of Mathematics, Moorhead State University, Moorhead, MN

51. Interpreting Tables of Correlations.
H. B. Slotnick, and Stephen Wonderlich, Department of Neuroscience, University of North Dakota School of Medicine, Grand Forks, ND

52. Multiple Meanings of The Multiple Coefficient of Determination.
Terry L. Shaffer, U.S. Fish and Wildlife Service, Jamestown, ND

53. Abusing The T-Test.
L. K. Johnson*, USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND

54. Choosing Appropriate Sums of Squares in Unbalanced Analysis of Variance.
T. Robert Harris, Statistics Consulting Center, Department of Mathematics, University of North Dakota, Grand Forks, ND

55. Nonlinear Models in a Nonlinear World.
Douglas H. Johnson, U.S. Fish and Wildlife Service, Jamestown, ND

56. The Joy or Nightmare of Analyzing Sandy Data.
M. B. Rao, Department of Statistics, North Dakota State University, Fargo, ND

(50)

SCIENTIFIC VERSUS STATISTICAL SIGNIFICANCE: SIGNIFICANCE TESTING WITH LARGE SAMPLES

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Despite the fact that significance testing is frequently under attack, it continues as a mainstay of scientific inquiry. Among the list of criticisms voiced against the use of significance tests is the fact that "with enough replicates nearly any null hypothesis can be rejected." As computers now facilitate collection of massive amounts of data, it is critical that investigators have a clear understanding of the effect of increasing sample size on significance test results.

For example, suppose the mean yields for two varieties of wheat actually differ by .05 bu/acre. An experiment with 10 plots for each variety probably will not have sufficient power to detect such a small difference. But eventually, by increasing the size of the experiment that difference of .05 bu/acre will be detected as "statistically significant". This phenomena is not unique to the t-test. Analyses of contingency tables with identical relative proportions but samples of 100 versus 400 can result in no significant association in the first case versus a highly significant association in the case of the larger sample.

Application of the one-sample chi-square test for goodness-of-fit to the distributions of red blood cell volumes from healthy individuals showed no significant lack-of-fit to the lognormal model in distributions containing approximately 5000 cells [1] but a statistically significant lack-of-fit in distributions containing at least 30,000 cells. [2] Graphical analyses showed no discernible difference between those distributions containing smaller sample sizes and showing a good fit to the lognormal model, and those distributions containing larger sample sizes and exhibiting a significant lack-of-fit to the lognormal model. Examination of the data revealed that for a distribution with a large sample size, a small discrepancy between the observed and fitted distributions may be detected by the chi-square goodness-of-fit test, yet this same discrepancy may go undetected in a distribution with a smaller sample size. Thus when using significance tests, it is important to take into account the sample size noting that larger samples will detect smaller discrepancies from the null hypothesis where they exist.

In addressing this concern, Cox and Hinkley [3] concluded: "The central point is that statistical significance is quite different from scientific significance and that therefore estimation, at least roughly, of the magnitude of effects is in general essential regardless of whether a statistically significant departure from the null hypothesis is achieved." This recommendation to report an estimate of the magnitude of the effect is straightforward in our first two examples. Techniques for constructing interval estimates for the true difference in means, (appropriate for the first example) and for the odds ratio (appropriate for the contingency table example) are well documented. Such is not the case for the goodness-of-fit problem.

However, an approach for measuring the degree of lack-of-fit has been developed and applied successfully to the evaluation of the distribution of red blood cell volumes. A measure of the degree of lack-of-fit is given by Moore [4]. For π_i = the true cell probability in the ith class and P_i = the expected proportion in the ith class under the null hypothesis, the discrepancy, d, is defined as

$$d = \sum_{i=1}^{k} \frac{(\pi_i - P_i)^2}{P_i}$$

While the theory for the construction of interval estimates for d, the true discrepancy, is not complete, it is reasonable to calculate the observed discrepancy, $\hat{d}$, and compare it to other observed discrepancies at a given sample size. Another suggestion for the goodness-of-fit test is to test the data against a number of different hypothesized models and to compare the resulting P-values. Plotting every possible discrepancy, d, against the resulting P-values results in a P-value function or consonance distribution [5]. Proponents state that this technique circumvents the artificial dichotomy imposed by significance testing and confidence intervals and is suitable to scientific investigation where the aim is knowledge and description, not decision making.

Thus, for scientific investigations, clearly sample size must be taken into consideration when significance tests are performed. As well as testing the statistical significance of the observed data, the estimation of the magnitude of the difference from the null hypothesis is critical where a judgement of "scientific significance" is needed.

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(51) INTERPRETING TABLES CORRELATIONS

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Introduction Phenomena of scientific interest are often multifaceted, and, in the process of studying them, we need to examine the relationship between each pair of facets. Using variables to represent facets, and Pearson product moment correlations to describe the relationships, we are presented with tables of correlations to examine and interpret. The examination is complicated by the number of correlations (e.g., there are 45 correlations for ten variables), which leads to difficulties we will call the problems of significance and interpretation.

If there is no relationship between the variables in a pair, there is still a nominal 5% chance of rejecting the true null hypothesis--a type I error. The problem of significance reflects the fact that as the number of correlations tested grows, the likelihood of at least one type I error increases markedly (at $\alpha = .05$, the likelihood is .60 when there are five variables, and .90 when there are 10). The obvious solution to the problem of significance is reducing the number of tests.

It is not necessarily true that all correlations with probabilities less than α present an immediately interpretable pattern. The problem of interpretation, then is one of finding some way to facilitate identification of theoretically important (i.e., meaningful) patterns among the correlations in the table.

Methodology We present an algorithm describing one way to address problems of significance and interpretation. It begins with graphic display of the variables individually and in pairs to allow visual examination of relationships among them, and proceeds to testing the entire correlation matrix to see if it departs from an identity matrix (i.e., a matrix where each variable correlates perfectly with itself and not at all with any other variable). If the correlation matrix under examination differs by no more than chance from an identity matrix, our work is done regardless of whether any individual correlations have small probabilities.

If the hypothesis that the correlation matrix is an identify matrix is rejected (i.e., at least two variables correlate in a markedly non-zero manner), our interest shifts to dealing with the problem of interpretation. We propose doing this by seeking clusters of measures (i.e., if several variables correlate well with each other in a pair-wise manner, the variables can be viewed--at least initially--as aspects of some construct of potentially theoretical importance). We propose this be done by identifying the principal components in the matrix, a procedure that (for our purposes) can be said to seek clusters of related variables. Further, these clusters (=components) are mutually independent, and the correlation of each variable with each component is also reported--a feature making it easy to see how each variable is related to whatever it is the component represents.

This methodology avoids testing individual correlations though, clearly, there are situations requiring specific correlations be examineded (e.g., theory building requires examining the relationship between specific variables). Under those circumstances, we recommend two related procedures, both protecting against type I errors. The first tests correlations as usual, but sets a stringent α level (e.g., if we wanted no more than a 5% chance of type I errors and had five correlations of theoretical interest, each would be tested at $\alpha = 0.01$). The second constructs a confidence interval around each correlation with an increased confidence coefficient (.e.g, if five intervals were to created, each would provide 99% confidence that the true population correlation fell within the interval in order to be 95% confident that no more than one of the five population correlations fell outside its interval).

Results and Discussion The above algorithm will be demonstrated with a data set to show both how it works and how to resolve problems associated with it (e.g., when variables appear related to one another based on the principal components analysis but have correlations failing to reach significance). A longer paper will also be available describing the statistical underpinnings of the procedure and explaining in more detail the methodology described.

(52) MULTIPLE MEANINGS OF THE MULTIPLE COEFFICIENT OF DETERMINATION

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The multiple coefficient of determination (R^2) is widely used by data analysts as a measure of the goodness of fit of a regression model to a set of data. It is so popular that most regression programs routinely calculate the statistic. Two recent papers (1,2) have demonstrated how careless reliance on the values produced by these programs can lead to erroneous conclusions. This paper will examine circumstances under which R^2 is commonly misused.

In a typical problem the analyst has a number of observations, each consisting of a response variable (Y_j) and K explanatory variables ($X_{1j}, X_{2j}, \ldots, X_{Kj}$), where j denotes the observation number. The object of the analysis is to uncover relations between the response variable and the explanatory variables. Often a linear relation of the form $Y_j = \beta_0 + \sum_i \beta_i X_{ij} + \epsilon_j$ is considered, in which ϵ_j represents a random error term. If b_i denotes an estimator for β_i, and we define $\hat{Y}_j = b_0 + \sum_i b_i X_{ij}$, then the b_i's are typically chosen so that $\Sigma(Y_j - \hat{Y}_j)^2$ is minimized. This method of estimation is commonly known as the method of ordinary least squares (OLS).

Much of the confusion surrounding the use of R^2 as a measure of goodness of fit stems from the lack of a consistent definition for it. Kvålseth (1) found eight different definitions appearing in the literature, but reported that six of them were equivalent for the above model using OLS, provided the intercept β_0 was included in the model. If we denote the mean of the Y_j's by $\bar{Y}$, then one definition is:

$$R_1^2 = 1 - \Sigma(Y_j - \hat{Y}_j)^2/\Sigma(Y_j - \bar{Y})^2.$$

R_1^2 takes on values between zero and one and can be interpreted as the proportion of variation in the Y_j's about the mean $\bar{Y}$ that is accounted for by the model.

A common misuse of R^2 occurs when comparing the fit of the above model in which $\beta_0 \neq 0$ with that of the no-intercept model (in which $\beta_0 = 0$). For no-intercept models R^2 is often calculated as the proportion of the variation in the Y_j's about zero, instead of about $\bar{Y}$, that is accounted for by the model. If $\bar{Y}$ differs from zero, then R^2 defined in this way may be substantially larger than R_1^2 for the intercept model even though the latter provides a better fit.

Transformations of the Y_j's pose another danger for the unwary analyst. Here, R^2 is expressed in terms of the variation in transformed Y values, and comparisons with models based on the untransformed Y values will not be valid. The same caution applies to models fit by the method of weighted least squares (2). In this situation R^2 measures the proportion of variation in weighted Y accounted for by weighted X.

Kvålseth (1) recommended the consistent use of R_1^2 as a means for comparing alternative models. Because of the numerous ways R^2 has been calculated, researchers should be certain that they understand how their regression program handles the calculation for various situations. Caution is also advised when comparisons are made with published material and the method of calculation is not specified.

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(53) ABUSING THE T-TEST

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Student's t-test is easy to calculate and, if used correctly, can be an effective research tool. Although the t statistic can be used to test numerous statistical hypotheses, this discussion will be limited to the use of the t-test for making inferences about sample means.

Suppose you obtain two random samples from two normally distributed populations having variances σ_1^2 and σ_2^2, respectively. Let $\bar{x}_1$ and $\bar{x}_2$ denote the sample means, s_1 and s_2 represent the sample standard deviations, and n_1 and n_2 be the sample sizes for groups 1 and 2, respectively.

The correct method of calculating the t statistic and its associated degrees of freedom depends upon the characteristics of the samples (1). If one can assume that the variances of the two samples are equal ($\sigma_1^2 = \sigma_2^2$), then a weighted average of the two sample variances, s_1^2 and s_2^2, provides the needed estimate of the population variance. The calculated t can be compared to the t distribution using $n_1 + n_2 - 2$ degrees of freedom. If the variances of the two samples are not equal ($\sigma_1^2 \neq \sigma_2^2$), then s_1^2 and s_2^2 can not be pooled. The resulting t statistic, t^*, does not have an exact sampling distribution. However, critical values for t^* can be found in tables of Student's t using Satterthwaite's approximation (2) to determine the appropriate degrees of freedom.

When the observations in a sample are measured or observed under both treatments, then the observations are not independent and are generally correlated. The difference between the two measurements obtained for each sample is an estimate of the relative effectiveness of the treatment. Under this design, each sample serves as its own control. To test whether there is a systematic treatment effect, one first calculates a difference score for each sample and then computes a mean ($\bar{d}$) and standard deviation ($s_{\bar{d}}$) of these n difference scores. To test whether there is a treatment effect ($\bar{d} \neq 0$), one calculates a paired (or dependent) t which has n-1 degrees of freedom.

A study of reported statistics in medical journals reported that the t-test is the most commonly used statistical test (3). Unfortunately, it is frequently misused. Common errors include violating the assumptions underlying the t-test and using the wrong form of the t-test given the sample data. Assuming equal variances in the two samples, if in fact the variances are not equal, may lead to incorrectly concluding that a treatment difference exists. In contrast, failing to use a paired t for correlated data may result in concluding that there is no difference between the two treatments when there actually is a systematic treatment effect within each sample. The paired t may be more efficient for some studies because intraindividual variability is excluded from the error estimate.

When an experiment involves more than two groups, an analysis of variance (ANOVA) should be used to analyze the data instead of multiple t-tests. If one simply tests all possible pairwise comparisons using a t-test, the probability of concluding an overall significant difference among means is greater than the alpha level used for each individual t-test. For example, an experiment which consists of 4 groups would require 6 t-tests to compare all possible pairs of means. If each t-test were performed using $\alpha = 0.05$, the overall error rate for the experiment would actually be $\alpha = 0.26$. The likelihood of incorrectly concluding that a significant difference occurred is substantially increased. An ANOVA should be performed to ensure the simultaneous correctness of a set of comparisons in order to guarantee a correct overall decision regarding the experiment.

Researchers must understand the statistical principles underlying the t-test before applying it to their research data or they risk the possibility of interpreting their data incorrectly.

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(54)

CHOOSING APPROPRIATE SUMS OF SQUARES IN UNBALANCED ANALYSIS OF VARIANCE

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Analysis of variance is a popular method of studying the dependence of a continuous response variable on one or more factors (categorical variables). If the amount of variation in the response variable related to a factor, computed as a sum of squares, is sufficiently large, one can conclude that the relationship is statistically significant (unlikely to have occurred by chance). If there are two or more factors, sums of squares are usually computed to test for interaction effects as well as the main effect of each factor.

Formulas for computing sums of squares given in many textbooks and computer software packages are correct (and computationally efficient) for one-way (single-factor) analysis of variance, and for balanced two-way and higher order treatment structures; e.g., an equal number of observations for each treatment combination. In the case of unbalanced treatment structures, there are several different ways to compute the sums of squares, leading to different results. None of them are necessarily "wrong," but they correspond to differing conceptions of the hypotheses to be tested. Two methods correspond to "sequential" and "unique" effects in multiple regression, but there are other methods as well because of the testing for interaction effects. Additionally, some formulas and computer software algorithms for comparing two levels of a factor give at best misleading results in unbalanced designs.

An overview of the concepts involved in these differences will be given, as well as advice for choosing a method and implementing it using one or more popular statistical computing packages.

(56)

THE JOY OR NIGHTMARE OF ANALYZING SANDY DATA

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Wind blown sands create sand dunes. It is not clear why sand collects into dunes at all, instead of scattering evenly over the land as do fine grains of dust, and how the dunes assume and maintain their own especial shapes. Sand dunes occur in two distinct environments; along the coasts of seas and rivers, on the one hand, and on the barren waterless floors of deserts, on the other. It is natural to enquire how the composition of sand particles and shape of a dune in a particular desert differ from those of a dune formed at a particular sea coast. Professor R. A. Bagnold made remarkable contributions towards a good understanding of formation of dunes.

This talk is concerned with some statistical problems that arose in the study of composition of sand particles in a sand dune. A random sample of a lump of sand particles taken from a sand dune constitutes as data. This sample of particles of sand is usually analyzed with respect to particle sizes by dividing the sample into subsamples by means of a series of sieves of diminishing size (i.e., mesh width) and then weighing, rather than counting the number of particles in, each subsample. The size distribution so obtained is not a frequency distribution in the usual sense.

In this talk, we consider the problem of fitting a parametric distribution to the observed mass-size distribution. We point out the difficulties that cropped up when we attempted to fit a particular parametric family of distributions, namely, "hyperbolic distributions" to the observed mass-size distribution. We will also point out the importance of this model fitting exercise in discriminating various sand dunes from sea coasts and deserts.

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(55) NONLINEAR MODELS IN A NONLINEAR WORLD

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A statistical model describes a relationship between a response variable Y and one or more explanatory variables $\mathbf{X} = (X_1, X_2, \ldots, X_k)$, as mediated by a set of unknown parameters $\boldsymbol{\theta} = (\theta_1, \theta_2, \ldots, \theta_p)$:

$$Y = f(\mathbf{X}, \boldsymbol{\theta}) + e,$$

where e represents an error term. Most of the commonly used statistical techniques available involve linear models, which can be represented as

$$f(X, \boldsymbol{\theta}) = \theta_1 X_1 + \theta_2 X_2 + \ldots + \theta_k X_k.$$

Linearity refers to the parameters that are to be estimated, not to the explanatory variables included in the model. For example, the polynomial model $f(X_1, \boldsymbol{\theta}) = \theta_1 X_1 + \theta_2 X_1^2$ is nonlinear in the variable X_1 but linear in the parameters and can be estimated with linear regression. Given a set of n observations of the response variable (Y_j, j=1, ..., n) and the corresponding explanatory variables (X_j, j=1, ..., n), the 'best' (i.e., least squares) fit to a linear model can be readily obtained.

Despite their power and ubiquity, linear models are not always the most appropriate. Many relationships in science are nonlinear. Nonlinear models are more difficult to deal with than linear models; least squares estimates of the parameters must be obtained iteratively. This paper discusses several concepts involved in nonlinear regression:

Choice of method Several numerical routines for arriving at least squares estimates of nonlinear models are available, often within a single regression program. Sometimes one method will find a solution when another method fails.

Specifying the function Unlike a linear model, which always has the same form (linear) with the user needing to indicate only the explanatory variables, a nonlinear model can take a variety of forms, which the user must specify. Some algorithms require also the partial derivatives of the function with respect to each parameter.

Specifying initial values Nonlinear regression routines require an initial value of each parameter to begin iteration. This step is possibly the most critical task in nonlinear regression. Five methods of arriving at initial values are described: 1) using prior knowledge from similar studies; 2) estimating from the linearized form, if the model can be transformed to linear; 3) calculating the error sum of squares for values on a grid of possible parameter values and using values that provide the smallest error sum of squares; 4) solving k nonlinear equations in k unknowns based on k representatively chosen observations; and 5) examining the function analytically. If the method fails to converge, or converges to nonsense values, with the initial values specified, others should be tried.

Bounds on parameters Often a nonlinear model involves a parameter that must be within a prescribed range. Some nonlinear regression programs allow the user to specify bounds. Otherwise problems can arise if the program attempts to evaluate a function for an inappropriate parameter value.

Overparameterization A routine may fail to converge if the model involves more parameters than can be estimated from the data. Careful analysis can indicate possible problems.

Residuals As with linear regression, results from model fitting should be closely examined. Particularly useful are graphs of the residuals, the differences between observed values of Y and values estimated by the model.

SYMPOSIUM

ON

POLYMER RESEARCH AND POLYMER CHEMISTRY IN THE CHEMISTRY CURRICULUM

Presiding: Martin Jones, Department of Chemistry, University of North Dakota, Grand Forks, ND

75. Formation of Hard, Tough Materials by Cross-Linking Low-Tg Liquid Crystalline Slide Chain Acrylic Copolymers.
Frank N. Jones and Der-Shyang Chen, Polymers and Coatings Department, North Dakota State University, Fargo, ND

76. Polymer Films as Matrices for the Electrodeposition of Metal Microparticles.
Duane E. Bartak, Sonja Semmens, Yizhong Sun, and Kent Kost, Department of Chemistry, University of North Dakota, Grand Forks, ND

77. Reactions of Polynaphthalenes in $AlCl_3$/NaCl Molten Salts.
Jeffery H. Banning and Martin B. Jones*, Department of Chemistry, University of North Dakota, Grand Forks, ND

78. Polymers in the General Chemistry Sequence.
Martin B. Jones*, Department of Chemistry, University of North Dakota, Grand Forks, ND

79. The Study of Polymers in the Undergraduate Organic Chemistry Class.
Randolph F. Rodewald*, Chemistry Department, Minot State University, Minot, ND

80. Physical and Engineering Aspects of Polymer Chemistry.
Dana T. Grow*, Department of Chemical Engineering, University of North Dakota, Grand Forks, ND

81. Polymer Chemistry in the Laboratory.
Milton Hanson*, Department of Chemistry, Augustana College, Sioux Falls, SD

81a. Polymer Chemistry at Bemidji State University.
Kirk P. Manfredi*, Department of Chemistry, Bemidji State University, Bemidji, MN

(75) FORMATION OF HARD, TOUGH MATERIALS BY CROSS-LINKING LOW-Tg LIQUID CRYSTALLINE SIDE CHAIN ACRYLIC COPOLYMERS

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Liquid crystalline (LC) acrylic copolymers were synthesized by grafting oligomeric p-hydroxybenzoic acid (PHBA) side chains to various methyl methacrylate/butyl acrylate/acrylic acid terpolymers. Mixtures of LC copolymers with hexakismethoxymethyl melamine (HMMM) were cast as films and baked at 150 °C, a temperature below the clearing points (152 to 183 °C). Adherent, solvent resistant films formed. Cross-linking presumably occurred by reaction of -COOH groups at the end of each PHBA side chain with HMMM. The ungrafted terpolymers were cross-linked similarly to provide controls. FT-IR indicated consumption of most of the -COOH groups in all cases; conversion of -COOH groups on PHBA residues appeared slightly lower than that of -COOH groups on acrylic acid residues. Birefringent domains were visible in the cross-linked materials; their population was much lower than the population of birefringent domains before cross-linking.

Both the hardness and the impact resistance of films made from liquid crystalline copolymers were invariably far superior to those of films made from their amorphous counterparts.

The potential utility of such LC copolymers as binders for thermosetting coatings was assessed. Variables studied were HMMM content, average length of PHBA grafts, Tg and Mn of the acrylic copolymer backbone, and functionality. When backbone Tg and functionality are both low (< 0 °C and < 7.5 mol %, respectively) coatings having an extraordinary combination of high hardness (> 30 KHN) and high impact resistance (> 80 in-lb) can be prepared.

The results have implications beyond the area of coatings. A new principle has been discovered: that cross-linking of low-T_g, liquid crystalline copolymers produces low-T_g materials that retain the elasticity associated with low-T_g but are substantially hardened and toughened. Possible applications in thermoset plastics and elastomers will be discussed.

(76) POLYMER FILMS AS MATRICES FOR THE ELECTRODEPOSITION OF METAL MICROPARTICLES

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The use of polymer films as matrices for metal deposition is an interesting and potential useful application in electrocatalytic processes. We have been interested in the deposition of catalytically active noble metals as very small particles (e.g., diameters less than 1000 A) in polymer films for important electrochemical reactions. A key factor in this work is the design and preparation of polymer films on electrode surfaces in order to achieve a combination of desirable properties. These polymer properties include: 1) chemical stability toward degradation reactions, 2) mechanical stability, 3) permeability relative to ion transport, and 4) electronic conductivity.

We have utilized poly(4-vinylpyridine) (PVP) as a matrix for the electrodeposition of platinum and other catalytic metals (1). In order to achieve chemical and mechanical stability of the PVP as a film (1.0 to 10μm thick) on glassy carbon surfaces, it was necessary to crosslink the spin-coated linear polymer. Two crosslinking procedures were used: 1) a free radical process which employed benzoyl peroxide and triallyl-substituted benzene and 2) a nucleophilic reaction using 1,6-dibromohexane to quaternize the pyridine's nitrogen. Both procedures produce a crosslinked polymer, which was stable and adhered to the carbon surface for days in 1 M sulfuric acid solution. However, because of PVP's relatively poor charge-transfer characteristics, we have initiated work on the deposition of metals in conducting polymer films.

We have found that polyaniline, which is a polymer with a wide range of conductivities that are dependent upon potential and pH (2), is an excellent matrix for the deposition of metal particles (3). The polyaniline films, which were prepared by the electrochemical oxidation of aniline in sulfuric acid solution, were found to have an ideal morphology in the form of a fibrillar structure. This open morphology with polymer fibers having a diameter of approximately 2000 A will allow effective mass-transport to the interior of the film in electrochemical experiments (Fig. 1). Polyaniline films which were 1.0 to 10 μm thick, were prepared on polished glassy carbon electrodes by cyclic voltammetric techniques.

Three-dimensional dispersion of platinum and palladium in the form of microparticles with a diameter of approximately 1000 A was accomplished by controlled-potential step electrodeposition experiments. Larger particles with diameters up to 3000 A were obtained in the electrodeposition of ruthenium. Characterization of the polymer films containing metal particles was accomplished by scanning electron microscopy (SEM) and elemental X-ray fluorescence analysis (EDAX).

The electrocatalytic activities of the polyaniline films containing metal microparticles were evaluated using them as a cathode material for the evolution of hydrogen or as an anode material for the oxidation of methanol. The platinum/polyaniline film exhibited good activity for the catalytic reduction of hydrogen and catalytic oxidation of methanol, respectively. In addition, the polymer film contributed a substantial amount of charge for the oxidation of methanol and offered a protecting matrix for the metal against loss of the particles. Finally, the polyaniline films containing metal particles will be compared to the PVP films in terms of their ability to effectively obtain three-dimensional dispersions of the metal particles and the overall stability in acid media.

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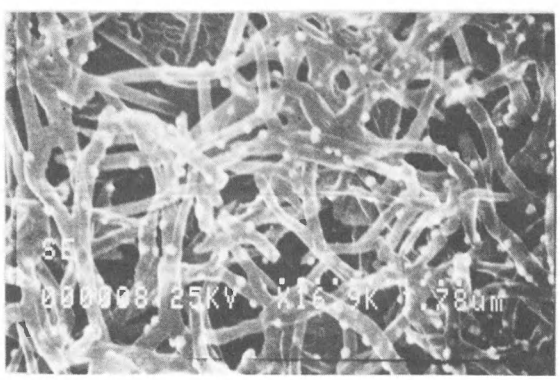

Fig. 1 SEM photomicrograph (x 16.9 K) of polyaniline film containing metal microparticles. The polyaniline film was prepared by cyclic voltammetry (six scans, 20 mV/s, -0.1V to 1.0 V vs. Ag/AgCl). The platinum was deposited by potential-step from K_2PtCl_6/H_2SO_4. The distance on the bar represents 1.78μm.

(77)

REACTIONS OF POLYNAPHTHALENES IN $AlCl_3$/NaCl MOLTEN SALTS

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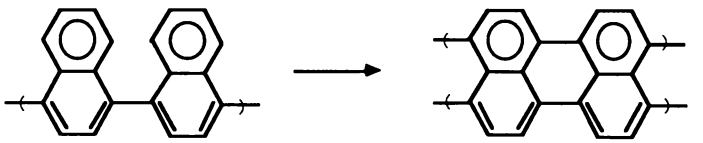
Poly(peri-naphthalene) (PPN, **1**) is a thermally stable organic polymer which possesses intrinsic electrical conductivity (1), and therefore does not need to be doped with electron acceptors or donors to achieve the conductive state. The physical and electrical characteristics of PPN make it an interesting target polymer. However, the current method of preparation of **1**, vapor-phase pyrolysis of 3,4,9,10-perylenetetracarboxylic dianhydride at temperatures greater than 500°C (1, 2), combined with its lack of solubility, greatly limits the processing of this material into useful forms. To address this problem, we have adopted a two-stage approach, involving synthesis of a processible precursor polymer followed by a curing reaction to give PPN (eq. 1).



2 **1** (1)

Poly(1,4-naphthalene) (**2**) and poly(1,5-naphthalene) (**3**) were prepared via Grignard coupling polymerizations, as previously described. (3) Prior research with binaphthyls (4) had shown that the desired ring fusion (e.g., conversion of 1,1'-binaphthyl to perylene) could be accomplished in moderate yield by treatment with a molten salt comprised of a 2/1 molar ratio of $AlCl_3$/NaCl at 125-150°C. Two samples each of **2** and **3** were subjected to the 2/1 $AlCl_3$/NaCl molten salt at 150°C for four hours. All four reactions yielded black, high melting (>400°C) products. Microanalyses of these materials revealed the presence of Cl, which could arise from nuclear chlorination or from molten salt or quenching solution (aq. NH_4Cl) residue. C/H molar ratios calculated from the analytical data ranged from 1.77-2.18 (assuming no chlorination of the aromatic nuclei) to 1.69-2.06 (assuming all chlorine is present as nuclear chlorine). These values are less than the theoretical value of 2.5 for PPN, but greater (except for one sample) than the C/H molar ratio for terrylene (three naphthalene rings fused at the peri posiitons). Thus, analytical data indicate that ring fusion of the naphthalene rings has taken place during the molten salt treatment. The initially obtained products were Soxhlet extracted with CH_2Cl_2, THF, and 1-methyl-naphthalene to remove low molecular weight components. Ultraviolet spectra of these extracts revealed the presence of perylene, terrylene, and quaterrylene, which may have arisen from ring fusion of binaphthyl, ternaphthyl, and quaternaphthyl present in the original polynaphthalene samples or from chain degraadation of the PPN formed in the reaction. A scanning electron photomicrograph of one of the extracted samples reveals that the bulk of the material is amorphous, granular substrate. However, pieces of nearly flat, ribbon-like material are also present. This offers further evidence that ring fusion has taken place.

Because of the degradation that evidently occurs during Lewis acid treatment of polynaphthalenes at 150°C for four hours, this method is not preferable as a means of preparing good yields of PPN. However, this method has demonstrated the validity of the two-stage approach for the synthesis of **1**.

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(78) POLYMERS IN THE GENERAL CHEMISTRY SEQUENCE

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The importance of a course in polymer chemistry to a chemistry major (undergraduate or graduate) who is planning a career in industry is well-established. Equally important, however, is an introduction to polymer chemistry for nonchemistry majors, who represent the future general populace of the country/world. A familiarity with composition/properties/uses of polymers will be valuable in the future to decide important issues such as disposal/recycling of polymeric products, use of alternative materials for packaging, construction, etc. and allocation of natural resources (particularly petroleum) between fuel and chemical manufacture. Most nonchemistry majors will not be able to take a separate course in polymer chemistry. Our chance to introduce the greatest number of students to polymers will come in the general chemistry sequence. Recognizing this, in 1980, the American Chemical Society established a Polymer Core Course Committee in General Chemistry, as one of six Polymer Core Course Committees. (1) This Committee was charged with investigating methods of incorporating polymer-related topics into the general chemistry sequence without requiring additional, polymer-specific time for the course. The Committee's report (2), copies of which will be available at the symposium, includes general topics that should be contained in a course, examples of how polymers can be used as tools for discussing important chemical principles and concepts, timely illustrations, references, summary statements regarding coverage of polymers in textbooks, and sample exam questions. Suitable laboratory experiments are not discussed in the report.

Most general chemistry textbooks include a little discussion of polymer chemistry in the back of the book within the chapter(s) which covers organic chemistry. Few texts have more than a cursory treatment of polymer chemistry (a notable exception being Chang's text, which has an entire chapter devoted to the topic (3)), which is understandable, given the time/material constraints. The location of polymer chemistry within the textbook, whether a separate chapter or not, often presents a problem. Few instructors in a typical two-semester course are able to cover the entire general chemistry text. Topics which are considered to be optional (i.e., not covered) are in the back of the text and frequently include organic/polymer chemistry. Aspects of polymer chemistry must therefore be introduced early in (and throughout) the course. One shortfall of most texts is the lack of use of polymers to illustrate concepts early in the text. The instructor, then, becomes responsible for giving the students appropriate examples during the course. For instance, in a description of solids, the contrast between crystalline and amorphous materials can be illustrated by comparison of sodium chloride and glass, which is a mineralogical-type of inorganic polymer (polysilicate). The principles of thermodynamics can be discussed using a rubber band. (3) Several other examples in addition to those in the Core Committee's report will be given in the presentation.

Lest the hands-on aspects of polymer chemistry be neglected, several experiments suitable for a general chemistry laboratory are available. In addition to the familiar nylon rope trick, students can prepare a polymeric allotrope of sulfur, polymerize dichlorodimethylsilane on a piece of filter paper, and synthesize poly(methyl methacrylate). (4) Demonstrations of experiments will be presented.

In times of decreasing enrollments in chemistry courses, the relevance of chemistry to everyday life cannot be ignored. Polymer chemistry is essential to life (and our lifestyle) and if discussed appropriately, can motivate/interest students in the study of chemistry.

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(79) The Study of Polymers in the Undergraduate Organic Chemistry Class

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A number of authors of Organic Chemistry textbooks (1) treat the study of macromolecules in a special topics chapter late in the text but introduce the concept rather early in the course when considering chemical reactions of alkenes. Students have been introduced to free radicals during the study of alkanes and are familiar with radical mechanisms involving initiation, propagation and termination steps as well as knowing the order of stability of alkyl radicals. Radical addition to alkenes is first shown while explaining the anti-Markovnikov addition of HBr to alkenes in the presence of peroxides. The free radical polymerization of ethylene, propylene, styrene and other familiar monomers is easily understood mechanistically in terms of already familiar steps. It is shown that chain termination may occur by a) combination of radicals , b) disproportionation of radicals and c) chain transfer. Head to tail addition of monomers is understood in terms of free radical stability.

The students are told that free radical polymerization is a form of **Chain Reaction Polymerization** , where each step produces another reactive particle and each step depends upon the previous one. It is further explained that such chain reaction polymers also may be formed through reactions initiated by either cations (usually acids) or anions (bases such as alkyl metal or amide ion). This presents an opportune time to review factors which tend to stabilize trivalent carbon intermediates. Students generally have little trouble understanding that alkenes undergoing cationic or anionic polymerization need functional groups which can stabilize intermediate carbocations or carbanions respectively. A great example of anionic polymerization is the base induced reaction of methyl 2-cyanoacrylate to form the familiar superglue. It is shown that the usual chain termination steps involved with the free radical or cationic polymerization are frequently unavailable in anionic reactions and the concept of a "living polymer" is presented.

The importance of stereochemistry in polymers is discussed when considering the polymerization of isoprene (a conjugated diene). Students are informed that natural rubber, a soft and tacky substance, is a polymer of isoprene where almost every double bond has **cis** geometry whereas the all **trans** isomer (gutta percha) is quite hard and brittle and is used in covering golf balls. It makes sense to most students that the softness and pliability of natural rubber is due to the mobility of the polymer molecules and their ability to slide by one another. The vulcanization process, in which polymer molecules are cross-linked by sulfide bonds is shown to restrict this motion and lead to a much harder and more durable rubber. Synthetic rubber made from the free radical polymerization of isoprene is quite different from the natural polymer because the geometry of the double bonds is randomly cis and trans. Students are generally interested to learn that either pure cis or pure trans polyisoprene can be formed if a **coordination catalyst** is used. The Ziegler-Natta titanium catalysts allow either isomer to be formed under the proper experimental conditions. These catalysts also permit the formation of linear polymers. These molecules form plastics with greater strength, stiffness and resistance to cracking than their branched counterparts which are formed in the radical process. Propylene can polymerize in several different stereochemical forms; isotactic, syndiotactic and atactic; all of which have different properties. Again, the Ziegler-Natta catalysts will selectively yield the isomeric polymers.

The concept of copolymerization is discussed and exemplified using the well known commercial plastic saran, an alternating copolymer of CH_2=CHCl and CH_2=CCl_2, and the idea of random and alternating copolymers is brought out.

Macromolecules resulting from reaction between two difunctional molecules are called **Step Growth Polymers**. Each bond in the polymer is formed independently of all others. the best known examples of these are the polyamides (Nylons) resulting from the reactions of diamines with diacids. Nylon-66 is the classic example of this and is formed from reaction of 1,6-hexanediamine (H_2N-$CH_2CH_2CH_2CH_2CH_2CH_2$-NH_2) and adipic acid ($HO_2CCH_2CH_2CH_2CH_2CO_2H$). Also well known (by name) to students are the polyester molecules. The study of these types of functional group polymers is normally reserved for the latter portion of the course after the chemistry of carboxylic acids and derivatives has been covered.

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(80) PHYSICAL AND ENGINEERING ASPECTS OF POLYMER CHEMISTRY

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Polymer chemistry affords the opportunity to reinforce fundamental ideas from thermodynamics and statistical thermodynamics in chemistry and chemical engineering courses[1]. Many of the ideas are similar to calculations in the kinetic theory of gases. For example, the probability, W(r), of one end of a polymer chain being in the region r to r+dr when one end is centered at the origin is

$$W(r)dr = (\beta/\pi^{1/2})^3 \exp(-\beta^2 r^2) 4\pi^2 dr \quad (1)$$

This is similar to a Maxwell-Boltzmann distribution of velocities. This probability distribution is then used to compute the average value of the end-to-end distance of the chain using the formula

$$\bar{r} = \int_0^\infty rW(r)dr / \int_0^\infty W(r)dr \quad (2)$$

The final answer is $\bar{r} = ln^{1/2}$, where l is the link length and n is the number of links. The same calculation is conducted to determine the average speed in a gas. This way of thinking about probability is also like finding the radial distribution function for the hydrogen atom by integrating over angular coordinates.

Another important example is the calculation of the elastic force due to the entropy of the chains. As is common in physical chemistry, there is an approach based solely on thermodynamics and an approach based on a molecular model and statistical thermodynamics. The change in the Gibbs free energy, dG, is

$$dG=VdP-SdT+fDL, \quad (3)$$

where V is volume, P is pressure, f is force, and L is the length of extension. The force is found as the derivative of the entropy

$$f = -T(\partial S/\partial L)_{T,V}. \quad (4)$$

In addition, there is a thermodynamic equation of state

$$f = (\partial E/\partial L)_{T,V} + T(\partial f/\partial T)_{V,L}. \quad (5)$$

The starting point of the statistical approach is the Boltzmann relation,

$$S = k \ln \Omega \quad (6)$$

where k is Boltzmann's constant and Ω is the probability. The entropy is lowered when the chain is stretched and the change is

$$\Delta S = -(k\nu_e/2)(\alpha^2 + 2/\alpha - 3) \quad (7)$$

where ν_e is the crosslink density and α is the extension.

Polymer solutions are also treated using statistical thermodynamics. The enthalpy, entropy and Gibbs free energy of mixing all have to be modified to account for the polymer chains. There are three types of interactions, solvent-solvent, polymer-solvent and polymer-polymer. The entropy of mixing formula is similar to that for gases if the proper variables are used

$$\Delta S = -k(n_1 \ln v_1 + n_2 \ln v_2). \quad (8)$$

Here n_1 is the number of solvent molecules, n_2 is the number of solute molecules, and v_1 and v_2 are the volume fractions

$$v_1 = n_1/(n_1 + xn_2) \quad (9)$$
$$v_2 = xn_2/(n_1 + xn_2) \quad (10)$$

where x is the number of links in a chain. The enthalpy of mixing is written in terms of the Flory parameter, χ,

$$\Delta H_M = kT\chi_1 n_1 v_2 \quad (11)$$

The Flory parameter characterizes the interaction energy per solvent molecule.

Topics in heat transfer, mass transfer, and fluid mechanics are also reinforced by examples from polymer technology. The design of extruders provides an exercise in the solution of flow equations. Heat transfer is also important in the design. Reactor design may also employ polymerization reactions.

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(81) POLYMER CHEMISTRY IN THE LABORATORY

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The laboratory experience in polymer chemistry is vital in teaching the unique aspects of macromolecules. More chemists are involved directly or indirectly with the polymer industry than are involved in any other aspect of the chemical industry. As students are prepared to enter the profession of chemistry, laboratory experience involving macromolecules is an important part of their preparation. Laboratory exercises to investigate the preparation and properties of polymers which do not require special equipment or great expense are available. Sources of these exercises will be given and some of them will be discussed.

(81a) POLYMER CHEMISTRY AT BEMIDJI STATE UNIVERSITY

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Reports from various ACS education committees have suggested the need to increase the amount of polymer chemistry taught to undergraduates (1,2,3). These suggestions have motivated the chemistry department at Bemidji State University to implement a new course in polymer chemistry. The initial course was taught for the first time in the fall of 1987. Its success has prompted the department to offer polymer chemistry as an upper division elective to be taught in alternating years.

The course was taught by the speaker who has no formal training in polymer science and spent a summer becoming a self taught polymer educator. The speaker will discuss his experiences with textbook selections, classroom demonstrations, and course content. A great deal of assistance was obtained from the education committee of the ACS Division of Polymer Science and industrial contacts. Inclusion of an extensive survey of inorganic polymers will be discussed. A number of simple classroom demonstrations will also be presented.

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SYMPOSIUM

ON

NORTH DAKOTA GRADUATES:

EXCELLENCE IN RESEARCH AND RESEARCH ADMINISTRATION

Presiding: Dr. Forrest Nielsen, President, North Dakota Academy of Science, Director, USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND

82. Hybrid Wheat Research - an International Perspective.
Ian B. Edwards*, Pioneer Hi-Bred International, Inc., Johnston, IA

83. University-Sponsored Research in North Dakota's Economic Development, and the Role Played by Graduate Students.
J. L. Ozbun*, President, North Dakota State University, Fargo, ND

84. Direct and Indirect Pathways of Hepatic Glycogenesis: Roles of Glucose-6-Phosphate.
Robert C. Nordlie*, Department of Biochemistry and Molecular Biology, University of North Dakota Medical School, Grand Forks, ND

85. The University of North Dakota Energy and Mineral Research Center, a Key Element in Economic Development and Higher Education in North Dakota.
Gerald Groenewold*, Director, Energy and Mineral Research Center, University of North Dakota, Grand Forks, ND

86. The Metabolism and Function of Platelet Activating Factor and Related Ether Lipid Mediators.
Fred Snyder*, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN

(82) HYBRID WHEAT RESEARCH - AN INTERNATIONAL PERSPECTIVE

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Since the first report of cytoplasmic male sterility (CMS) in wheat in 1951 and the discovery of a usable system for hybrid production in 1962, significant progress has been made in hybrid wheat breeding. The first decade of hybrid wheat development was devoted largely to perfecting the genetic mechanism, while the second decade has addressed the questions of agronomic improvement in inbreds and an increase in testing for heterosis and for general and specific combining ability. The basic problem of wheat requiring a high seeding rate and having a low seed multiplication ratio compared to other crops has imposed a limitation on testing. The complexity of the pollen fertility restoration system also slowed progress in male inbred development. This problem has since been overcome.

The first viable chemical hybridizing agent (CHA) was identified in 1973, and improved products have been developed since that time. CHA's offer a number of advantages over the CMS system and breeders have been able to produce and test more hybrids than was previously possible. The first commercial hybrids developed with a CHA were released by Rohm & Haas Company in the Hard Red Winter and Soft Red Winter wheat areas in 1982. Pioneer Hi-Bred International has been engaged in hybrid wheat research since 1968, and marketed Hard Red Winter wheat hybrids (developed by the CMS system) in Kansas and Oklahoma from 1975-81. Small quantities of HRW hybrid seed continue to be marketed by two companies in the U.S.

In Europe, the first commercial CHA hybrid wheat seed was sown in France in 1983-84. Interest in hybrid wheat production remains strong, and chemical manufacturers are currently awaiting full label registration on new CHA compounds. However, until that time commercial production will be highly restricted. There are four factors that affect farmer acceptance of hybrid wheat namely: 1) the extra seed cost per acre compared with varietal seed; 2) the anticipated yield increase of the hybrid over standard varieties; 3) the grain market price of wheat; and 4) the cost of credit to the farmer. There are two factors that favor the use of hybrid seed in Europe compared to the U.S., namely: 1) higher yields - the result of more favorable climatic conditions and intensive management practices; and 2) higher grain prices - the result of EEC subsidies on wheat.

Pioneer commenced international wheat research operations in Europe in 1985. Three years of testing in France revealed a yield advantage of the top five hybrids over the check variety mean of 12-23%, 5-14% and 11-16% in 1986, 1987 and 1988 respectively. In Spain, the 1988 hybrid yields from multi-location testing were 6-14% above the cultivar "Cartaya" and 27-34% above "Yecora Rojo". Experience with experimental CHA hybrid production indicates that hybrid seed can be economically produced and the use of hybrid wheat will provide a satisfactory return on investment to both the farmer and the seedsman. Research is now directed towards the effective use of heterotic pools in hybrid breeding, including the development of winter x spring hybrids for several European locations.

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(83) UNIVERSITY-SPONSORED RESEARCH IN NORTH DAKOTA'S ECONOMIC DEVELOPMENT, AND THE ROLE PLAYED BY GRADUATE STUDENTS

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One of the most difficult problems faced by colleges and universities in gaining public support for research and other scholarly activity is to communicate, in clear and persuasive ways, the value of research.

To a very large segment of the general population, research is an esoteric and largely incomprehensible activity, one requiring substantial infusions of public money, with relatively few tangible, understandable returns.

In a few cases, such as the development of specific crop varieties that can quickly be shown to return new dollars, or medical breakthroughs (Salk Polio Vaccine, for example) which have dramatic and measureable effects on public health, the benefits are obvious.

But in many, many more, by far the majority of instances, it is difficult if not impossible to demonstrate direct returns to the public.

As a consequence, even in dealing with fairly sophisticated mass media, what, on the surface, may appear to be frivolous research undertakings are often held up to public ridicule. Examples include Sen. William Proxmire's "Golden Fleece Award," Ralph Nader's report on agricultural research entitled "Hard Tomatoes, Hard Times," and the episode of the CBS program "60 Minutes," a few years back, which characterized faculty research grants at a neighboring institution as "Welfare for eggheads."

A related matter is the concern that legislators, and to a lesser degree, members of the public sometimes express about the high cost of operating graduate programs, particularly when a high proportion of graduate students appear to be other than U.S. citizens.

Reluctant, though many of us may be to acknowledge that public skepticism about the things we do, it is a reality with which we must deal. Failure on our part to do so constitutes hiding our heads in the sand.

So how do we deal with the problem of communicating clear, understandable research values to an often-doubting public, thereby gaining greater confidence and support? It is not easy. The most obvious solution is not necessarily the right one.

It is human nature, I suppose, presuming you believe there is such a thing as human nature, to believe the solution is simply to talk a little harder and a little louder to persuade people of the validity of our position--that research is inherently valuable. You simply have to take our word for that. But communication research has shown that's not how people are persuaded. They are persuaded, as are you and I, when we are shown it is in our best interests to adopt a different point of view. Then, and only then.

All of us have had the experience of watching and listening to political campaigns. The folks who put those ads together are experts at persuasive communication. Yet how many of us are actually convinced to vote just the opposite of the way we had originally intended to vote? My assumption is very, very few. People see what they want to see; hear what they want to hear; do what they want to do--selective exposure, selective perception, selective retention. That's the social scientific description of such behavior. As scientists, it is our responsibility to understand that process and to put it to work in gaining public support for the vital work we do.

There are obvious hazards, of course, to putting research, especially basic research, into the context of economic development. Sooner or later, we will be called upon to demonstrate in measurable ways that the result was worth the investment. So, before we climb on board the wagon of economic development, we need to be prepared for that day of reckoning. But I'm convinced we can deal with that. I'm equally convinced it's the only route to go. We simply can't go on expecting the public to maintain its support, based mostly on blind faith.

The intent of this presentation is to explore ways in which we, as scholars and scientists can more effectively communicate the value of scientific research to the general populace, thereby gaining vitally needed support.

(84) DIRECT AND INDIRECT PATHWAYS OF HEPATIC GLYCOGENESIS: ROLES OF GLUCOSE-6-PHOSPHATASE

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Classically, a poised balance between hepatic glucose phosphorylation and glucose-6-phosphate hydrolysis has been considered a major determinant of the direction and rate of flux of glucose between mammalian liver and blood (1). When the glucose phosphorylation rate exceeds that of glucose-6-phosphate hydrolysis, the liver takes up glucose from the blood and deposits it as potential energy in the form of glycogen. When blood glucose is low, the rate of glucose-6-phosphate hydrolysis exceeds glucose phosphorylation and a net flux of glucose from the liver to the blood occurs.

Within the past ten years, the universality of this concept has been questioned (2). This questioning was triggered by the apparent limited availability, or total absence, of the enzyme classically believed to catalyze hepatic glucose phosphorylation---glucokinase. This enzyme is low or absent in livers of the majority of species examined, and in livers of acutely fasted or diabetic mammals. Glycogenesis in response to a glucose load occurs in all, however. Based on these and similar observations, others (2) have postulated that to resolve this "glucose paradox", an alternative pathway for conversion of glucose to hepatic glycogen must exist. This has been termed the "indirect pathway" to contrast it with the classical "direct pathway" described above. It involves glycolysis (including glucose phosphorylation) in some non-hepatic tissue, followed by hepatic gluconeogenesis and ultimate flux of glucose-6-P to glycogen.

We have for 25 years advocated an hypothesis alternative to the above (1). Glucose-6-phosphatase, we have shown, is a bifunctional enzyme capable of potent synthetic as well as hydrolytic function. For example, inorganic pyrophosphate or carbamyl-phosphate, may, in the presence of this enzyme, transfer a phosphoryl group to glucose to produce glucose-6-phosphate (1). Unlike glucokinase, this activity is present in the livers of all species examined, and in livers of diabetic or acutely fasted mammals.

The specific inhibitor of glucokinase, N-acetylglucosamine (GlcNAc), has been used as an experimental probe in our laboratory to quantitate the portion of hepatic glucose phosphorylation which is attributable to glucokinase and that portion which is not. Isolated, perfused livers (3) and isolated hepatocytes (4) served as models for study. Preparations from fasted, fed, and diabetic rats were employed. Net glucose uptake, glycogenic rate, steady-state glucose-6-P concentration, and rate of hepatic glucose phosphorylation measured by the release of ^{3}H from 2-^{3}H-D-glucose were the parameters examined.

Our experimental results indicate that even in the nearly complete absence of glucokinase, hepatic glucose uptake, glycogenesis from glucose, and glucose phosphorylation all occur. This glucose phosphorylation appears to be due to synthetic function of glucose-6-phosphatase (3,4). With our perfusion system, a maximum of 25% of total glycogenesis from glucose can be accounted for by the indirect pathway wherein the erythrocyte serves as the site of requisite glycolysis (3).

We have also focused recent studies on the effects of inhibition of glucose-6-phosphatase, a requisite of both the "indirect" and "direct" pathways, and on the consequences of such inhibition upon net glucose utilization and hepatic glycogenesis. The metabolic inhibitor 3-mercaptopicolinate, as well as the metabolite fructose-1-P arising with fructose feeding or infusion, have been shown to inhibit glucose-6-phosphatase competitively. Both also serve to enhance net glucose uptake and, especially, to promote flux from glucose to hepatic glycogen. Details of both of these studies will be discussed, by Drs. Ann Bode and Brian Robbins, elsewhere at these meetings. (Supported in part by Research Grant AM/DK 07141 from NIH, and by grants from the North Dakota Affiliate, Inc., American Diabetes Association, and the Dakota Aerie of Eagles).

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(85) UNIVERSITY OF NORTH DAKOTA ENERGY AND MINERAL RESEARCH CENTER, A KEY ELEMENT IN ECONOMIC DEVELOPMENT AND HIGHER EDUCATION IN NORTH DAKOTA

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The Energy and Mineral Research Center (EMRC) at the University of North Dakota, embraces an integrated systems approach to energy, environmental, and mineral research, beginning with fundamental evaluation and characterization of earth resources, followed by research and development of innovative technologies to efficiently utilize those resources, and culminating in the utilization and/or safe disposal of wastes generated in natural resource consumption.

Originally a federal coal research facility, the Center became part of the University of North Dakota in 1983. Since that time, the EMRC has expanded its coal research programs, and is the leading low-rank coal research facility in the world. In addition, the EMRC has the leading groundwater research program in this region and has major and expanding research programs in waste management, air emissions control, fluidized-bed combustion, and geothermal resources, as well as growing programs in oil and gas, and the development and application of innovative analytical procedures and technologies for waste-site evaluation and cleanup. A major emphasis of the current EMRC coal research program is research and development focused on "clean" technologies for utilizing our vast coal resources.

Today's energy, environmental, and mineral research needs typically require the expertise of a total systems team which can focus on specific details while retaining a broad perspective. More than 150 professional full-time scientists, engineers, and technicians are available at EMRC to address current problems and assess future needs. The total EMRC staff at the end of 1988 numbered 204 individuals. This reflects a consistent growth from a total staff of approximately 160 in July of 1987. EMRC integrates the talents of full-time research chemists, physicists, biologists, geologists, groundwater hydrologists, chemical engineers, civil engineers, mechanical engineers, electrical engineers, geological engineers, and many others, to provide a unique research group dedicated to client needs and complemented by a broad academic community. The EMRC permanent professional research staff is dedicated entirely to contract research and receive no state appropriated funds.

The key to a productive research environment is in providing high-quality researchers with the equipment and environment to be creative. The EMRCs engineering and scientific research staff is extensively equipped with state-of-the-art analytical and engineering facilities. The main EMRC facilities, with over 120,000 square feet of laboratory and pilot-plant space, are located on the southeast corner of the University of North Dakota campus. Additional laboratories are located within the engineering, geology, and chemistry departments. Research capabilities span the range of sophisticated laser diagnostic tests on milligram samples to ton-per-hour process development units and pilot plants. High-severity processes can be developed from conceptual ideas through proof-of-concept demonstration in the flexible EMRC reactor systems. Laboratory- and pilot-scale combustors up to 1.4 million BTU/hr, as well as diesel and gas turbine simulators, are available for evaluating new fuels and assessing new emissions control technologies. Test equipment is available to address the entire waste management system from wastewater treatment to benign disposal or use of solid residues. Analytical laboratories at EMRC are equipped for the chemical and physical characterization of a wide variety of naturally-occurring and synthetic materials. Analyses are conducted using a combination of spectroscopic, chromatographic, and physical methods. Available analytical techniques can be applied for the identification and characterization of solid, liquid, and gaseous materials. Quantitative and qualitative analyses of organic and inorganic materials, mineralogical and surface characterization of complex crystalline solid samples, along with microbiological and thermal analyses, provide data used in investigations being conducted by various scientists and engineers at EMRC. Thus, the EMRC contains facilities providing a total system assessment of a wide variety of energy, environmental, and mineral resource research topics.

Although the EMRC is the primary research entity within the University of North Dakota School of Engineering and Mines, it also has a commitment to participating in the educational programs of the University and the state by providing research support, scholarships, and fellowships to undergraduates, graduates, and post-doctoral students. Therefore, EMRC research programs are providing intelligent energy, environmental, and mineral-resource-utilization strategies and training opportunities for current and future generations, as well as providing major opportunities for natural-resource-based economic development in North Dakota.

(86) THE METABOLISM AND FUNCTION OF PLATELET ACTIVATING FACTOR AND RELATED ETHER LIPID MEDIATORS

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One of the most potent lipid mediators ever discovered (1-3) is a novel phospholipid class possessing the chemical structure 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine. It is commonly referred to as platelet activating factor (PAF) but this is a misnomer in view of the diverse biological activities PAF possesses. A diglyceride-type analog (1-alkyl-2-acetyl-sn-glycerol) also exhibits properties similar to PAF, but it is less active (4). These ether-linked lipid mediators are capable of eliciting a variety of biological responses (see 5 for recent review) such as (a) hypotension, (b) aggregation and degranulation of platelets and neutrophils, (c) chemotaxis, (d) increased Ca^{2+} influx, IP_3 production, and glycogenolysis, (e) bronchoconstriction, and (f) numerous other effects. The biological potency of PAF can best be envisioned by realizing that 1 oz. of PAF dissolved in 1.42 billion gallons of water (3,310 football fields covered 1 foot deep with water) is a concentration sufficient to aggregate platelets as well as generating other PAF-induced cellular responses.

PAF is generally considered to be an undesirable autocoid responsible for severe pathological consequences and it has been strongly implicated as a major contributing factor in asthma and a variety of other diseases. On the other hand, it should also be stressed that PAF appears to be an important physiological mediator; the latter has been brought to the forefront in studies documenting PAF is required for the successful implantation and development of the fertilized egg in the uterus. The two enzymatic pathways for PAF production described by our laboratory have provided a strong biochemical basis for supporting the concept that the PAF involved in pathological versus physiological processes is derived via different biosynthetic routes.

PAF can be synthesized enzymatically by either remodeling or de novo reactions. Until recently, the most extensive studies in this area have dealt with the remodeling route since the enzymes involved are stimulated by a variety of agents that provoke inflammatory responses. When certain cell types are stimulated, alkylacylglycerophosphocholines (a common membrane storage form of ether lipids) can be remodeled to form PAF via the combined actions of phospholipase A_2 and an acetyltransferase. In contrast, the de novo pathway for PAF synthesis is responsible for the direct formation of PAF from 1-alkyl-2-lyso-sn-glycero-3-P via a sequence of steps involving acetylation, dephosphorylation, and the transfer of a phosphocholine moiety from CDP-choline to alkylacetylglycerols. The de novo enzymes do not respond to inflammatory stimuli, but can be stimulated by certain physiological factors such as neurotransmitters and fatty acids. Most tissues possess the enzymes required for the de novo synthesis of PAF, whereas only a limited number of cells (e.g., neutrophils, macrophages, platelets, basophils, endothelial cells) appear to be capable of producing PAF via the remodeling reactions.

Recent studies (5) of the properties of the acetyltransferase, phosphohydrolase, and cholinephosphotransferase in the de novo pathway of PAF biosynthesis have shown they are distinctly different from the corresponding enzymes that utilize 1-alkyl-2-lyso-sn-glycero-3-phosphocholine, phosphatidate, and diacylglycerols, respectively, as substrates. These experiments have also clearly demonstrated the specificities of the enzymes that catalyze the final reaction steps in both the remodeling and de novo pathways determine the type of PAF species produced in various cells. Regulatory enzymes responsible for catalyzing the rate-limiting steps in the de novo synthesis of PAF have been identified as cytidylytransferase (activated by fatty acids) and acetyl-CoA:1-alkyl-2lyso-sn-glycero-3-P acetyltransferase. Results obtained with 20:4-depleted and 20:4-supplemented HL-60 cells (differentiated) in conjunction with the use of inhibitors of phospholipase A_2 and enzymes that produce bioactive eicosanoid mediators suggest an arachidonoyl specific phospholipase A_2 catalyzes the rate-limiting step for PAF production in the remodeling route. The high degree of specificity of the enzymes in PAF metabolism makes them attractive candidates for the development of specific inhibitors for pharmacological intervention of disease-related processes.

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(100) LASER FLASH PHOTOLYSIS OF BIS[TRICARBONYL(CYCLOPENTADIENYL)TUNGSTEN]

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Extensive continuous photolysis studies of single-bonded metal carbonyl dimers propose mechanisms involving two different pathways: a homolytic cleavage of the metal-metal bond and a carbonyl-loss process (1). Nevertheless, whether those photoproducts arise from different excited states or are originated from a single upper level has not been elucidated since time resolved experiments have mostly been performed under UV irradiation.

We are using a flash-lamp-pumped dye laser that is tunable at a narrow linewidth to irradiate a tungsten dimer solution in pure toluene in order to establish any partitioning in the formation of the photoproducts. Differential spectra of the two intermediates were obtained at several different times after the pulse (μs to ms range) and for different wavelengths of irradiation (yellow-green region); the results are in good agreement with those reported for a similar molybdenum compound (2). They show evidence for a short lived species assumed to be a seventeen-electron radical and a longer-lived photoproduct attributed to a carbonyl-loss intermediate, each following a second order recombination decay under non-photochemical conditions. Figure 1 reports spectra plotted at 1μs (radical) after a 590nm pulse and at 200μs (when the second species has grown to a maximum) compared to the ground state spectrum of a 1mM solution of $Cp_2W_2(CO)_6$ in neat toluene. On the other hand, a quadratic dependency of each process over the light intensity has been observed. Figure 2 plots the overall change of absorbance as a function of the light intensity (quadratic fit) and the squared light intensity (linear fit). Both fits have correlation coefficients of 0.99. This feature indicates a simultaneous two-photon absorption to populate higher excited states which would contribute to the observed photochemistry. Single photon regime conditions (lower light intensity or longer excitation wavelength) are currently being investigated in order to clarify the partitioning of the different pathways as well as the possible threshold in energy for the carbonyl labilization. Those results will be presented along with kinetic data.

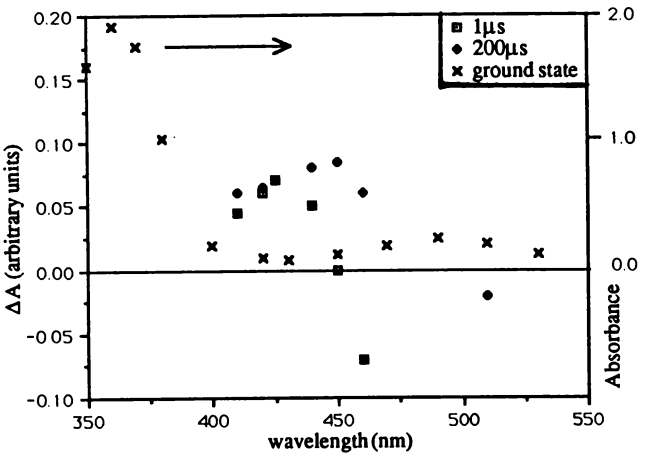

Figure 1

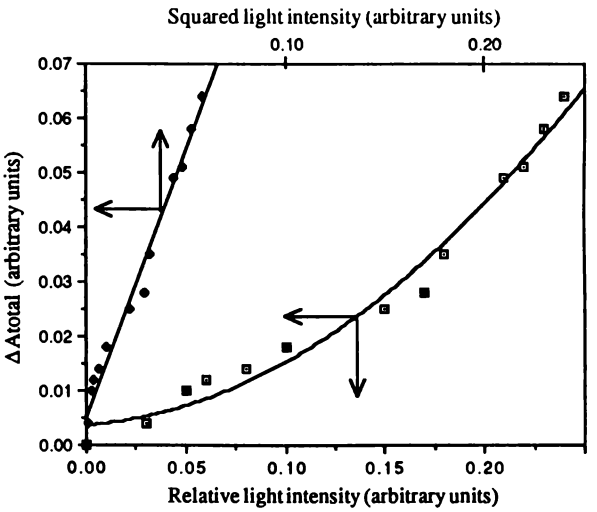

Figure 2

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(101) USE OF AN ADVANCED IMAGE ANALYSIS SYSTEM TO DETERMINE THE EFFECTS OF DIETARY BORON ON BONE MORPHOLOGY IN THE CHOLECALCIFEROL-DEFICIENT CHICK

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Previous research with chicks has shown that interactions between dietary boron and magnesium significantly affect the rate of proximal tibial growth plate (PTGP) mineralization in the cholecalciferol (vitamin D_3)-deficient chick (1). Thus, the enhanced penetration of the marrow sprouts into the PTGP observed in magnesium-adequate chicks deprived of boron could result from 1) increased activity of serine proteases such as plasminogen activator or 2) decreased resistance of the cartilage extracellular matrix to degradation. Therefore, an experiment was designed to determine the effects of dietary boron deprivation on PTGP structural degradation in vitamin D_3-deficient chicks fed adequate dietary magnesium.

Day-old cockerel chicks (16 per group) were housed in all-plastic environmental chambers (2) and fed a diet based on ground corn-casein-corn oil and containing 0.200, 0.248, 0.334, 0.481, 1.231, 2.095 or 3.973 mg boron (as orthoboric acid)/kg, and vit. D_3 at 125 (inadequate) IU/kg. At 28 days of age, PTGP sections were prepared (3) for image analysis (IA) using a Cambridge 970 Quantimet Image Analysis System*. In each section, three separate microscopic fields were selected for analysis at prescribed distances distal to the articular cartilage-PTGP junction. The optical images were received by a Chalnicon scanner positioned over the free camera port of a Zeiss III Photomicroscope. The "live" digitized signals were then sent to a video processor for shading correction and subsequently to an IA processor which converted the images into an array of 704 lines each of 896 picture elements (pixels). The grey level of each pixel was converted to a six or eight bit binary number. An integral DEC LSI 11/23 microprocessor was used to control the image analyzer, and to handle data generated by the IA system. Working routines were constructed as needed from menus of operational commands for image detection, field and feature measurements, distribution of data and storage or printing of results; the software thus facilitated both the interactive creation and automatic execution of IA routines.

Table 1. Effects of Graded Levels of Dietary Boron on Cholecalciferol-Deficient Chick PTGP Chondrocytes and Extracellular Matrix (ECM) in Histological Field 3

Treatment	Field 3 Chondrocytes			Field 3 ECM	
	Total Number	Total Perimeter	Number Deviating from Horiz. Orientation	Distance Between Chondrocytes	Area
B, mg/kg		μm	%	μm	%
0.200	124	4611.0	24.3	14.98	86.5
0.248	122	5135.8	21.7	12.99	80.8
0.334	124	4569.6	19.6	14.70	86.8
0.481	104	3826.1	18.8	17.80	85.8
1.231	95	4132.0	21.6	15.60	79.2
2.092	119	4602.0	23.5	15.34	84.8
3.973	118	4653.6	24.8	12.73	79.6
Quadratic Regression					
P values	0.0006	0.0006	NS	0.006	NS
R^2	0.30	0.30	0.07	0.22	0.01

Significant effects of dietary boron were most evident in histological Field 3 (Table 1), an area which usually consisted of the maturation zone of the PTGP. The number and total perimeter of chondrocytes in Field 3 decreased, then increased, as a function of dietary boron concentration. Thus, vitamin D_3-deficient chicks fed diets containing 0.481 and 1.231 mg boron/kg exhibited fewer, but larger chondrocytes in Field 3, an indication that concentrations of dietary boron near 1.0 mg/kg diet stimulated the maturation of the PTGP. Dietary boron near 1 mg/kg also tended to decrease the abnormal orientation induced by vitamin D_3-deficiency. Dietary boron did not affect the chondrocyte-extracellular matrix ratio. However, the horizontal distance between chondrocytes increased, then decreased, as a function of dietary boron concentration. The findings suggest that maturation of the PTGP in the vitamin D_3-deficient chick is sensitive to changes in dietary boron.

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

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(66) HYDROGEOLOGY AND GROUNDWATER CONTAMINATION AT THE FLYING J REFINERY, WILLISTON, NORTH DAKOTA

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The Flying J Refinery at Williston, ND refined various crudes into conventional fuels for approximately 30 years. Product and crude from several sources that include the tank farm, loading docks, flare pit, and API (American Petroleum Institute) separators were spilled or leaked into the environment. Four solid-waste impoundments collected waste from the API separators and were closed under provisions of the Hazardous and Solid Waste Act of 1984. During drilling operations associated with the preparation of the Closure Plan for the impoundments, hydrocarbons were detected above and below the water table (1). The presence of those hydrocarbons resulted in a need for additional characterization of the refinery site to define the extent and direction of movement of the groundwater and hydrocarbons.

The stratigraphy of the site was described from the drill cuttings and cores taken during the installation of monitoring wells. The hydrogeology and hydrogeochemistry were defined by measurements of water levels and water quality in these wells.

The refinery site is located on a glacial outwash terrace. The terrace deposits consist of two glaciofluvial sand bodies separated by till. These deposits overlie bedrock of the Sentinel Butte formation and form a complex hydrogeologic system through which the groundwater flows.

The upper glaciofluvial sand forms an unconfined aquifer up to 28 feet thick and is exposed at the surface. The lower glaciofluvial sand forms a confined aquifer up to 20 feet thick. Both sands appear to be poorly sorted river deposits. The till between the sand bodies forms a confining unit. Water table conditions exist in the till and in the upper sand. The till consists of nearly equal parts of clay, silt and sand with occasional pebbles and cobbles (2). It is often fractured and contains thin lenses of sand and gravel.

Flow in the two permeable glacial sand bodies is primarily lateral and is vertical in the confining till. Water table flow is generally toward the southwest. Flow in the upper sand can be either toward the northwest or toward the southwest, while flow in the lower sand is toward the south. Heads in the upper sand are greater than heads in the lower sand. Heads in the Sentinel Butte are slightly greater than heads in the overlying lower sand.

Several hydrocarbon plumes have been defined in the sands and till. The greatest accumulation of product is in the two sand units. The product source for the sands probably includes releases from the processing plant, loading docks, and flare pit. Some crude and waste oil were found in the till downgradient from the crude unloading docks. Hydrocarbons in the upper sand are present at the water table and within the overlying capillary zone and flow toward the northwest and southwest. Hydrocarbons in the lower sand are confined and flow toward the south but in response to local permeability conditions. Considering the potential for downward flow in the glacial deposits it is likely that some migration occurred along fractures in the confining till from the upper sand to the lower sand. Hydrocarbons in the till occur at the water table, and in parts of the unsaturated, and saturated zones as well. The Sentinel Butte was determined to be free of hydrocarbons. This is probably due to the greater head in the Sentinel Butte than the lower sand.

The extent, location, and movement of the hydrocarbon plumes are controlled by the complex geology and hydrogeology of the site. The suite of hydrocarbon constituents in each plume is related to the source(s).

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(14)

3-MERCAPTOPICOLINATE PROMOTES HEPATIC GLYCOGENESIS BY INHIBITING GLUCOSE-6-PHOSPHATASE

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The direction and net rate of glucose flux between the hepatic cell and the blood are ultimately determined by the relative rates of glucose phosphorylation and opposing glucose-6-phosphate hydrolysis (1). Regulation of the glucose/glucose-6-phosphate cycle would be important in determining (i) the fate of hepatic glucose-6-phosphate which can arise from at least four different pathways and (ii) whether glycogen production occurs via a direct or indirect pathway.

To study regulation at this site, we used a recycling liver perfusion system containing erythrocytes to study net glucose uptake, glycogen production, and steady-state concentration of glucose-6-phosphate all measured as a function of glucose concentration. Livers from 48 h fasted rats were perfused with 7.5, 15, or 30 mM glucose concentrations. The inclusion of 4 mM 3-mercaptopicolinate (3-MP) in the perfusate inhibited gluconeogenesis from lactate produced by erythrocyte glycolysis, as well as inhibiting any gluconeogenesis from endogenous substrates entering the gluconeogenic process distal to the reaction catalyzed by P-enolpyruvate carboxykinase (PEPCK), the gluconeogenic enzyme inhibited by 3-MP (2). The hypothesis was that the inclusion of 3-MP should increase net glucose uptake.

Net glucose uptake was increased as expected; however, the inclusion of 3-MP also led to an unexpected enhancement in glycogenesis as well. The effects were greatest with low, near physiologic concentrations (7.5 mM) of glucose. Perfusions were then performed with no added glucose but with 20 mM dihydroxyacetone present to obtain observations of the effect of 3-MP with a gluconeogenic substrate thought to be immune to inhibition by 3-MP. Without 3-MP, marked net production of glucose was observed, with little glycogen deposition. In the presence of 3-MP, however, glucose production was curtailed and glycogen deposition was markedly enhanced.

The concomitant reduction in glucose production, resultant enhanced net glucose uptake, and elevated glycogenesis in the presence of 3-MP suggested that 3-MP might be inhibiting glucose-6-phosphatase. This inhibition would lower metabolic cycling at the glucose/glucose-6-P site in liver, would selectively inhibit the rehydrolysis of glucose-6-P formed from either glucose phosphorylation or gluconeogenesis, and would thus enhance flux through the glucose/glucose-6-P cycle toward glycogen. Results of inhibition kinetic studies with isolated intact and disrupted liver microsomes showed 3-MP to be an effective competitive inhibitor of glucose-6-P hydrolysis. Additional studies showed no effect by 3-MP on glucokinase activity.

These experiments suggest that regulation at the glucose/glucose-6-phosphate site through a selective inhibition of glucose-6-phosphatase is important in the control of blood glucose levels and in glycogen production via the direct pathway.

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(67) MELTWATER FLOOD ORIGIN FOR GREAT PLAINS DRAINAGE NETWORK

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Introduction: The Redwater-Yellowstone, Yellowstone-Little Missouri, Powder-Little Missouri, and Little Missouri-Missouri drainage divides all show evidence of having been crossed by large floods (1, 2). Evidence includes anastomosing channel complexes crossing the divides and coarse-grained alluvium from western sources on the divides. This evidence suggests a glacial meltwater floodway existed between continental ice sheet(s) and the Rocky Mountains. The floodway would have been active at times of continental ice sheet maxima and would have served as a flood route for meltwater from the Laurentide Ice Sheet(s), Cordilleran Ice Sheet(s), and Rocky Mountain alpine ice caps. Meltwater floods may have been enhanced by catastrophic failure of glacial lakes and, possibly, immense jokulhlaups associated with igneous activity in the Yellowstone Plateau region. Floodwaters would have flowed southward across the Great Plains into the Gulf of Mexico. If correct, no modern Great Plains river valley, which crosses the floodway path, can predate the immense meltwater floods. This paper describes the hypothesized floodway, reviews supporting evidence for the flooding, and presents a model for systematic formation of Great Plains river valleys by catastrophic meltwater floods.

Great Plains Floodway Description: The floodway extended from Alberta southward to Texas. The floodway surrounded Rocky Mountain outliers --including the Black Hills. Today, the floodway region is drained by a series of river systems which extend across the Great Plains from the Rocky Mountain front to the Gulf of Mexico-Mississippi River-Missouri River line. Rivers crossing the floodway path are characterized by narrow, elongate drainage basins. Drainage divides are asymmetrical. Many of the narrow, elongate drainage basins do not originate in the mountains, but instead begin on the Great Plains --a short distance in front of the mountains.

Upstream Record: Mapped Laurentide Ice Sheet margins and topographic relationships of Rocky Mountain outliers and other uplands in central Montana and southern Alberta suggest formation of numerous large, ice-dammed lakes whenever ice advanced and retreated across the region. Repeated catastrophic failure of ice and soft sediment dams, which held these immense lakes, was not only possible, but probable. Evidence for large ice-marginal lakes in the ice-free corridor of southern Alberta has been described by Bretz (3), Horberg (4), and others. Kehew and Lord (5) have described evidence for recognition of spillways characteristic of sudden, rapid drainage of glacial lakes. This evidence includes deeply incised inner channels flanked by broad, scoured outer zones. Further evidence includes large boulder bars within channels. Topographic map evidence and preliminary field observations suggest many, if not all, of the outlet channels described by Bretz record sudden, rapid drainages of large lakes. Similar evidence for sudden, rapid drainage of large ice-marginal lakes is found in central Montana. For example, Calhoun (6) and Alden (7) describe the Shonkin Sag, north of the Highwood Mountains, as the outlet of a large ice-marginal lake. Evidence from recent topographic maps supports sudden, rapid drainage of a large lake through the Shonkin Sag spillway.

The Downstream Record: Evidence for immense floods into the Gulf of Mexico has been reported by Kennett and Shackleton (8), Emiliani, Rooth, and Stipp (9), and others. Major influxes of glacial meltwater into the Gulf are recorded by deep-sea cores collected from all areas of the Gulf. All workers report massive floods of glacial meltwater entered the Gulf during melting phases of the late Wisconsin Laurentide Ice Sheet. Dates proposed for peak floods vary, although some workers suggest massive flooding may have continued for a period as long as several thousand years. Similar floods probably occurred during melting of earlier ice sheets. The Great Plains floodway, described here, would have been the only open drainage route for much of western and central North America at times of Laurentide Ice Sheet maxima.

Model for Formation of Great Plains River Valleys: The model proposed here was suggested by Shepherd and Schumm (10) and brief observations of drainage networks developed in a small clay pit near Chicopee, Massachusetts. Meltwater from the Laurentide Ice Sheet, Cordilleran Ice Sheet, and Rocky Mountain alpine ice caps moved as giant sheets of water southward through the ice-free corridor. At the maximum extent of the Laurentide Ice Sheet floodwaters were forced west of the Ozarks and reached the Gulf of Mexico by crossing Texas. Huge sheets of water moved across the Great Plains scouring easily eroded bedrock. Erosion by these sheets of water produced grooves now seen as streamlined residuals and oriented secondary drainages on the upland surfaces. Flow down the Great Plains floodway was progressively cut off by systematic development of deep trenches --each of which extended across the floodway route. The trenches grew by rapid headward erosion. The first trenches to form were in the south and may have included segments of the Rio Grande and Pecos valleys. Systematically the Colorado (Texas), Brazos, Canadian, Cimmarron, Arkansas, Smoky Hill, Republican, Platte, Niobrara, White, Cheyenne, Belle Fourche, Moreau, Grand, Cannonball, Heart, Little Missouri, Powder, Tongue, Yellowstone, Musselshell, Missouri (upper), and Milk River valleys were formed. Formation of this progressive sequence of trenches was made possible by headward erosion of a main Mississippi-Missouri valley trench. As each secondary trench extended across the floodway path the trench captured and channeled floodwaters into the main trench. Once formed a secondary trench would prevent use of the floodway route to the south. Each secondary trench is younger than the trench immediately downstream on the floodway route. (At times several trenches may have formed simultaneously with the downstream trenches extending further across the floodway route than the upstream trenches.) Additional work is needed to correlate formation of each trench with specific flood events and glacial advances.

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(68) ## ORIGIN OF THE KILLDEER MOUNTAIN LAKE BASIN, DUNN COUNTY, NORTH DAKOTA

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Introduction: The Killdeer Mountains are located in Dunn County, North Dakota. Published and unpublished reports include: Quirke (1), Stone (2), Delimata (3), and Forsman (4). Bluemle (5, 6) summarizes prevailing ideas. Previous work establishes a lacustrine origin for the 400-foot thick caprock. This paper addresses the origin of the basin in which the lacustrine sediments were deposited.

Discontinuous lacustrine strata extend eight miles in a southwest-northeast direction with a maximum width of three miles. The irregular distribution of lacustrine deposits as shown on geologic maps (6) is similar to the shape of modern reservoirs. Total area covered by lacustrine strata approximates ten square miles. Limited debris at the base of the uplands suggests modest erosion of the caprock.

A conglomerate at the southwest end of the lake deposits contains cobble-size igneous clasts. This conglomerate is found only in lower lacustrine strata. Similar igneous clasts can be found on Yellowstone River terraces and are found in conglomerates (mapped as White River Group) in the Little Badlands, Rainy Buttes, and Chalky Buttes areas of North Dakota. Rock types can be traced to the Beartooth Mountain area and elsewhere in the modern Yellowstone River drainage basin. This investigation found no evidence to support the Black Hills source suggested by Quirke, Stone, and others.

The presence of coarse-grained conglomerate in the lacustrine sediments provides a southwest limit for the margin of the lake. No evidence suggests the lake was significantly larger than the area outlined by present-day lacustrine strata. The depth of the lake basin can be determined by the thickness of lacustrine sediments.

Origin of the lake basin: The Killdeer Mountain lake basin would have been surrounded by easily eroded claystones which underlie western North Dakota. Smith (7) outlines mechanisms which can form lake basins. The Killdeer Mountain lake basin, like every lake basin, was formed by at least one of these mechanisms:

Eolian processes: Lake basins can be formed by deflation. Deflation basins are found in the Great Plains region. However, a deflation origin is not consistent with cobble-size clasts which imply a major river initially flowed into the Killdeer Mountain lake basin.

Fluviatile processes: Lake basins can be formed by fluviatile processes. Most fluviatile processes produce shallow lakes. The Killdeer Mountain lake basin was at least 400 feet deep. Plunge pools at the base of waterfalls can be deep. A 400-foot deep plunge pool is unlikely in North Dakota.

Glacial processes: Glacial erosion and deposition can result in lake basins. The Killdeer Mountain lake basin is located along a recognized glacial margin. The shape and size of the lake are consistent with direct or indirect glacial blockage of a deep river valley. Ages which have been proposed for the lacustrine sediments are not consistent with ages proposed for continental glaciation(s) which reached the Killdeer Mountain area.

Gravity processes: Lakes form in valleys dammed by landslides. The Killdeer Mountain lake basin could have formed in a landslide-blocked river valley. A 400-foot high landslide dam does explain the size, shape, and depth of the basin. If correct, the landslide dam had to stop and probably diverted a major river.

Impact processes: Lake basins can be created by meteorite impact. The probable shape of the Killdeer Mountain lake basin is not consistent with an impact origin. The presence of a major river makes the impact origin unlikely. Further, evidence of other impact features has never been reported.

Organic processes: Lakes can be formed by accumulations of organic matter in rivers. Such lakes are shallow. Lakes also form as a result of dams built by higher organisms. The lake predates man and is probably too large to have been formed by other complex organisms --such as beavers.

Shoreline processes: Lake basins can be produced by coastal processes. There is no evidence the Killdeer Mountain lake basin was near a shoreline.

Solution processes: Lake basins can be formed in soluble rocks. No evidence suggests soluble bedrock surrounded the Killdeer Mountain lake basin.

Tectonic processes: Lake basins can be produced by crustal warping, folding, or faulting. A tectonic depression of the size, shape, and depth indicated for the Killdeer Mountain lake basin should be bounded by recognizable structures. Such structures have never been reported.

Volcanic processes: Lake basins can develop in volcanic craters or in valleys blocked by lava flows. While the lacustrine sediments contain volcanic ash and rounded clasts of igneous rock there is no evidence of local volcanic craters or lava flows which could create a lake basin.

Conclusions: The Killdeer Mountain lake basin was 8-10 miles long (southwest-northwest), less than three miles wide, approximately 400 feet deep, and fed by a river from the southwest. The basin was formed by blockage of a deep narrow valley. The dam stopped and probably diverted a river capable of transporting cobble-size clasts.

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(69) PYROMETAMORPHISM IN UNDERGROUND COAL GASIFICATION: ROCKY MOUNTAIN 1 TEST PROGRAM

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Underground coal gasification (UCG) results in the formation of a cavity as the coal is gasified. Conditions in the cavity are typically characterized by high temperatures (in excess of 1000°C) and low pressures (10-15 bars). The metamorphism of overburden materials under these conditions is pyrometamorphism (1). With increasing temperature, thermal effects include dehydration, calcination (dehydroxylization, decarbonation), sintering, and ultimately fusion. The strength, stability, and leachability of materials, as well as the chemistry and flow of groundwater, are affected in the pyrometamorphic halo. Knowledge of UCG-related pyrometamorphism is important in modeling the growth and stability of the cavity, the potential for, and mitigation of, adverse UCG-related impacts on groundwater, and the geothermometry of the UCG process and must, in part, be grounded in a fundamental understanding of the formation, character, and behavior of altered materials. Although nearly thirty UCG tests have been conducted among eleven sites in the United States, investigations of UCG-related pyrometamorphic materials have been limited mainly to geothermometry. Consequently, researchers at the North Dakota Mining and Mineral Resources Research Institute (NDMMRRI) at the University of North Dakota and the Chemistry Department at North Dakota State University have begun work on a generic model of solid state UCG-related residues, including pyrometamorphic materials, that will take into account the variability of coal-bearing strata, the variability of organic materials in coal, the variability of UCG temperature and redox conditions, and the effects of post-burn restoration activities. This effort includes the investigation of the chemical, mineralogical, and physical character of unaltered and altered overburden materials from UCG settings as well as a review of the literature concerning analogous processes, particularly spontaneous in situ coal-combustion events. This research is part of NDMMRRI's Gas Research Institute-funded program associated with the Rocky Mountain 1 (RM1) UCG test at Hanna, Wyoming.

Research to date has focused on the characterization of unaltered and altered materials from the Tono I UCG test site (Centralia, Washington; Skookumchuck Formation, Eocene, marginal marine deposits; subbituminous coal), which was excavated and sampled following gasification. On the basis of six samples of altered overburden, mineral phase assemblages and melting relations were successfully modeled using phase diagrams for the system $(FeO+MgO)-Al_2-SiO_2$ constructed from those available in the literature (2). Further, a hitherto unrecognized precursor to the formation of Fe-cordierite from SiO_2 and spinel at high temperatures was identified (2). Preliminary results from the characterization of cores of unaltered and altered materials from the RM1 site (Hanna, Wyoming; Hanna Formation, Eocene, alluvial plain environment; bituminous coal), as well as the characterization of RM1 materials thermally altered under controlled conditions in the laboratory, indicate general agreement with the Tono model.

The review of more than 70 references on such topics as mineralogy, physical character, combustion dynamics, environmental effects, and age-dating of natural coal burns and their products provided a basis for comparison between natural burns and UCG-related systems (4). Information from these sources and from UCG residue characterization and modeling efforts indicated that natural coal burns constitute the closest analogues to UCG, since both produce similar metamorphic environments capable of producing a similar suite of pyrometamorphic materials. Indeed, both processes can produce hornfels (low grade, sintered material) and buchite (high grade, fused material) that are isochemical with parent materials. However, important differences in setting and combustion dynamics result in differences in occurrence and distribution of altered materials (4). Low grade alteration suites (calcined, sintered) predominate in natural coal burns where combustion occurs in the unsaturated zone at low rates under variable conditions. Alteration halos in these settings can be several meters thick. Buchites are confined to surface-connected vents where higher temperatures result from combustion of product gas. UCG-related systems, however, are typically set in the saturated zone below the water table and are characterized by high rates of combustion under relatively uniform combustion conditions. Altered materials are confined to a rubble pile in the cavity and to a thin layer on the cavity roof. Buchite is the dominant alteration product. Research in the coming year will focus on continued characterization of the cores of altered materials from the RM1 site, investigation of the leachability of RM1 altered materials, and continued development of the generic model for solid state residues.

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(91) CALLUS GROWTH ON CULTURE FILTRATE AS A TECHNIQUE FOR MEASURING SUNFLOWER HYBRID RESISTANCE TO PHOMA MACDONALDII

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Phoma macdonaldii causes leaf and stem lesions on sunflowers. The pathogen sometimes causes girdling stem lesions and plant death prior to normal maturity (1). Selecting plant resistance using the fungus directly is difficult. The objective of this research was to evaluate indirect screening using P. macdonaldii culture filtrate as a selecting agent to locate resistance in sunflower germplasm. In this study culture filtrate was prepared by growing P. macdonaldii in one liter Fernbach flasks with Czapek-Dox broth (Difco) supplemented with sunflower tissue extract (2). Cultures were incubated at 25 C in shake culture (200 rpm) for one month. Mycelial growth was removed by coarse filtration and the filtrate was filter sterilized prior to use.

The filtrate was incorporated into sunflower tissue culture media SF3 or SN (3,4). Sunflower calli derived from hypocotyl tissue were manipulated with standard procedures (1). One month old calli of two sunflower hybrids, 'Interstate 894' and 'Sigco 475,' were placed on the media with filtrate of P. macdonaldii at concentrations of 200 to 800 ml/L of medium. One hundred calli of each hybrid were tested at each concentration on each medium.

After one month, calli were observed for necrosis and weighed as a measure of growth. Weight was significantly less with filtrate in the medium. Interstate 894 appeared smaller in size when grown in the presence of the filtrate. Sigco 475 was less affected. When weights of cultivars were compared, differences were not significant; however, calli from susceptible lines were clearly necrotic and severity of necrosis was related to filtrate concentration. The difference was sufficient to identify plant resistance. A toxin, possibly zinniol (2), may be involved in symptoms. Supportive evidence for a toxin was obtained when 75 surface sterlized seeds of Interstate 894 were germinated on water agar (control) or on water agar amended with culture filtrate. Seedling radicle length was significantly (P=0.05) less (40%) on filtrate. Tests with 50 seeds of each of the two cultivars and a more susceptible line ('Sigco 432') showed the resistant cultivar (Sigco 475) developed radicle lengths (average 8 mm) which were not significantly different from the Sigco 475 control (average 12 mm). The susceptible lines had shorter roots (Sigco 432=4 mm, Sigco 432 control=10 mm; Interstate 894=4 mm, Interstate 894 control=8 mm). Apparently radicle length in this test can be used as a measure of plant resistance.

Laboratory results were compared to results from greenhouse and field trials. In the greenhouse sunflower seedlings 'Interstate 894' and 'Sigco 475' were grown to the V4 (four true leaf) stage in pasteurized soil mix. Plants were inoculated with conidia taken from cultures grown in petri dishes on potato dextrose agar (PDA) under fluorescent light (50 $\mu Em^{-2}sec^{-1}$) for 14 days at 26 C $\pm$ 5. Conidia were collected by flooding the plates with sterile distilled water, and after 18 to 20 hr, scraping the surface with a rubber policeman to recover conidia resting on the agar surface. The suspension was adjusted to 2.6 X 10^7 conidia/ml by haemocytometer counts. The conidia were atomized onto plants and the plants were placed in an unlighted 25 C chamber at 100% RH for 48 hr (5). After incubation, plants were returned to the greenhouse and one week later seedlings with lesions were counted. In the greenhouse, the two hybrids had similar percentages of plants infected, percentages of plants with only leaf lesions and percentages of plants with both stem and leaf lesions; however, Interstate 894 had twice the percent plants with only stem lesions.

To determine field responses, the hybrid lines were planted in an experimental plot near Fargo in May and ten plants of each line were evaluated in September 1987 for number and size of lesions at the lowermost nodes. Infection was dependent upon natural inoculum. Interstate 894 plants had a high percentage of plants with lesions while 'Sigco 475' plants had no Phoma lesions.

The field, greenhouse and callus test results were similar. This is a good indication that greenhouse screening and callus screening may be valid procedures for testing plant resistance in this fungus-pathogen system.

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(15) COPPER DEFICIENCY ALTERS THE RESPONSE AND CYTOSKELETAL ORGANIZATION OF THROMBIN-ACTIVATED PLATELETS

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Recent reports indicate that copper deficiency can alter the protein composition of cell membranes and their underlying cytoskeleton (1,2). Because the cell membrane, with its variety of receptors, transport proteins and enzymes, is an important mediator between external stimuli and cell response, alteration of the cell membrane or underlying cytoskeleton may contribute to the pathophysiological consequences of copper deficiency in the cardiovascular, hematopoietic, reproductive, nervous and immune systems. The objective of the present study was to investigate the possibility that copper deficiency can interfere with normal stimulus-response coupling in thrombin-activated platelets.

Male, weanling Sprague-Dawley rats were fed diets containing either <1 ppm Cu (CuD) or 6 ppm Cu (CuA) for 42 days. Blood was withdrawn from these rats to obtain platelet-rich plasma through low-speed centrifugation (160xg for 20 min). Platelets were obtained by centrifuging the platelet-rich plasma (730xg for 10 min). The platelets were washed three times and suspended at a concentration of $5x10^8$ platelets/ml in buffer containing 0.138 M NaCl, 0.0029 M KCl, 0.012 M $NaHCO_3$, 0.00036 M Na_2HPO_4, 0.0055 M glucose, 0.001 M EDTA, pH 7.4.

Platelet response was assessed by measuring ATP release following stimulation with 0.1 Unit of thrombin/ml of platelet suspension. The rate of ATP secretion from platelets of rats fed CuD was 34±9 $nmolesxmin^{-1}x10^{-9}$ platelets which was significantly greater than the 14±4 $nmolesxmin^{-1}x10^{-9}$ platelets from platelets of rats fed CuA (values are means±SEM, N=8, $P<0.05$, t-test).

The cytoskeletal reorganization that normally occurs following thrombin activation was also altered in the platelets of rats fed CuD. Whole platelets were solubilized with 1% Triton X-100 at various times following activation with 0.1 Unit of thrombin/ml of platelet suspension. The Triton X-100 insoluble cytoskeletal proteins were then isolated by centrifugation (8700xg for 4 min). Electrophoretic analysis of these proteins indicated that the time-dependent association of myosin with the cytoskeleton following thrombin activation was greater in platelets from rats fed CuD than in platelets from rats fed CuA (Table 1).

Table 1. Effect of Copper Deficiency on the Association of Myosin Heavy Chain With the Cytoskeleton of Thrombin-Activated Platelets (mean±SEM)

Time Following Activation(s)	Relative Increase in Myosin Heavy Chain	
	CuD (N=8)	CuA (N=7)
0	1.0	1.0
10	1.1±0.1	1.0±0.1
30	2.0±0.1	1.4±0.1
60	2.2±0.2	1.5±0.1
Source of Variation	Analysis of Variance P Values	
Dietary Cu	0.005	
Time	0.0001	
Dietary Cu x time	0.007	

The change in intracellular free calcium concentration following thrombin activation was monitored using Fura-2, a sensitive calcium indicator. Following activation with 0.1 Unit of thrombin/ml, the rise in intracellular free calcium concentration was 47±7 nM in platelets from rats fed CuD and 72±9 nM in platelets from rats fed CuA (values are means±SEM for N=6, $P<0.05$, t-test).

The lower rise in intracellular free calcium following thrombin activation of platelets from copper-deficient rats suggests that copper deficiency enhances the utilization of free calcium released from intracellular stores following activation. This may explain how copper deficiency leads to increased association of myosin with the cytoskeleton of thrombin-activated platelets, because incorporation of myosin into the cytoskeleton is regulated by myosin light chain kinase, a calcium-dependent protein kinase (3). Because secretion is dependent on the contractile cytoskeleton, increased myosin content may alter the function of the cytoskeleton and lead to the higher secretory rates observed in the thrombin-activated platelets from copper-deficient rats.

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(70)

MODELING SHORELINE EROSION TRENDS ON LAKE SAKAKAWEA

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Bank erosion along reservoir shorelines results in loss of land and reservoir storage capacity. After completion of Garrison Dam in 1953 water levels on Lake Sakakawea continued to rise until 1969 when a maximum pool level of 564.3m was reached (1). Since then, bank erosion has claimed a considerable amount of land. Previous attempts to model bank erosion rates on this lake were unsuccessful (2,3). The purpose of this study, begun in early 1983, has been to measure current bank erosion rates, erosion factors, and to develop a more accurate model for predicting erosion rates. Sandberg (4) developed a statistically significant bank erosion model based on data from the first two and one half years of this study. Sandberg's model contains two equations: one incorporates warm season bank erosion and the other, cold season erosion. Analyses of data since 1985 indicate that erosion rates are strongly influenced by lake level, and less so by changes in seasonal erosion processes (Figure 1).

Detailed measurements at 20 sites along eastern Lake Sakakawea (1983-1988) reveal an average erosion rate of 1.16m/yr. Erosion rates for warm and cold seasons and high and low lake levels were also compared. The average high lake-level erosion rate was approximately 30cm/month, while the average cold and low lake-level rates were only about 5cm/month. As can be seen on figure 1, the average erosion rates are significantly higher during extended periods of high lake levels, but low lake-level erosion rates are similar for both warm and cold seasons.

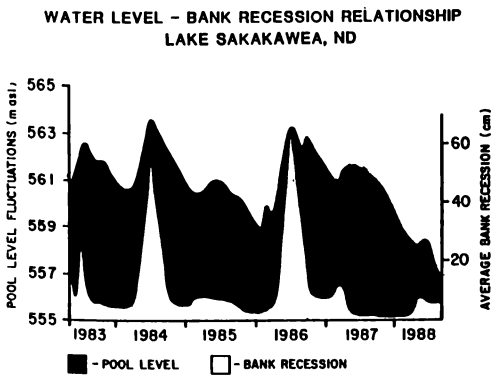
Two sets of processes and passive erosion factors must be considered to account for the large difference between high and low lake-level erosion rates. During high lake-levels wind-driven wave action is the most important process; during low lake-levels oversteepened banks continue to fail due to instability and thaw failure, but at slower rate. Many factors contribute to the magnitude of bank erosion at any one site during both low and high lake-levels. However, only a few were used to develop the bank erosion model described below. These included factors that were relatively easy to measure and which showed a strong correlation with erosion rates. The procedure involved using the monthly bank erosion rates for each station and the most significant associated variables. These were subjected to a regression analysis to test the statistical significance of each variable and to derive an equation for predicting site-specific erosion rates. Because the processes are different during high and low lake-levels, two separate equations were derived. The high lake-level equation, which has a resulting (r) value of **59%** is:

$$RH = 29.59\sqrt{A} + 0.44\sqrt{B} + 1.64\sqrt{C} - 14.65\sqrt{D} - 34.33,$$

where **RH** = the monthly high lake-level erosion rate, **A** = the effective fetch (km), **B** = bank height (m), **C** = percentage of coarse beach clasts, and **D** = beach slope. The low lake-level equation, which has a resulting (r) of **78%** is:

$$RL = 0.14E + 0.03B - 0.62,$$

where **RL** = the monthly low lake-level erosion rate, **E** = angle between the bank orientation and the sun, and **B** = bank height (m). The total erosion, then, is **HP(RH)+LP(RL)**, where **HP** = the number of high lake-level months and **LP** = the number of low lake-level months. The successful application of these two equations for modeling future bank erosion on Lake Sakakawea depends on our ability to predict future lake-level fluctuation patterns accurately.



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(71) WIND EROSION ON MARS: IMPLICATONS OF ATMOSPHERIC CUSHIONING

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The planet Mars is known to have wind speeds in excess of 300 mi/hr. Such high wind speeds accompany the periodic global dust storms of Mars. Wind erosion through sandblasting might therefore be expected to be more effective on Mars than on Earth. This hypothesis especially follows from the additional observation that Mars is a dry planet, without bodies of water or vegetated areas that might serve as traps for windblown sand. However, several workers have suggested that many landforms on Mars appear to be not eroded by wind, in spite of the activity of global dust storms. They presume that sand-size material is required for wind erosion to occur, and so suggest that sand-size material is lacking on Mars (1). Although, on Earth, saltatory motion of sand grains normally precedes and initiates the suspension transport of clay-size material, on Mars, dust devils may be responsible for the lifting of clay-size material directly.

Is it correct that sand grains are necessary for wind erosion, or is it also possible for suspended clay-size grains to "dustblast" surfaces? Kuenen (2) suggested (for Earth) that particles below sand size impinge with insufficient force in air to cause erosion. He offered no clear explanation for the lower size limit, except to suggest that an independent cause is that such grains are carried in suspension.

McCauley (3) suggested that wind erosion on Mars should be highly effective because of the combination of great wind speeds and reduced cushioning of grain impacts in the thin Martian atmosphere (~6 mb). This was the first suggestion of atmospheric cushioning, even though Kuenen had used the term "cushioning" with regard to the erosive ability of grains carried in water. If atmospheric cushioning is a significant process, then it must be considered in comparisons of wind erosion processes on Earth and Mars. Perhaps on Mars even silt and clay-size grains are capable of effecting wind erosion under conditions of reduced atmospheric cushioning.

In order to determine whether atmospheric cushioning is real and, if so, to compare its effectiveness under different atmospheric pressure conditions, experiments have been conducted using a small race track air tunnel and fine sand to fine silt-size grains made to impact repeatedly against obsidian obstacles. Experiments conducted under conditions of controlled air velocities and air pressures revealed that atmospheric cushioning is a significant process. Grains carried under conditions of low air velocities and pressures (158 mb) caused much more rapid and extensive wear of obsidian obstacles that did grains carried under conditions of higher air velocities and pressures (1013 mb). Further, cushioning was found to reduce the rate at which erosion occurs but not to cause a lower size limit for erosive grains. Under Martian dust storm conditions, even grains of such small size as to be normally carried in suspension may act as significant erosive tools. Sand-size grains are not required for wind erosion on Mars.

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(72) COMPARISON OF THERMAL PROFILES AND THERMAL HISTORY MODELS FOR THE ANADARKO, BIG HORN, DENVER, MICHIGAN AND WILLISTON BASINS

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Present-day temperature profiles for the Anadarko, Big Horn, Denver, Michigan, and Williston basins were calculated using the relationship

$$T = T_o + \sum_{i=1}^{n} Z_i \, (Q/K_i) \qquad \text{Eq. 1}$$

where T is temperature, T_o is surface temperature, Z is depth, Q is surface heat flow, and K is thermal conductivity. This expression is the integral of Fourier's law of heat conduction in one dimension for steady-state conditions. The temperature profiles are significantly different (Fig. 1) even though surface heat flow values in the basins are not substantially different. Surface heat flow is of the order of 60 mW m^{-2} in the Anadarko, Denver, Big Horn and Williston basins and is of the order of 50 mw m^{-2} in the Michigan Basin. Each basin has a characteristic thermostratigraphy, a temperature profile that reflects the thermal conductivity structure of the stratigraphic section, that is different from the others. The differences among the temperature profiles are caused by the different thermal conductivities of the lithologic units in the basins. This distinction is important because it shows that the thermal structure of a basin is uniquely determined by its lithology and its stratigraphic history. This implies that the thermal history as well as the present day thermal structure of a particular basin may be unique.

Thermal history models for each basin were calculated from the present day thermal conductivity structure using Waples' method (1) for Lopatin's (2) time-temperature index (TTI) for thermal maturity of kerogen bearing sediments. The relationship is

$$TTI = \sum_{n\,min}^{n\,max} (t_n)(r^n) \qquad \text{Eq. 2}$$

where t is time in M.A. the formation has spent at a particular temperature and r^n is a value corresponding to temperature based on Lopatin's (2) application of the Arrhenius equation to reaction rates for kerogen. The thermal maturity data indicate a unique thermal history and formation rate for petroleum from kerogen in each basin. In the general case, the onset of significant petroleum occurs when the temperature rises above 50 °C and ends when the temperature rises above 160 °C. Examination of Figure 1 shows that each of the basins investigated has a distinctly different petroleum generation zone.

We conclude that each basin is unique in its temperature profile and its thermal history. Thermal history models of a basin must be based on the thermal conductivity structure as well as the subsidence history.

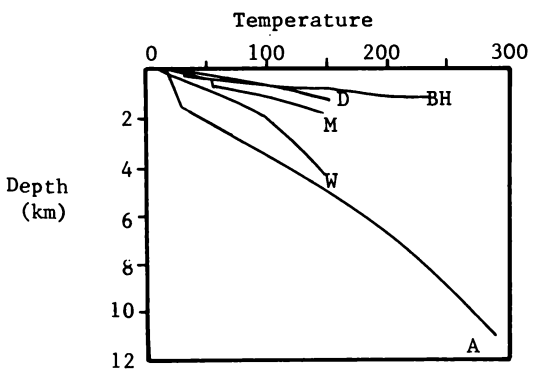

Figure 1. Temperature vs. depth. Basin designations are Anadarko (A), Big Horn (BH), Denver (D), Michigan (M), and Williston (W). Temperatures are in deg. C and depths are in metres.

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(87) EFFECTS OF ZINC DEFICIENCY ON TEMPERATURE REGULATION BY RATS IN THE COLD

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Zinc is an essential trace element that is an important component of many metalloenzymes. The importance of zinc in biology is emphasized by its role in carbohydrate, protein, and lipid metabolism (1). Because of its general role in macronutrient metabolism, it has been suggested that zinc may play a role in regulating energy expenditure and basal metabolism (2). Some recent observations indicate that circulating thyroid hormone concentrations may be influenced by zinc nutritional status (3). Because thyroid hormones are important in regulating thermogenesis and energy metabolism, we undertook the present study to determine the effect of zinc deficiency on temperature regulation in animals.

Thirty weanling, male Sprague-Dawley rats weighing 50-60 g were housed individually in a controlled temperature environment with a 12-hr light-dark cycle. The rats were matched by weight into three groups (n=10). One group was fed ad libitum a semipurified diet containing all essential nutrients with a zinc intake of 16 ppm (AL). The second group was fed a similar diet but with a deficient zinc intake of 0.8 ppm (ZD). The third group was pair-fed the AL diet in amounts equal to that consumed by a paired ZD animal (PF). The animals were given distilled deionized water ad libitum.

The rats were fed for 28 d, fasted, then placed in individual plastic cages in an environmental chamber at 4°C for 4 hrs. Rectal temperature was measured under thermoneutral conditions and after each hour of cold exposure. Then the rats were anesthetized using barbituate and sacrificed. Blood was obtained by cardiac puncture and analyzed for thyroxine (T_4) and triiodothyronine (T_3).

Table 1
Effects of Zinc Deficiency on Body Weight, and Rectal Temperatures and Thyroid Hormone Concentrations During Acute Cold Exposure

		Rectal Temperature, °C					T_4	T_3
	Weight, g	0 hr	1 hr	2 hr	3 hr	4 hr	µg/dl	ng/dl
AL	229[a]	38.2[a]	37.7[a]	37.5[a]	37.6[a]	37.6[a]	6.9[a]	112[a]
PF	126[b]	37.9[a,b]	37.4[a]	37.4[a]	37.6[a]	37.6[a]	4.9[a]	110[a]
ZD	94[c]	37.8[b]	37.1[b]	36.8[b]	36.8[b]	36.6[b]	3.1[b]	79[b]

[a,b,c]Values in a column with different superscripts are different ($p < 0.05$).

Zinc deficiency and pair-feeding, or caloric restriction, depressed growth. At room temperature (23°C), ZD rats had lower rectal temperatures than AL or control animals. During cold exposure, ZD rats were unable to maintain core temperature. Both T_4 and T_3 concentrations were depressed in ZD rats after cold exposure (Table 1).

A second experiment was performed using 40-50 g weanling, male Sprague-Dawley rats that were matched by weight into three groups (n=10) and fed diets as described above for 42 days. The animals were exposed to a cold environment (2-3°C) for six hours. After cold exposure, the animals were sacrificed and blood and liver specimens were obtained.

Table 2
Effects of Severe Zinc Deficiency on Body Weight, and Rectal Temperatures and Thyroid Hormone Concentrations During Acute Cold Exposure

		Rectal Temperature, °C							Liver Zn	T_4	T_3
	Weight, g	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	µg/g	µg/dl	ng/dl
AL	270[a]	38.0[a]	37.8[a]	37.6[a]	37.6[a]	37.4[a]	37.5[a]	37.4[a]	103[a]	6.9[a]	112[a]
PF	118[b]	37.8[a,b]	37.7[a]	37.5[a]	37.6[a]	37.4[a]	37.1[a]	37.2[a]	100[a]	5.8[a]	103[a]
ZD	84[c]	37.6[b]	37.1[b]	36.8[b]	36.5[b]	35.4[b]	34.8[b]	34.0[b]	86[b]	3.2[b]	77[b]

[a,b,c]Values in a column with different superscripts are different ($p < 0.05$).

Severe ZD and PF markedly restricted growth. Liver zinc content, an index of zinc status, was depressed in ZD. At thermoneutral conditions, ZD depressed rectal temperture. The ZD animals also demonstrated significantly reduced rectal temperatures throughout the cold tolerance test. Post exposure thyroid hormone concentrations were depressed by ZD (Table 2).

These findings indicate that zinc deficiency impairs cold tolerance in rats and exerts this effect by influencing thyroid hormone metabolism.

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(73) THE T CROSS COAL BED (PALEOCENE, NORTH DAKOTA): THE IMPORTANCE OF REEVALUATING HISTORIC DATA IN GEOLOGIC RESEARCH

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The T Cross coal bed is significant as a widespread marker in the Williston Basin and formational boundary unit between the Ludlow and Slope Formations in the Paleocene of western North Dakota. Each new study has built upon previous work involving the T Cross without reevaluation of its original usage. This review and reevaluation of the original observations regarding the correlation of the T Cross coal bed show that its current usage and importance as a marker is difficult to verify on the basis of existing information.

The T Cross coal bed was named by Hares in 1928 (1) in one of the more extensive and detailed coal surveys performed by the U.S. Geological Survey in North Dakota. The focus of this work was coal land classification. Almost all of the work in North Dakota was done during the field season of 1911 by two geologists and untrained helpers. The coal bed derives its name from the "old T Cross ranch" where the bed was measured and described from exposures along Bacon Creek about 300 m west-southwest of the still-standing ranch house in sec. 20, T. 133 N., R. 104 W. The thick (7.3-8.5 m) coal bed, in this area of poor exposures and low relief, was noted earlier by Leonard in 1908 (2) in his extensive study of the coals in the valley of the Little Missouri River in southwestern North Dakota. Leonard did not name this bed but stated that the bed "perhaps be correlated with bed F, but [that] this could not be determined with any degree of certainty" (bed F appears to be equivalent to the Yule coal bed of Hares). In his summary of the T Cross coal bed, Hares (p. 47) stated: 1) that the T Cross is a thick coal in the area of T Cross Ranch, 2) the bed thins substantially to the north (T. 134-135 N., R. 105 W.), and 3) it extends to the latitude of Yule (sec. 25, T. 136 N., R. 105 W.). Note that elsewhere Hares' stated that this coal thickens instead of thins in township T. 135 N., R. 105 W. These summary statements are not, however, supported by Hares' geologic map (pl. 14) and township-by-township coal descriptions. The T Cross coal bed is not mapped as occurring in the river bluffs of the Little Missouri in townships north of T. 134 N., nor is the coal occurrence discussed in appropriate sections of the text. Reasons for these rather obvious discrepancies in stated versus documented occurrence are not clear, but Hares' published text and annotations to field maps indicate uncertainty in the correlation of some coal beds outside of their most representative outcrops. These problems in bed correlation and general interpretation of the stratigraphic framework led Hares to a determination of the thickness of the Ludlow Member (after Hares) that is approximately 58 m less than the amount established by his own criteria in recognizing formational contacts.

On the basis of reevaluation of Hares' unpublished and published geologic data, subsequent surface and subsurface stratigraphic work, and new field observations in western Slope County, the T Cross coal thins, and may split to the north from the T Cross Ranch occurrence, and thus cannot be reliably correlated into T. 134 N. Assuming Hares' observations on the T Cross in T. 134 N., R. 105 W. are correct, a T Cross correlation from the T Cross Ranch occurrence is possible. On the basis of Hares' own observations, however, from T. 134 to 135 N., the correlated stratigraphic horizon of the T Cross coal (of Hares) occurs within the interval of the Upper-Coal-Pair and Oyster lignite (nomenclature after Moore [3]). Use of the name T Cross by later workers for a coal in T. 135 N., R. 105 W., is substantially lower in the section than used by Hares, and apparently has no validity or attendant significance. For example, the lower brackish tongue of the Cannonball Formation has been associated with the T Cross coal bed in T. 135 N., R. 105 W. to the point of defining the relationship as the "T Cross bed" (3,4). Hares' occurrences of the T Cross and "Ostrea" localities south of this township do not indicate this association.

The problems associated with properly utilizing the name T Cross for coal correlation in Slope County are complex and not resolvable without additional subsurface and surface studies that specifically address this interval of the section. The coal bed name "T Cross" has been widely used in building a framework for other field observations in the lower part of the Fort Union Group of the Williston Basin. T Cross usage subsequent to Hares' is based largely on unsupported assumptions and face value considerations that may have led to significant error in correlation. Markers, as important components of a stratigraphic framework, require review of their historical basis and special consideration to justify new applications. Future researchers need to know the strength of the stratigraphic control before the information can be generalized for other purposes. This research is part of a program supported by the U.S. Bureau of Mines and NSF (EAR-8804881).

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(96) SPATIAL POPULATION REDISTRIBUTION TRENDS AND MIGRATION REGIONS IN NORTH DAKOTA

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A century ago, when North Dakota achieved statehood, its population of less than two hundred thousand was basically rural and scattered in homesteads or small settlements. With each wave of immigration the population increased to an all time high of 680,845 in 1930. The current population is lower than this record high and is projected to decline in the coming decades (1). This paper examines trends in population redistribution employing several spatial concentration measures and using the fifty-three counties as the geographic units of analysis. The first measure used to analyze population redistribution is the Hoover Index (H):

$$H = 1/2 \sum_{i=1}^{n} |Pi - Ai| * 100,$$

where Pi is that proportion of the state's population living in county i and Ai is the proportion of the state's land area in county i. If each county has the same proportion of the state's people and land area, then population would be evenly distributed and the index would be 0. If everybody lived in a single county, concentration would be at a maximum and the index would equal 1. The Hoover Index was calculated for each census from 1920 to 1980 and updated to 1986 (Fig. 1). At the beginning the index changed slowly, but accelerated since 1940s--a sign of population concentration trends in the state. Between 1920 and 1986, only nine counties gained while forty-four lost population (Fig. 2). Sioux and Rolette counties' modest growth is primarily generated by high Native American fertility. However, in-migration is the major component of growth in cities of the remaining seven counties. The second measure of population concentration is the Urbanization Index (U), which is the proportion of the state's inhabitants who live in places of at least 2,500. Less than ten percent of the population lived in urban centers at the turn of the century (Fig. 1). Currently over half of the state's population lives in the urban places, a shift in spatial population distribution caused mainly through the process of rural-urban migration.

To gain further insight into the process, intercounty migration flow was analyzed between 1975-1980. Data were copied from 1980 census tapes and organized into a 53 X 53 destination-origin gross migration matrix. All terms on the main diagonal of the matrix are assumed to be zero, implying that migration within a county is zero. Application of a Q-mode factor analysis reduces the 2,756 flows to a smaller and manageable set of factors. Factor scores identified five counties which represent the focus of origins or migration producing regions. The five counties with highest factor scores (5>) are: Burleigh, Cass, Grand Forks, Stark, and Minot (Fig. 2). Their associated migration origins are determined by the highest factor loadings (≥0.5). These migration regions are clusters of counties that manifest a high degree of similarities in the way they generate migration, and are grouped based upon their similarity of destination. Cartographic portrayal of scores reveals five functional migration regions focused on five largest cities. These growth poles are sites of North Dakota's largest universities, major health facilities, and two military bases, and comprise over seventy percent of the state's total urban population (Fig. 1). Their sociopolitical dynamism and high accessibility, along with changes in the state's agricultural economy, may be suggested as causes of population concentration demonstrated in this study. The trend is likely to continue decades into the second century of North Dakota's existance.

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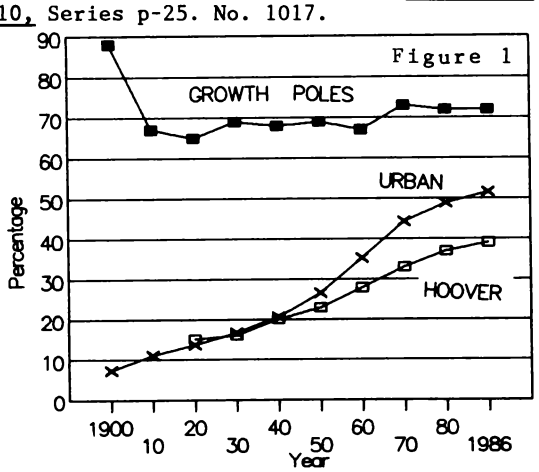

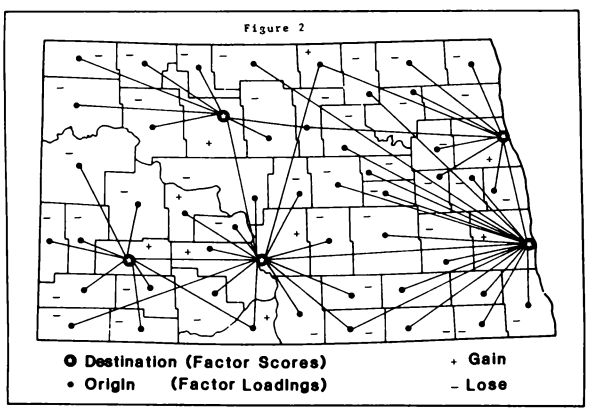

(97) RECENT FEMALE GEOGRAPHIC MOBILITY IN NORTH DAKOTA

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Recent achievements made by women in socioeconomic and political arenas are impressive throughout the United States and especially in North Dakota. There are more women with income earning employment, college education, and professional careers than ever before (1). The number of widowed, divorced, and female head of households has also increased. An accelerated rate of female geographic mobility is the major cause of noteable change in North Dakota's sex ratio, which declined from 122 men per 100 women in 1910 to 102 in 1984. These gains in socioeconomic and political dimensions have often been realized by moving from rural to urban areas, where over half of the women now live. The "mobility transition" theory predicts convergence in female and male rates as the development proceeds (2).

This study examines the fifty-three counties of North Dakota to see whether the female in-migration rate (Y) to them can be predicted from structural attributes of each county. An exploratory regression analysis of in-migration rates and eighteen independent variables pertaining to social, economic, and demographic structure of counties resulted in the selection of three variables: Crude death rate (X1), Median income (X2), and Divorce rate (X3). Reduction of the mortality rate is the ultimate goal of development. It is expected that migrants would not go to high mortality counties, everything else being equal. Economic prosperity, on the other hand, is expected to attract migrants. North Dakota's divorce rate increased 345 percent from 1950-80 (1). Divorce generally leads to residential mobility of at least one adult member. Female in-migration rate (Y) was regressed against the above predictor variables (Equation A).

A. $Y = 10.09 - 0.87(X1) + 1.14(X2) + 0.001(X3)$

(2.11) (3.49)** (2.11)* (3.49)**

$R^2 = 0.60$ F = 24.29** N = 53

B. $Y = 12.29 - 0.92(X1) + 1.11(X2) + 0.001(X3)$

(2.45) (4.10)** (2.26)* (3.41)**

$R^2 = 0.65$ F = 28.52** N = 51

Level of Significance: * = 0.05 ** = 0.01
t-ratios for regression coefficients appear in the parenthesis

Sixty percent of the variation in female in-migration rate is explained by the combined influence of the three selected variables. Regression coefficients are all significant at .05 or better and have the expected signs. Divorce rates are often higher in urban than rural areas. It is also shown that many divorced women migrate to urban areas seeking employment. An examination of the standardized residuals revealed Golden Valley and Mercer counties as two extreme outliers--counties fundamentally unlike the others in our study. Mercer has experienced severe economic fluctuations due to changes in oil and gas prices. The coal gasification plant in Beulah caused rapid population increase in the county. Its unusual circumstances justifies its removal from the study. Golden Valley was also eliminated because it received 141 men for every 100 women in-migrants from 1975-80. Apparently its farming/ranching economy has not been attractive to female migrants. Further analysis of the internal structure of outliers remains to be done. The same regression model was calculated without these counties which improved the coefficient of determination by five percent (Equation B).

Our analysis indicates that mortality, median income, and the divorce rate are important variables affecting female in-migration in North Dakota. Regression coefficients measure change in in-migration rate to any county by substituting the values of independent variables in Equation B. This exploratory analysis could be improved by further disaggregation of migration data by considering different age groups and whether they moved for schooling, military, economic, or personal reasons. The dependent variables could also be made more female specific (3). This study shows that health care availability, spatial economic inequality, and high divorce rates are three major factors which influence women's migration decisions. These are issues and challenges for North Dakota to address as it celebrates its centennial year, if it wishes to offer better quality of life for all its residents.

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(102) SEMI-CLOSED, TEFLON TUBE, WET-ASH DIGESTION FOR THE DETERMINATION OF BORON IN BIOLOGICAL SUBSTANCES BY INDUCTIVELY COUPLED ARGON PLASMA SPECTROPHOTOMETRY

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Recent findings suggest that comparatively low concentrations of dietary boron affect several aspects of mineral metabolism (1,2). It therefore seem appropriate to determine precisely the concentration of boron in foodstuffs and biological tissues. However, accurate determinations of small concentrations of boron have proved exceptionally difficult to obtain mainly because of the volatility of boron; the U.S. National Bureau of Standards (NBS) does not currently certify the concentration of boron in any substance. Boron concentrations in biosubstances are presently determined in this laboratory (3) by a wet-ash, low-temperature, Teflon* tube digestion followed by inductively coupled argon plasma spectroscopy (ICAP). That method gives acceptable results but is time-consuming (10 days/sample), requires substantial amounts of high-purity reagents and is prone to accidental contamination because of the open-vessel design. Thus, an attempt was made to overcome those difficulties.

Certified citrus leaves (batch 1572) and wheat flour (batch 1567a) standards from the U.S. National Bureau of Standards and an in-house freeze-dried diet pool were used as test materials. Teflon tubes (40 ml) with screw caps were modified by inserting a section of Teflon tubing (2.54 cm x 2.0 mm) in the middle of the screw cap. The standards and diet pools (0.3 g triplicates) were weighed directly into the acid-washed Teflon tubes. Blanks were prepared for each batch. The tubes were capped subsequent to the addition of 2.0 ml of 16.1 N HNO_3 ("Ultrex" grade, J.T. Baker Chemical Co., Phillipsburg, NJ) and allowed to digest at room temperature for 72 hours. They were subsequently placed in a sand bath heated to 160°C and were refluxed continuously for 48 hours near the constant boiling point of nitric acid (120°C). The samples were then injected with 1.0 ml 30% H_2O_2 ("Aristar" grade, Gallard/Schelsinger Chemical Mfg. Corp., Carle Place, NY) and heated for four hours. The samples never reached dryness during any part of the procedure. Samples and blanks were then diluted to 5.0 ml with 0.1 N HCl ("Ultrex" grade, J.T. Baker Chemical Co., Phillipsburg, NJ) in glass volumetric flasks, transferred immediately to 15.0 ml plastic tubes and stored until analysis by ICAP.

Table 1. Mineral Content of NBS Wheat Flour and Citrus Leaves Standards and In-House Diet Pool Performed by ICAP Analysis after Semi-Closed, Teflon Tube, Wet-Ash Digestion

Sample	B	Cu	Fe	Mn	P	Zn
NBS Citrus Leaves	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Mean	76.40	16.29	77.48	20.99	1290	28.20
St. Dev.	2.85	0.08	7.17	0.98	68	0.48
Cert. Value	-	16.50	90.00	23.00	1300	29.00
+/-	-	1.00	10.00	2.00	200	2.00
NBS Wheat Flour						
Mean	2.21	2.16	13.51	9.35	1238	12.14
St. Dev.	0.47	0.11	0.67	0.33	46	2.06
Cert. Value	-	2.10	14.10	9.40	1340	11.60
+/-	-	0.20	0.50	0.90	60	0.40
In-House Diet Pool						
Mean	1.71	2.32	20.60	9.29	2237	15.60
St. Dev.	0.65	0.06	0.34	0.31	63	1.10

The ICAP analyses (Table 1) indicate that this method of reflux digestion yields values within the range of certified values for copper, manganese, phosphorus and zinc. The variability of boron analyses was satisfactory for citrus leaves but unacceptably high for wheat flour and the diet pool. Because citrus leaves contain substantially more boron than either wheat flour or the diet pool, variability for the latter samples could probably be reduced by increasing sample size. Our boron concentration values for citrus leaves were in the range of those (64.3±0.6 µg B/g) obtained by neutron activation (4). Thus, the method described here uses less time and materials and gives reproducible values for boron and several other elements.

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

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(58)

EFFECTS OF BORON, STREPTOZOTOCIN AND THEIR INTERACTION ON INTERMEDIATE METABOLISM AND BONE TURNOVER IN RATS

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Previous research with chicks has shown that dietary boron significantly decreases the abnormally high concentrations of plasma glucose induced by cholecalciferol (vitamin D_3)-deficiency (1). Also, chicks are apparently sensitive to small changes in dietary boron; plasma glucose was found to decrease, then increase, as a function of dietary boron fed over a twenty-fold range (0.200 to 3.973 µg B/g diet) (2). In general, the effects of dietary boron seem more marked in the vitamin D_3-deficient chick than the vitamin D_3-adequate chick (3) and suggest that boron deficiency perturbs a vitamin D_3-dependent system. Furthermore, pancreatic insulin release is known to be impaired in the vitamin D_3-deficient rat (4). Thus, a 2x2 factorially arranged experiment was designed to determine the effects of dietary boron on the intermediate metabolism of glucose in the vitamin D_3 deficient rat made diabetic by streptozotocin injection.

Male, weanling, Sprague-Dawley rats were assigned to groups of 24 and were fed a ground corn-casein-corn oil based diet which was vitamin D_3-deficient and contained 0.034 mg boron/kg. The diet was supplemented with either 0 or 3 mg boron (as orthoboric acid)/kg. After 49 days, 12 rats from each of the two dietary groups were placed in metabolic cages for urine and fecal collections. At 57 days, one-half of the rats in each dietary group, including those in the metabolic cages, were injected with 75 mg streptozotocin/kg body wt. At 60 days, the rats were fasted for 16 hours, weighed and decapitated subsequent to pentobarbitol anesthesia and cardiac exsanguination with a heparin-coated needle and syringe.

Table 1. Effects of Boron, Streptozotocin, and Their Interaction on Selected Indices in the Rat

Treatment		Blood Plasma						24-hour Urinary	
B	Strepto-zotocin	Glucose	Albumin	Uric Acid	Insulin	Aspartate Transaminase	Ionized Calcium	Hydroxyproline (HP)	HP/Creatine Ratio
		mg/100ml			µIU/ml	U/l	mmol/l	mg	
0	-	116	2.58	0.85	6.15	110	1.00	0.65	74000
3	-	121	2.28	0.70	5.30	102	0.96	0.70	69000
0	+	504	2.53	1.33	5.26	173	1.10	4.59	614000
3	+	505	2.34	0.73	4.38	115	0.93	5.77	1029000
		Analysis of Variance - P Values							
Boron		NS	0.004	0.008	0.003	0.038	0.0004	NS	NS
Streptozotocin		0.0001	NS	0.066	0.002	0.020	NS	0.0001	0.0006
B x Strepto.		NS	NS	NS	NS	NS	0.02	NS	NS
Sq. Root EMS		24	0.27	0.40	0.77	47	0.08	112.63	439000

All rats injected with streptozotocin exhibited the classic signs of acute phase diabetes mellitus, including substantial elevations of plasma glucose, triglycerides, β-hydroxybutyrate, pyruvate, acidosis, and urinary glucose; boron affected none of those measures (Table 1). Compared to the non-injectioned rats, the injected animals also exhibited a five-fold increase in urinary loss of hydroxyproline, an amino acid primarily associated with bone collagen. The streptozotocin-injected rats also exhibited elevations in plasma uric acid and aspartate transaminase (an enzyme often measured to assess liver function), especially in rats deprived of boron. The boron supplement of 3 mg/kg diet significantly depressed plasma albumin, uric acid, insulin, and aspartate transaminase. Furthermore, an interaction between dietary boron and streptozotocin-injection affected ionized calcium levels; the boron supplement of 3 mg/kg diet depressed ionized calcium concentrations only slightly in non-injected rats but substantially reduced the abnormally high concentrations of ionized calcium in the streptozotocin injected rats. The results indicate that dietary boron has a significant impact on the plasma ionized calcium and hydroxyproline turnover imbalance induced by streptozotocin; further research is needed to determine whether or not the impact of boron on mineral metabolism is beneficial.

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(57) THE EFFECT OF DIETARY PROTEIN INTAKE ON ZINC REQUIREMENTS AND BONE ZINC IN THE GROWING RAT

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Dietary protein from various food sources has been associated with enhanced absorption of zinc from meals (1, 2). Surprisingly, rats fed a diet with 45% rather than 15% lactalbumin had higher tibia zinc concentrations after 30 days (3), despite a dietary zinc concentration (23.7 mg/kg) that was twice the normally required 12 mg/kg. Although the increase in bone zinc concentration was attributed to improved zinc bioavailability, it could instead reflect altered zinc metabolism, accompanied by increased zinc requirements and bone zinc deposition. The purpose of the present investigation was to determine zinc requirements at two moderate levels of protein intake, using weight gain and tibia zinc concentrations in growing rats as criteria.

Weanling, male, Long-Evans rats were fed, ad libitum, purified diets containing either 15 (low-protein) or 30% (high protein) egg white and 2.5, 5, 7.5, 10, 12.5, 15, 20, or 25 mg Zn/kg for 46 days. The two protein levels were chosen to provide adequate and luxuriant amounts of nitrogen and essential amino acids. Minerals were adjusted to be similar in the diets with the two different protein levels. For each protein level, a broken-line model (4) was used to determine the minimum zinc intake required for a maximum response in either body weight gain or tibia zinc concentration.

At dietary zinc concentrations higher than 10 mg/kg, low protein rats ate similarly, grew less initially, and then grew faster, achieving weights similar to high protein rats in weeks 5-6. At low zinc intakes, low protein rats ate more, weighed more, and at the lowest Zn intake, converted food into body weight more efficiently than high protein rats. Absorption of Zn-65, added to the diet on day 20 and monitored by whole body counting, was unaffected by protein. Protein affected the biological half-life of Zn-65 only at the lowest zinc intake, with a mean (± SD) of 83 ± 7 days for the low protein rats and 163 ± 8 days for the high protein rats. Weight gain and tibia zinc were correlated with dietary zinc at low zinc intakes, reaching similar maximum weight gain, but different maximum tibia zinc values (Table 1). The low dietary protein level resulted in a lower zinc requirement using either weight gain or tibia zinc concentration as criterion (Table 1). Low protein rats required less total zinc (µg/day) than high protein rats for a similar maximum weight gain, and required similar total zinc to achieve their lower maximum tibia zinc.

TABLE 1
Effect of Dietary Protein on Tibia Zinc Concentrations and Dietary Zinc Requirements

	15% Egg white Diet	30% Egg white Diet
Maximum tibia zinc (µg/g dry)	225 (217-233)*	276 (263-288)
Zinc requirement (mg/kg diet)		
based on weight gain	5.3 (5.1-5.5)	6.8 (6.5-7.1)
based on tibia zinc concentration	12.0 (11.6-12.5)	13.5 (13.0-13.9)
Zinc requirement (µg/day)		
based on weight gain	89 (85-93)	174 (165-185)
based on tibia zinc concentration	233 (224-241)	222 (213-230)

*Mean followed by 95% confidence interval.

These results suggest that high dietary protein intakes are associated with increased zinc requirements and greater deposition of zinc into the bone of growing rats. Tibia concentrations of calcium, iron, potassium, magnesium, sodium, and phosphorus were unaffected by dietary protein level (data not shown). It is not known whether the difference in bone zinc concentrations may be associated with any functional advantage. If so, the effect of dietary protein on bone zinc concentration could be of practical importance in infant nutrition because the protein content of infant formulas and milks varies considerably, from 10.5 g/liter in mature human milk to 34 g/liter in whole cow milk.

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(16) EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL AND COCAINE ON ENERGY SUBSTRATE UTILIZATION IN THE RAT TESTIS.

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Utilization of substrates like glucose and fructose is an important source of energy for normal functioning of different tissues in the body. This is particularly true in case of brain and testis where the demand of these substrates is much greater. Marihuana and its psychoactive constituent, delta-9-tetrahydrocannabinol (THC) have been known to inhibit different gonadal functions including testosterone synthesis, nucleic acid and protein synthesis, spermatogenesis and sperm motility (1,2,3,4). In investigating the mechanism (s) of these effects, we suggested that THC causes these reproductive insults by inhibiting the metabolism of energy rich substrates in the testis (5,6,7).

In recent years, the abuse of cocaine (COC) has become widespread in the younger population of the society. At this point, not much information is available as to the effects of COC on the energy metabolism of the testis. Therefore, the following studies were conducted to compare the THC effects with COC on the metabolism of glucose in rat testis. Groups of rats were treated acutely with 10 mg/kg, po, THC or 40 mg/kg, ip, COC. Control rats received 2 ml/kg of sesame oil or saline. THC treated rats were sacrificed 2 hr post injection whereas COC animals were killed 15 and 30 min following the drug administration. Their testes were removed and sectioned into small pieces. With these tissues, radio-respirometric studies were conducted using 5.5 mM radiolabled glucose as the substrate. Testes from rats treated with THC showed a 29% inhibition ($p<0.001$) in glucose metabolism as compared to their controls. On the other hand, testicular tissue of rats treated with COC and sacrificed either 15 or 30 min had no significant difference in glucose metabolism from their respective controls (3.76 ± 0.59 vs. 3.68 ± 0.64 and 2.89 ± 0.26 vs. 2.84 ± 0.32 μmol CO_2 x g dry tissue x 100^{-1}. These initial observations suggest that COC has no effects on glucose metabolism in the rat testis. However, recent studies indicate that effects of COC on glucose metabolism in brain are dependent on its route of administration (8,9). Therefore, experiments with intravenous COC are now planned to check this possibility. (Supported by NIDA grant DA03595).

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(103) THERMAL IONIZATION MASS SPECTROMETRY OF ZINC IN BIOLOGICAL SAMPLES

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Thermal ionization mass spectrometry (TIMS) has been used widely by nuclear chemists and geochemists. It is used to determine isotopic abundances of the elements because of its great sensitivity and precision. Stable isotopes are of interest to the biomedical research community because they can safely be used as metabolic tracers in human subjects. Thus, TIMS has become of interest to life scientists. This paper describes methodology used for isotopic analyses of biological zinc by TIMS.

Organic matter in feces was destroyed by ashing in a muffle furnace. The ash was dissolved in 12N HCl, then Zn was separated by chromatography on Dowex AG 1-X8 resin. The Zn-containing fraction was evaporated to dryness and redissolved in 100 µl of 2% HNO_3. Sample application to degassed Re filaments was as follows: 5 µl Zn solution (500-2500 ng Zn) was applied to the filament and dried under a heat lamp with a current of 1.1 A applied to the filament. When the Zn had dried, two 10 µl portions of colloidal silica gel (1) were applied, followed by 1 µl of 0.75 N H_3PO_4. After 5 minutes at 1.1 A, the filament current was increased to 1.6 A at a rate of 0.05 A/min. At the end of this heating program, the heating lamp was turned off and the current increased to 2.2 A until fumes of H_3PO_4 no longer evolved. Then current was increased until the filament glowed dull red for 1 sec.

Mass spectrometry was done with a thermal ionization mass spectrometer equipped with a single Faraday cup detector. Sample filaments were heated at 1.6 A for 20 minutes, and then to 2.4 A (approx. 1300°C) over 30 minutes. At this point a detectable signal at m/z 64 was obtained. Heating was continued until a filament temperature of 1550°C was reached after 85 min. At that time the m/z signal ranged from 100 mv to 2V(10^{-12} A to $2x10^{-11}$A), and data collection was begun.

Repeated analyses of a natural abundance standard and a ^{70}Zn-enriched standard gave results which did not differ from theoretical or calculated values and with relative standard deviations usually in the range of 0.5-1.0%. Samples prepared with 3-10 µl 0.75 N H_3PO_4 according to the procedure of other investigators (2,3) gave much weaker signals (≤100 mv from a 2500 ng Zn sample at 1500°C). The H_3PO_4 picks up water during sample preparation, thus it took up to an hour to dry samples prepared with larger amounts of the acid.

Preliminary data indicate that traces of organic matter in the sample, in addition to any traces of ion exchange resin, may increase ionization efficiency, resulting in stronger signals. Samples were refluxed with m-tetraphenylporphyrin (TPP) in dimethylformamide after the ion exchange step, and evaporated to dryness; then 3 drops of concentrated HNO_3 were added with heating to destroy the TPP. The HNO_3 was evaporated, then the residue was dissolved in 100 µl 2% HNO_3. A clear pale yellow solution resulted, in contrast to solutions from samples not treated with TPP, which are colorless. Samples were applied to Re filaments using the procedure described above. Stronger and more persistent ion signals were observed with both standard samples and fecal Zn samples that had undergone the TPP treatment (Fig. 1).

Isotope ratios were not affected by the method of sample preparation, except that the m/z 70/68 ratio was slightly, but significantly ($p < 0.03$) lower than the standard when the TPP preparation was used (0.2694±0.0125 vs 0.2772±0.0013). However, the number of standard samples prepared by the TPP procedure was small (n=3); the existence of a systematic effect on the measured ratio needs further confirmation.

The development of a sample preparation procedure which produces stronger ion beams will reduce the number of samples that need to be reanalyzed because of failure to obtain a beam strong enough and stable enough for precise determination of Zn isotope ratios.

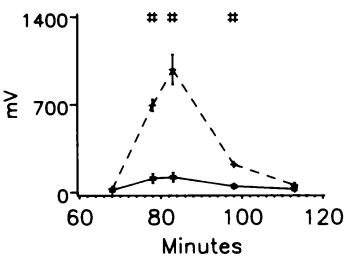

Fig 1. Ion beam intensity at different times during heat-up and measurement for ^{70}Zn standard samples prepared conventionally (—) and samples prepared with the TPP procedure (--). Points indicated with # are significantly different ($p < 0.0005$).

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(17) CHARACTERIZATION OF GLYCOLYTIC ENZYME INTERACTIONS WITH MICROTUBULES

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INTRODUCTION: Electron microscopy has revealed a network of proteins pervading the cytoplasm (1). This network, called the microtrabecular lattice (1), appears to be a polymeric structure composed of cytoplasmic proteins including those commonly considered cytosolic (1), such as the glycolytic enzymes (2). The nature and specificity of the interactions between the proteins making up the lattice have not yet been established. The microtrabeculae is proposed to interact with the cytoskeletal proteins F-actin and microtubules. Although interactions with tubulin have been established (3), interactions with microtubules have not. Therefore glycolytic enzymes were treated for interactions with microtubules.

METHODS: Taxol stabilized microtubules were prepared using a modification of Keates' procedure for tubulin preparation (4). Stable microtubules at a final concentration of 3 mg/ml were mixed with enzyme at a final concentration of 0.3 mg/ml at various concentrations of KCl in one-third strength resuspension buffer (33 mM MES, 0.33 mM EGTA, 0.42 mM Mg acetate pH 6.6 adjusted with KOH) with or without 7 percent polyethylene glycol (PEG) approx mol. wt. 8000. After incubation and centrifugation the supernatant and pellet fractions were separated. The pellet fraction was resuspended in one-third strength resuspension buffer. Both fractions were assayed for enzyme activity.

RESULTS: Glycolytic enzymes were subdivided into three groups as determined by their interactions with microtubules when tested in the presence and absence of PEG. The first category exhibiting minimal activity in the pellet fraction both in the presence and absence of PEG, includes triose-phosphate isomerase, lactate dehydrogenase type-H, enolase, and phosphoglycerate mutase. The second category, including glucose-6-phosphate isomerase and phosphoglycerate kinase, showed an intermediate amount of activity in the pellet fraction. Finally, the third category consisted of aldolase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase type-M which had pellet activities greater than 40 percent in the presence or absence of PEG.

This latter group was investigated further by testing for pelleting in the presence and absence of 7 percent PEG at various concentrations of KCl. In each case the activity found in the pellet fraction increased with the addition of PEG and decreased with increasing concentrations of KCL.

Phosphofructokinase was studied independently of the other enzymes because of its instability in the buffers used to test the other enzymes. However by using a modified phosphofructokinase stabilization buffer (5) this enzyme was found to associate with tubulin that had been crosslinked to a Sepharose column.

DISCUSSION: The interactions of the glycolytic enzymes with both tubulin and microtubules are consistent with a hypothesis that glycolytic enzymes are part of the microtrabecular lattice, and that the microtrabecular lattice is anchored to the cytoskeleton by interacting with microtubules.

The decrease in interaction with increased ionic strength is consistent with the view that the interactions are electrostatic. Since PEG enhanced the interactions at low ionic strength when compared to its absence, and higher ionic strength solutions were required to reverse binding, PEG presumably enhanced the ionic interactions of microtubule with the enzymes.

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(59) THE EFFECTS OF ANIMAL PROTEIN ON ABSORPTION AND METABOLISM OF MANGANESE IN THE RAT

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Although it contains very little Mn, animal protein added to vegetarian diets has been reported to improve Mn balance in humans (1,2). This study examined the absorption and utilization of Mn in rats fed diets based on soy, casein, beef, chicken, or tuna.

In Exp. 1, diets were formulated to contain 20% protein; other components, including fat and mineral content, were adjusted to be identical in all diets. All diets contained recommended amounts of nutrients for rats, except that Mn was 5 mg/kg instead of the recommended 50 mg/kg. In Exp. 2, diets were the same as in Exp. 1 except that mineral content was not equalized among the diets; thus mineral composition varied depending on the amount contributed by the protein source. Dietary Mn was 5.6 - 8.0 mg/kg.

Male weanling Long-Evans rats were fed test meals of 4 g of their respective diets plus 3 μCi ^{54}Mn after they had eaten the experimental diets for 2 wk. Retention of ^{54}Mn was measured by whole-body gamma counting. Mn balance was measured in Exp. 1 only. Biochemical indices of metabolism were determined in tissues when rats were killed after 5 wk.

Table 1
Effect of Dietary Protein on ^{54}Mn Absorption, Biological Half-life (BH) and Mn Balance

	Experiment 1				Experiment 2	
Diet	^{54}Mn % Abs	BH[1] (d)	BH[2] (d)	Mn Balance (μg/d)	^{54}Mn % Abs	BH[2] (d)
Beef	4.57^{a}	5.5^{b}	18.5^{c}	3.2^{ab}	4.14^{c}	26.9^{a}
Casein	5.30^{ab}	6.2^{b}	15.4^{ab}	2.8^{ab}	5.88^{abc}	16.3^{b}
Chicken	6.57^{bc}	9.1^{a}	17.4^{bc}	3.5^{ab}	6.39^{ab}	17.4^{b}
Soy	5.46^{ab}	6.8^{b}	14.5^{a}	9.9^{a}	4.62^{bc}	16.2^{b}
Tuna	8.42^{c}	7.1^{ab}	15.0^{ab}	-5.2^{b}	7.71^{a}	16.7^{b}
Root MSE	1.33	1.5	1.8	9.3	1.34	1.66
ANOVA (p value)	<0.0001	<0.0004	<0.0002	<0.05	<0.0001	<0.0001

[1]from d 5-8 after ^{54}Mn dose. [2]from d 11-22 after ^{54}Mn dose.
Values in the same column not sharing a common superscript are significantly different by Tukey's test.

In both experiments (Table 1), ^{54}Mn absorption was greatest from tuna and lowest from beef. The ^{54}Mn BH rapid-turnover component (d 5-8) was lowest in rats fed beef and greatest in those fed chicken in Exp. 1. However, the BH of the slower-turnover component (d 11-22) was largest for beef and the smallest for soy in both experiments. Net Mn balance was most positive for soy-fed rats and least positive for rats fed tuna. In Exp. 2, the BH of ^{54}Mn in beef-fed rats was 66% greater than in soy-fed rats, but in Exp. 1 the BH in beef-fed rats was only 27% greater than in soy-fed rats. Biochemical indices of Mn status were essentially unaffected by protein source.

In contrast to reported effects of animal protein on Mn metabolism in humans (1,2), these experiments showed little change in Mn status in rats fed animal protein instead of soy protein. However, in Exp. 2, where mineral content of the diets was allowed to vary depending on the protein source, the BH of ^{54}Mn in rats fed beef was markedly longer than, in the first experiment, while the BH for rats fed other diets was similar between the two experiments. The beef diet in Exp. 2 contained about 88 mg Zn/kg, in contrast to 35-40 mg Zn/kg for other diets. The beef diet contained about 58 mg Fe/kg; soy, 69 mg Fe/kg; and other diets, 36-40 mg Fe/kg. High amounts of dietary iron are known to inhibit Mn absorption and to increase Mn turnover. The increased BH of ^{54}Mn in rats fed beef, and the lack of change in the BH in soy-fed rats, between the two experiments argues against the effects of beef being the result of differences in dietary Fe. Zn is not known to affect Mn metabolism. The effects of animal protein on Mn metabolism in rats are less marked than those reported for humans (1,2). The mechanism of those effects is not clear, but may be related to the amino acid composition, rather than the mineral composition, of the protein source.

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(10) THE EFFECT OF BUFFERS ON METAL ACTIVATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE

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The choice of buffer to be used in an in vitro study of an enzyme is too often made without consideration of its possible interaction with the enzyme or its substrates. Phosphoenolpyruvate (PEP) carboxykinase is an enzyme with a dual requirement for metals (1,2). Optimal activity is expressed when magnesium (Mg) is present in millimolar concentration to complex with the nucleotide substrate (GDP or GTP) and manganese (Mn) is present in micromolar concentration to complex with and activate the enzyme. The reaction catalyzed by PEP carboxykinase is reversible. Either iron or manganese may be dissociable activators of enzymes in vivo (e.g., see (3)). Below we compare the effects of buffers and metals on the specific activity of each isozyme of PEP carboxykinase in each direction of catalysis at pH 7.4.

We have purified to homogeneity both the mitochondrial and cytosolic isozymes of PEP carboxykinase from rabbit liver. In order to study the iron (Fe) activation of these isozymes over a wider range of pH than possible with spectrophotometric assays, we used an HPLC-based assay developed by Jacoby (2) in which the nucleotide substrate and product (GTP and GDP) are quantitated. The following buffers adjusted to pH 7.4 were used at a concentration of 50 mM in the assay: imidazolium-chloride, Tris-chloride, potassium phosphate, and sodium-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). For each buffer and each isozyme, the following four sets of metals were used: 2.1 mM Mg(II) 2.1 mM Mn(II) 2.0 mM Mg(II) + 0.1 mM Mn(II), and 2.0 mM Mg(II) + 0.1 mM Fe(II). Mg and Mn were added as the chloride salts while Fe was added from a freshly prepared solution of $Fe_2(NH_4)_2(SO_4)_2$. All buffers, substrates, and metals were purified in order to further reduce the concentrations of adventitious metals. Each isozyme was equilibrated with 10 mM Hepes, pH 7.4, by centrifugation through Sephadex G-25. The concentration of this buffer in the final assay mixture, occurring as a result of carryover, was 2 mM. The concentrations of other substrates were 1.0 mM GTP and 2 mM oxaloacetate (OAA) in the direction of PEP synthesis; 50 mM $NaHCO_3$, 2 mM PEP, and 1 mM GDP in the direction of OAA synthesis.

In the direction of OAA synthesis from PEP, essentially no activity for either isozyme was observed when Mg was the only divalent metal present. Appreciable rates were obtained in the presence of Mn only, but the best rates were obtained with the combination of Mg and Mn. These data can be explained by assuming that the Mg-GDP complex is a better substrate than Mn-GDP. Iron gave appreciable activation of both isozymes except when phosphate was used as the buffer. Phosphate is known to promote autoxidation of ferrous iron to ferric iron (4), and the form of Fe which activates PEP carboxykinase is the ferrous ion. Although imidazole is a known chelator of Mn and Fe, the highest activities conferred by either Fe or Mn was when imidazole was used as the buffer. Imidazole may have promoted Fe activation by stabilizing the ferrous state, however, this explanation does not apply to Mn since that metal is redox-stable.

In the direction of PEP synthesis from OAA, each isozyme showed appreciable rates with Mg alone, and somewhat higher rates with Mn alone. The highest rates, approximately five-fold greater than in the presence of Mg only, were obtained with Mg plus Mn. Iron gave two to four-fold activation of cytosolic PEP carboxykinase with the highest rate being in imidazole and the lowest in phosphate buffer. In comparison, the mitochondrial isozyme was weakly (about two-fold) activated by Fe in imidazole and Tris buffers while no activation or slight inhibition was observed in HEPES and phosphate buffers. (Supported by the EPSCOR grant from NSF to North Dakota.)

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(19) INCREASED EXPRESSION OF HUMAN RETINAL VESSEL BASEMENT MEMBRANE 110kD COLLAGEN IN AGING AND DIABETES

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Basement membranes (BMs) are usually thin continuous extracellular structures which limit the connective tissue space and function to give support and as a permeability barrier. When visualized by electron microscopy, normal BMs appear to be a fine felt-like fibrillar meshwork, while BMs from diabetics have been described as generally thickened with retinal vessels fitting into two classes (1). The BM of type A vessels has an extracellular matrix which is expanded by loose concentric pericytic matrix layers with thickened subendothelial and pericytic BM. Vessels with compact pericytic matrix and poorly recognizable endothelial and pericytic BM are of type B. Although these two vessel types have been described, no link has been made to a specific disease stage.

All BMs have been found to contain type IV collagen but collagens exist as a multigene family and the literature has suggested the existance of novel collagens which are yet to be fully characterized (3). One such collagen was partially characterized in our laboratory (J.C.S.). A novel 110kD protein (found as a helical trimer parent molecule) was initially isolated from purified bovine retinal vessel basement membrane (RVBM) and appeared to represent a significant portion of the BM (4). This novel collagen had not previously been elucidated because most investigators have concentrated their efforts on isolating the constituents of BM from the Englebreth-Holm-Swarm (EHS) tumor with the assumption that all BMs were essentially similar. EHS tumor was easy to work with because it consists of a large amount of a relatively soluble BM matrix. Unfortunately retinal basement membrane is very difficult to solubilize. We used a method developed by Carlson et al. (2) which utilized detergent extraction and limited pepsin digestion to purify the retinal vessel basement membrane.

After isolating 110kD collagen in bovine RVBM we wanted to determine whether it was species specific or could be found in human retinal vessels. Using 329 human retinas donated by the National Diabetes Research Interchange, we grouped them into diabetic or normal and pooled the retinas into age catagories of: 0-29, 30-50, 51-70, 71-100, or unknown age. Essentially, the pooled retinas were extracted by the above method (2) in order to purify the BM. Since we are unable to specifically quantify 110kD collagen at this time we analyzed equivalent amounts of the extracted basement membrane/retina by SDS-PAGE electrophoresis after which the resulting collagens were quantified using a coumputer enhanced image analysis densitometer.

A linear increase in 110kD collagen was shown with age ($r=0.97$) in normal human retinal vessels. Using a test of least squares linear fit it was determined that the regression of normal age was significant ($p<0.05$). Age was known only in two of the four groups of pooled diabetic retinas thus the determination of linear regression was not possible. Accordingly we attempted to fit the known and unknown ages of the diabetics to the normal age curve by dividing the observed quantity of 110kD collagen by the slope. The predicted ages were unreasonably high which suggested the diabetics had a greater than normal for age increase in the expression of 110kD collagen. In these determinations the diabetics showed a 3.5 fold increase of 110kD over normals ($p<0.05$).

These preliminary results indicate a significant increase in 110kD collagen in diabetic RVBM. In addition we demonstrated a high correlation in RVBM between increasing age and the expression of the 110kD protein.

In order to investigate the possible significance of 110kD collagen in the pathogenesis of the complications of diabetes monospecific antibodies will be generated and utilized to morphologically localize the presence and distribution of this protein in both normal and type A and B diabetic retinal vessel BMs.

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(25) MEASUREMENT, MIGRATION AND MITIGATION OF ENVIRONMENTAL RADON

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Environmental radon was measured with standard charcoal canisters and with a grab sample method employing 0.16 L Lucas cells. Concentrations in buildings ranged from approximately 1.5 pCi/L in a well-ventilated public building to greater that 1100 pCi/L in a sump pump chamber in a private home. Seventy-nine percent of nearly 600 canister measurements in the homes in this area (North Dakota and NW Minnesota) had radon concentrations above the EPA recommended maximum level of 4 pCi/L.

Radon concentrations have been found to be higher in the winter, presumably because radon travels beneath the frozen soil until it reaches a region where it can seep in/out. Such a region is normally below a heated concrete slab, beneath a basement floor or around the basement walls or footings. Radon enters the structure from the openings in the concrete such as annular spaces around sewer pipes through open sump pump chambers or cracks in the concrete. Representative radon concentrations found in the typical basement were 15, 100, and 300 pCi/L for hairline cracks, annular spaces around drains/sewer valves and sump pump chambers, respectively. Forced air heating tended to raise the levels of radon in the upper living quarters of a home.

Several representative cases in which effective techniques were used to reduce radon concentration levels are listed as follows:

House A. (finished basement, sump pump)
1. Initial radon concentration, 76 pCi/L.
2. Concentration after vent of sump, 15 pCi/L.
3. Concentration after installation of air-to-air heat exchanger, 7.5 pCi/L.
4. Concentration after installation of subslab ventilation, 4 pCi/L.

House B. (finished basement, accessible wall cracks)
1. Initial radon concentration, 350 pCi/L.
2. Concentration after filling space around floor drain, 38 pCi/L.
3. Concentration after sealing floor and wall cracks, 20 pCi/L.

House C. (finished basement, no sump pump, walls inaccessible)
1. Initial radon concentrations, in the fall season: master bedroom, 5 pCi/L, basement bedroom, 10 pCi/L; winter season: 12 pCi/L.
2. Concentration after installation of subslab ventilation system (withdrawal port in closet), 8.3 pCi/L (avg. of four tests).
3. Concentration in exhaust gas from ventilation system, 110 pCi/L.

House D. (furnace room unfinished)
1. Initial radon concentration in master bedroom (upstairs), 12.3 PCi/L.
2. Radon concentrations around "roughed in" shower drain and stool coupling (annular spaces), 1000 and 300 pCi/L, near seams in concrete floor, 20 pCi/L.
3. Concentrations in master bedroom after sealing seams and annular spaces, 9.3 pCi/L.

Each house was found to be unique with no particular reduction method working best. Generally, homes with sump pumps and associated drain tiles had an excellent "built-in" radon removal system and radon levels could be reduced with exhaust of the sump pump air.

(104) FROTH FLOTATION OF SELECTED NORTH DAKOTA LIGNITES

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The role and effectiveness of collector and frother-promoter reagents in froth flotation are fairly well understood in higher-rank coals (1). However, it has not been determined which reagents are most suited for effective froth flotation of low-rank coals (LRCs). Recent research at the University of North Dakota Energy and Mineral Research Center (UNDEMRC) has concentrated on the use of fuel oil (a collector) and Shur-Coal 164A (frother-promoter), manufactured by Sherex Chemical Company (2). Fuel oil (an alkane mixture; C_5H_{12}-C_8H_{18} and aromatics) has been shown to be a good collector, but its effectiveness is highly coal-specific (1,3). As a collecting agent, fuel oil coats coal particles, making them even more hydrophobic and floatable (3). Shur-Coal 164A, a frother-promoter, is necessary to stabilize bubble attachment to hydrophobic particles.

The purpose of this paper is to report the froth flotation cleaning yields and ash reduction efficiencies obtained using various concentrations of fuel oil and Shur-Coal 164A and two different grinds of two North Dakota lignites.

Fuel oil and Shur-Coal 164A were mixed in a ratio of four parts fuel oil to one part frother-promoter, on the recommendation of the Sherex Chemical Co. This solution was then added in varied dosages to a coal/water fuel mixture containing 8 wt% coal. Froth flotation testing was completed on combustion grind (80% <75 μm) and micronized (100% <50 μm) coals using a Denver froth flotation cell. Two North Dakota lignites, Beulah-Zap (Coteau Mine; 9.4 wt% ash) and Velva (Velva Mine; 12.5 wt% ash) were selected for testing. Dosages used were 2, 4, and 8 kg of the fuel oil: SC164A mixture per ton (metric) of coal.

Results of the froth flotation experiments predictably indicate that as the dosage increases, the yield (amount of float product) increases for each coal and each grind (Table 1). At 4 kg/ton, the yields are nearly equivalent to higher rank coals, and the dosage-yield relationship is conventionally thought to be economic (3). Ash reduction was not predictable. One would expect greater ash reductions with low dosages, but this was not the case. Overall, the ash reductions were not similar to those obtained using higher rank coals under similar dosages. The effect of grind is also inconclusive. Development of a more effective frother-promoter is needed for low-rank coals, and more research is needed on the effect of grind on ash reduction.

Table 1

Froth flotation results for combustion ground and micronized Beulah-Zap and Velva lignites using a mixture of fuel oil and Shur-Coal 164A. Weight percentages are on a moisture free basis.

	BEULAH-ZAP LIGNITE				VELVA LIGNITE			
	COMBUSTION		MICRONIZED		COMBUSTION		MICRONIZED	
Dosage (Kg/ton)	Yield (wt%)	Ash Red. (wt%)	Yield (wt%)	Ash Red. (wt%)	Yield (wt%)	Ash Red. (wt%)	Yield (wt%)	Ash Red. (wt%)
2	38.4	10.9	39.5	12.6	20.3	9.2	15.7	10.2
4	60.8	17.2	74.4	9.1	54.0	12.6	44.2	9.8
8	70.1	18.8	80.4	10.1	85.6	5.3	57.6	6.6

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SEASONAL WETLANDS AND LIVESTOCK GRAZING ON THE MISSOURI COTEAU : ABOVEGROUND BIOMASS

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Seasonal wetlands provide important breeding waterfowl habitat (1) and livestock forage. These wetlands and their surrounding matrix of upland habitat are commonly managed for livestock grazing. Seasonal wetlands are composed of wet meadow and shallow marsh vegetation zones (2). There are no published data on the relationship of livestock grazing and grazing systems and wetland forage production and disappearance within the Prairie Pothole Region.

This study was initiated to determine the influence of grazing management strategies on biomass production and disappearance from seasonal wetlands. Study sites were located on the Central Grasslands Research Station near Streeter, ND. Seasonal wetlands were sampled in each of the following grazing treatments: 1) ungrazed control (UN), 2) twice-over rotation (TO), 3) short duration (SD), and 4) seasonlong (SL). The UN wetlands were not grazed or mowed during the study. The TO system consisted of a single herd rotated among 4 pastures; each pasture was grazed for 20 days, rested for 60 days, then grazed for 20 days. The SD system had one herd rotated among 8 pastures, each grazed for 5 days, then rested for 35 days. The cycle was repeated for the duration of the grazing period. The SL grazing treatment was a single herd occupying one pasture for the duration of the season. Stocking rates were 0.30 ha/animal unit months (aum) for SD and TO, and 0.54 ha/aum for SL. The length of the grazing season was 160 days for 1985-1987 and 105 days in 1988.

Production and disappearance were estimated using paired 0.25m^2 quadrats. Production data were collected on ungrazed plots from late July through mid-August (3). Quadrats to estimate disappearance were clipped from similar grazed plots located in the same wetland immediately after the final grazing period. Samples were air dried at 65°C to a constant weight. Three wetlands were sampled in each treatment from 1986 to 1988 and two wetlands per treatment were sampled in 1985. Shallow marsh sites were not sampled during 1986 due to high water conditions. Annual precipitation on the station for 1985 through 1988 was 45.4 cm, 67.4 cm, 41.7 cm and, 21.8 cm respectively. These same annual totals expressed as a percentage of the long-term average were 101%, 148%, 92%, and 49% respectively. Standing water was present on all sites throughout 1986, and no standing water was present on the wetlands by July 1988.

ANOVA for all wet meadow sites indicated that treatment effects were not significant (P>.05), but that year effects were significant (P<.05) for both production and disappearance. ANOVA for all shallow marsh sites indicated that both treatment and year effects were significant (P<.05) for production and disappearance. High water availability during the early growing season characterized 1986 and 1987, and in Table 1 there is only one significant difference between means for these two years in both vegetation types. Disappearance for both vegetation types in 1985 was similar among treatments. Production was similar among treatments in the wet meadow, but highest for the UN treatment in 1985. Shallow marsh production and disappearance in 1988 were similar among treatments. During 1988, wet meadow production in the UN treatment was higher than in the SL treatment, while SD disappearance exceeded that in the SL treatment. There were few consistent differences among the grazed treatments in any year. Production in the UN treatment generally exceeded the grazed treatments only in 1985. The weather conditions associated with each year, rather than grazing treatments, appear to have the most significant influence on production and disappearance.

TABLE 1. BIOMASS PRODUCTION (PROD) AND DISAPPEARANCE (DIS) IN THREE GRAZED AND ONE UNGRAZED TREATMENT ON TWO SEASON WETLAND VEGETATION TYPES FROM 1985-1988 NEAR STREETER, ND (n=NUMBER OF QUADRATS, DATA ARE MEAN ± STANDARD ERROR).

	SHALLOW MARSH			WET MEADOW			
	1985	1987	1988	1985	1986	1987	1988
UN-PROD [1]	6829 ± 610a (n=8)	6961 ± 729 (n=12)	4074 ± 570 (n=14)	2286 ± 252a (n=8)	6558 ± 437 (n=24)	7270 ± 428 (n=12)	2922 ±332a (n=12)
DR-PROD	2912 ± 331b (n=8)	6098 ± 1347 (n=7)	2301 ± 228 (n=6)	3180 ± 238bc (n=8)	7164 ± 475 (n=12)	7610 ± 506 (n=7)	2495 ± 232ab (n=6)
DR- DIS [2]	1210 ± 243 (n=8)	2181 ± 577xy (n=7)	1565 ± 209 (n=6)	964 ± 250 (n=8)	2629 ± 363 (n=12)	3835 ± 444 (n=7)	1699 ± 286xy (n=6)
SD-PROD	4073 ± 541b (n=8)	7939 ± 1330 (n=8)	3240 ± 609 (n=7)	3349 ± 206c (n=8)	8143 ± 976 (n=12)	7391 ± 529 (n=7)	2417 ± 281ab (n=6)
SD- DIS	1152 ± 231 (n=8)	3854 ± 1118x (n=8)	2694 ± 628 (n=7)	1581 ± 221 (n=8)	2946 ± 576 (n=12)	3086 ± 642 (n=7)	2060 ± 262x (n=6)
SL-PROD	2708 ± 344b (n=8)	4220 ± 307 (n=7)	2809 ± 474 (n=6)	2504 ± 333ab (n=8)	7198 ± 624 (n=12)	9091 ± 1041 (n=6)	1483 ± 207b (n=6)
SL- DIS	1309 ± 332 (n=8)	725 ± 296y (n=7)	2535 ± 478 (n=6)	1284 ± 179 (n=8)	2169 ± 282 (n=12)	4744 ± 932 (n=6)	1053 ± 216y (n=6)

[1]PRODUCTION MEANS IN THE SAME COLUMN FOLLOWED BY DIFFERENT LETTERS DIFFER AT THE 0.05 LEVEL.
[2]DISAPPEARANCE MEANS IN THE SAME COLUMN FOLLOWED BY DIFFERENT LETTERS DIFFER AT THE 0.05 LEVEL.

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(98) AN ANALYSIS OF THIRD WORLD PLACE NAME KNOWLEDGE IN 1988 AT SCHROEDER JUNIOR HIGH SCHOOL OF GRAND FORKS, NORTH DAKOTA

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Renewed concern about low levels of place name knowledge among Americans has prompted geographers to reconsider the nature of geographic education (1). Nationally, increased attention is being given to examining how place names are learned (2). Studies in North Dakota dealing with this central issue of geographic education have focused upon using the International Geographical Union's (IGU) World Basic Place Vocabulary Test as a diagnostic instrument to assess place name learning abilities (3,4). This test of 50 basic locations consisting of five oceans, 32 countries, and 13 cities has been used most frequently in the Grand Forks (ND) Public School District.

Previous research involving junior high school students revealed that countries in the Third World are not readily identified correctly by students in grades seven through nine (5). However, there also appeared to be distinct variations in place name knowledge that reflect two factors: 1) the grade level of students; and 2) a nation-state belonging to a particular regional grouping of Third World countries.

Consequently, two hypotheses were selected for investigation in the 1988 study at Schroeder Junior High School of Grand Forks. First, ninth graders would have a drop in retention of Latin American locations learned in grade seven but would have the highest scores for all other Third World locations. Second, knowledge of locations could be predicted as ranging from lowest to highest according to the country's belonging to a recognized regional grouping. Those nation-states of Black Africa were expected to be in the range of 50-70% accuracy, Middle Eastern countries being known at 60-80% accuracy, Asian nation-states being moderately well-known with 70-90% accuracy, and Latin American countries being the best known at 80-100% accuracy.

The test was conducted during the first two weeks of the 1988-89 academic year. All junior high school students present on the test dates were included in the sample. The three sub-populations were 141 ninth graders, 162 eighth graders, and 182 seventh graders. When the 50-item test was completed and returned, locations of Third World countries were analyzed separately.

The results for the Third World country data do not appear to support either of the two hypotheses. The first hypothesis seems unproven because although the ninth graders did score higher than the other two grade levels for Black African, Middle Eastern, and Asian locations, in only two cases, that of Argentina and Chile, did the ninth graders show any loss of retention of place name knowledge of Latin American countries learned in grade seven.

The second hypothesis was not supported by the data for any grade level, and several key points should be made about each grade level. Grade nine students scored below 50% accuracy for two of the four countries representing Black Africa and averaged under 60% accuracy for three of the five Middle Eastern countries. Students in grade eight almost met the expectations for knowing Latin American place names with only Peru (79.01% accuracy) falling below the 80% minimum. However, eighth graders did quite poorly with Middle Eastern and Asian locations, e.g., all Middle Eastern place names were below the 60% minimum and three of the four Asian locations were under 70% accuracy. The seventh graders had the most unexpected scores. Considering that world history is covered in grade six, it was anticipated that the incoming seventh graders would do reasonably well. With the exception of Mexico (92.85% accuracy), no score was higher than 65.93% correct, this being the percentage accuracy for China. Indeed 13 of the 20 Third World locations were identified with 50% or less accuracy by the seventh graders.

There are two implications for this study. First, further general investigation needs to be conducted to determine how specific place names are learned and retained by junior high school students. Second, research must be undertaken in local middle schools to determine why there is unexpectedly low place name knowledge for incoming seventh graders. The process of place name learning is most critical during the middle school and junior high school (2), and the IGU World Place Vocabulary Test provides an excellent diagnostic for helping students deal with improving their geographic literacy.

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(99) A PRELIMINARY PROFILE OF SEVENTH GRADE GEOGRAPHY IN 1988 FOR NORTH DAKOTA

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According to Cirrincione and Farrell (1), social studies teachers in the United States support curriculum reform efforts that emphasize providing additional geographic content and improving geography instruction for pre-college students. This positive response indicates that professional geographers should be well-received locally when trying to help pre-college educators deal with what Natoli and Gritzner (2) have identified as dismally low levels of geographic literacy. In order to undertake such actions effectively, it is necessary to understand what aspects of geography are being taught, how that geographic content is being taught, and who is teaching the geography components of a social studies curriculum.

Such a study in curriculum reform for pre-college geography in social studies must first focus upon individual states because of the absence of a national comprehensive system of schooling for grades K-12. Geographic education in North Dakota's social studies classrooms has been examined previously by Anderson (3) and Willard (4) with Munski (5) having specifically examined geographic education in social studies at the grade seven level. Seventh grade social studies is the only grade level that specifies teaching a formal course in geography in the state.

Each of North Dakota's 279 public school districts was surveyed regarding the content, methods, and personnel involved in the seventh grade social studies/geography course. Using a random sampling technique, one school teacher per public school district was contacted to complete the 28-item questionnaire. Only 108 of the returned surveys were usable for the data analysis.

Preliminary analysis of this data has revealed five major results. First, the content of this course is a regional course that focuses foremost upon the geography of the United States; 91.7% of the respondents cover the United States. The inclusion of other world regions by respondents decreases in this order: Canada (85.7%), Central America (82.1%), Mexico (78.6%), Europe (67.5%), Soviet Union (58.3%), South Asia (56.0%), Africa (53.6%), Australia (52.4%), East Asia (52.4%), Southeast Asia (52.4%), Pacific Islands,(41.0%), and Antarctica/Arctic (36.9%). Second, maps and globes are not frequently used. Using a standard that maps and globes should be part of lessons at least every second day, only 47.3% of the teachers taught lessons that included using wall maps during presentations; no more than 33.3% of the respondents referred to a globe in any of their lessons. Third, microcomputers are used in any fashion by only 18.8% of the sampled teachers. Fourth, lectures and discussions are the dominant delivery mode of instruction with 48.1% of the respondents giving a daily lecture and 50.0% of them conducting discussions each day. Finally, geography instruction is provided overwhelmingly by non-geographers. Only 2.4% of the teachers polled have a geography major while 14.3% of respondents have a minor in geography.

This pilot study has revealed that certain changes must be undertaken in geographic education at different levels. First, there must be increased in-service programs to upgrade teacher background in geography ; 93.9% of the respondents supported this suggestion. Second, local teacher trainees should have more coursework in the geography of North America, Central America, and South America. Finally, local curriculum reform must be geared toward increased usage of maps, globes, and microcomputers if we are to have junior high school students better able to address the key questions of geography as outlined by Natioli and Gritzner (2): "Where, why, and of what significance are places on the earth's surface?"

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(20) GLUCOSE-6-PHOSPHATASE AS A SITE OF REGULATION OF BLOOD GLUCOSE IN TWO MODEL SYSTEMS: THE DIABETIC RAT AND THE TUMOR-BEARING MOUSE

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The increase in hepatic glucose production with the development of diabetes in the rat coincides with a change in the kinetic parameters of glucose-6-phosphatase, the enzyme which catalzyes the final step in glucose production (1). The in vivo growth of a tumor also results in enhanced hepatic gluconeogenesis as the host attempts to maintain blood glucose levels in the presence of the additional use of glucose by the tumor (2). Therefore, kinetic characteristics of hepatic microsomal glucose-6-phosphatase from intact and detergent-treated tumor-bearing mice and diabetic rats were determined and compared to values for the respective control animals.

Kinetic assays on hepatic microsomal glucose-6-phosphatase from tumor-bearing and control mice and diabetic and control rats were carried out at glucose-6-P concentrations ranging from 0.200 mM to 10.0 mM. Incubations were carried out at pH 7.0 for microsomal preparations which were treated with Triton X-100 and untreated, respectively. The kinetic parameters, K_m and V_{max}, for tumor-bearing and control mice and diabetic and control rats are given in Table 1. K_m values are in terms of mM and the V_{max} values are in terms of nmole Pi x min^{-1} x ug microsomal $protein^{-1}$. The values are means ± standard error (n = 5 and 7 for the mouse and rat, respectively). Statistical analysis of the data was done by Scheffe Test.

Table 1. Microsomal Glucose-6-phosphatase, K_m and V_{max} Values.

Animal Treatment	Intact Microsomes K_m	Intact Microsomes V_{max}	Disrupted Microsomes K_m	Disrupted Microsomes V_{max}
Normal Rat	1.78 ± 0.09	0.358 ± 0.027	0.859 ± 0.077	0.491 ± 0.044
Diabetic Rat	2.22 ± 0.10	0.422 ± 0.045	0.936 ± 0.062	1.32 ± 0.26
Control Mouse	2.70 ± 0.33	0.272 ± 0.021	1.03 ± 0.12	0.542 ± 0.045
Tumor Mouse	5.03 ± 0.67	0.391 ± 0.073	1.11 ± 0.12	0.472 ± 0.095

There is a decrease in the K_m and an increase in the V_{max} for microsomal preparations from the rat and the mouse upon detergent-disruption of the microsomal membrane regardless of the animal treatment. A significant increase in the K_m for the enzyme from intact microsomes ($p < 0.05$) occurs when the rats were made diabetic and when a tumor is present in the mouse. The V_{max} for the enzyme of disrupted microsomes from diabetic rats is greater ($p < 0.01$) than from disrupted microsomes of control rats. There is not an increase, however, in the V_{max} of disrupted microsomes in the tumor-bearing mouse over the control mouse.

The data from the diabetic rat agree with earlier findings which have been rationalized in terms of the translocase/catalytic unit concept of glucose-6-phosphatase structure (3). Findings in the tumor-bearing mouse are inconsistent with this particular concept but may be rationalized in terms of the conformational-constraint substrate transport concept (4).

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(26) ENVIRONMENTAL RADON UPTAKE IN HUMANS: AN ENIGMA?

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It has been demonstrated that radon is stored in fat and that the rate of loss of radon increases by a factor of approximately two over a two-hour postprandial period (1,2). Apparently, increased blood flow to the gastrointestinal tract required for digestion results in delivery of radon to the lungs from which it is exhaled.

In a series of tests, a subject entered a radon chamber containing radon at concentrations from 7.4 to 22 kBq/m^3 (200 to 600 pCi/L) and remained there in a seated position for 60 min. In some instances the air in the chamber was filtered to remove radon progeny, both attached and unattached. During these exposures the subject wore a filtering mask. In other instances the radon and progeny were allowed to approach secular equilibrium and no mask was worn. Twenty minute postexposure Rn-222/Rn-222 progeny body content, as determined by library-least-squares analysis of whole body counting data, ranged from approximately 0.7 to 3.0 kBq (20 to 80 nCi) after the filtered atmosphere had been inhaled, and approximately 1.1 to 5.2 kBq (30 to 140 nCi) after the unfiltered atmosphere had been inhaled. Regional whole body counting of Bi-214 (a radioactive decay product of Rn-222) gamma ray emissions showed that the greatest activities were in the nasal and thorax regions after the unfiltered atmosphere had been inhaled. Activity was greatest in the upper head (brain), and the second greatest activity was in the lower abdominal region, after filtered atmosphere had been inhaled.

Fifteen minute postexposure urine activities of radon progeny ranged from 0.5 to 16 Bq (14 to 420 pCi) after the filtered atmosphere had been inhaled, and from 0.6 to 37 Bq (16 to 1000 pCi) after the unfiltered atmosphere had been inhaled. Exposures under seemingly identical conditions did not yield reproducible urine data except that uptake was higher after unfiltered atmosphere had been inhaled. Apparently Rn-222 and Rn-222 progeny passed through the lungs to the blood and were filtered by the kidneys.

Body content of environmental Rn-222 progeny was measured in three free-living females who had been exposed to radon naturally present in their homes. Early morning, postprandial whole body counts under similar initial conditions determined their Rn-222 progeny body contents to be 0.67, 1.7 and 3.7 Bq (18, 45 and 100 nCi) for respective exposures to 0.41, 2.4 and 0.74 Bq/m^3 (11, 66 and 20 pCi/L) of radon.

Apparently Rn-222 uptake, storage and excretion is not a linear function of exposure but varies under seemingly identical conditions; both inter- and intra-subject variations are involved.

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(88) RELATIONSHIP BETWEEN ESSENTIAL TRACE ELEMENT NUTRITION AND SELF-REPORTED MOOD STATES

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The Profile of Mood States (POMS (1)), a standardized self-report measure of mood states, was administered weekly to 63 healthy adult women (aged 18-81 years) participating in six independent studies of trace element nutrition. Prior to each study, participants were determined to be free of psychopathology as indicated by the absence of elevated (>70) scaled scores on the Minnesota Multiphasic Personality Inventory (2). Each study lasted approximately six months and required 24 hour/day residency on a metabolic unit where diet and exercise were strictly controlled. Participants could not take part in more than one study. Anger (ANG), anxiety (ANX), confusion (CON), depression (DEP), fatigue (FAT) and vigor (VIG) subscale scores of the POMS, along with a derived measure, total mood disturbance (TMD), were related to dietary manipulation and to trace element concentrations in the blood.

Eleven menstruating women who were consuming only 4 mg Fe/day reported feeling significantly more DEP, FAT, and TMD, and marginally less VIG than when they were fed an Fe-adequate diet (15 mg/day) supplemented with 50 mg Fe/day. Compared to when they were supplemented with 2 mg Cu/day, 8 menstruating women consuming only 0.8 mg Cu/day reported greater CON, DEP and TMD, and marginally greater ANX and FAT. Compared to when they were fed 7 mg Zn/day, 5 postmenopausal women consuming a diet containing only 3 mg Zn/day reported decreased ANG and CON, which further decreased as the study continued. In a cross-over, double-blind factorial study of dietary Mg (100 & 300 mg/day) and Al (0 & 1000 mg/day), 12 postmenopausal women reported no significant effect of Mg on their mood, either alone or in interaction with Al. However, the presence of Al in the diet significantly increased reported VIG, and marginally decreased reported DEP and TMD. In a cross-over, double-blind factorial study with 13 postmenopausal women, the manipulation of dietary B (0.25 & 3.25 mg/day) and Mg (120 & 320 mg/day) showed a significant effect of Mg only, with greater Mg intake resulting in increased reports of ANG. Greater ANX was reported with the high Mg intake when dietary boron was high, but not when it was low. A cross-over, double-blind factorial study of dietary Mn (1 & 6 mg/day) and Ca (500 & 1200 mg/day) showed no significant effect of either element alone on the mood states reported by 14 menstruating women. A significant interaction affected VIG; it was lowest when women were fed the low Mn - high Ca diet.

The blood concentrations of several trace elements indicated numerous, but often inconsistent relationships, with mood states. Serum Fe was positively related to DEP and FAT in one study and to VIG in another. These relationships did not hold for other studies or when data analyzed across studies. Plasma Cu was negatively related to ANG, ANX, DEP and FAT when data from all studies were analyzed together; however, there were no statistically significant relationships when data from each study were analyzed individually. Plasma Zn was positively related to ANG, ANX, CON and TMD, and negatively related to VIG in one study, but these relationships were not found in any other study or across studies. Serum Mg was positively related to ANX, DEP, FAT and TMD, and negatively related to VIG in only one of the two Mg studies. Serum Ca was positively related to VIG in one study, but negatively related to VIG when data from all studies were analyzed together.

Dietary effects on mood states were evident in all six studies with higher intakes of Al, Cu and Fe, and lower intakes of Mg and Zn, associated with more positive mood states. However, possible seasonal effects on mood were poorly controlled in three of these studies. Correlations between mood states and the concentrations of Cu, Fe, Mg and Zn in the blood were also numerous. However, these effects were often inconsistent when different studies were compared, or data were analyzed across studies. The interaction between mineral elements and/or individual differences may be responsible for some of these inconsistencies. In conclusion, these data provide only weak support for the existence of a trace element-mood relationship. A more unambiguous test of this relationship will require studies which employ a cross-over, double-blind design, and which permits consideration of trace element interactions and differences in the characteristic mood states of individuals. A measure more sensitive to subtle fluctuations in mood states also may be required.

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(74)

TECTONICS AND METAMORPHISM IN THE SUPERIOR PROVINCE (WESTERN ONTARIO) AND THE TRANS-HUDSON OROGEN (MANITOBA/SASKATCHEWAN)

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Studies of Precambrian rocks in the Superior Province (SP) of western Ontario and Manitoba, and in the Trans-Hudson orogen (TH) of Manitoba and Saskatchewan reveal these terranes to be quite different. Both terranes include greenstone belts, dominated by low-grade metamorphosed sediments and volcanics, and sedimentary-plutonic belts, dominated by tonalites and psammites that have been metamorphosed to the upper-amphibolite and granulite facies (1,2,3).

In the Archean Superior Province, the greenstone subprovinces are long linear belts (up to 2000 km long) separated by sedimentary-plutonic subprovinces. Petrology, deformation and metamorphism are nearly uniform over large areas. Many high-grade (granulite facies) rocks are found, characterized by distinct gravity and magnetic anomalies (1,3,4).

In the Proterozoic Trans Hudson Orogen (a.k.a. Churchill Province), there has been a great deal of deformation. Nappes and well-developed shear zones are common. With few exceptions, petrologic terranes larger than a few 10's of km in maximum dimension are hard to identify. Granulite facies rocks are rare, and when present appear to be windows to the older basement (2,5).

Despite the variation in degree of deformation and metamorphism, it appears that the TH rocks were originally very similar to the SP rocks. Recent data suggest that they may be the remobilized remnants of Archean rocks equivalent to those found in Ontario. If so, the linear belts of the Superior Province once extended nearly 4000 km in an east west direction. These belts, which are interpreted to represent accreted island arcs and microcontinents, suggest that an already large continent had developed in North America prior to 3.2bya.

Studies of metamorphism in the SP English River Subprovince (ERSP) show that the granulite facies metamorphism, preserved in the center of the province, occurred at 6kbar and 750°C. Trend-surface analysis, using garnet-biotite temperatures, reveals a "thermal anticline" with temperatures of 600 °C at the north and south borders of the ERSP. A magmatic heat source must have been present (5,6). In the case of the ERSP, that source was probably the tonalitic magmas which have, until recently, been interpreted as anatectites. Major element chemistry suggests that there can be no genetic relationship between the "anatectites" and potential "restites", which further supports this theory (7). More geochemical studies, especially one involving trace elements and isotopes are needed.

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(60) STUDIES ON THE RELATIONSHIP BETWEEN NICKEL AND VITAMIN B_{12} IN THE RAT

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Evidence exists suggesting that nickel (Ni) is an essential nutrient for higher animals. However, conclusive demonstration of a biochemical function for Ni has been elusive. Findings reported by Nielsen (1) suggested that Ni has a biological role closely related to vitamin B_{12} (vit. B_{12}) metabolism. The following experiment was performed to confirm that relationship.

Male weanling Sprague-Dawley rats were assigned to groups of six in a three-way 2x2x2 factorially arranged experiment. Supplemented to the basal diet, based on acid-washed ground corn and skim milk, were Ni (as $NiCl_2$) at 0 and 1 µg/g, vit. B_{12} at 0 and 5 ng/g, and either glycine (Gly) at 10 g/kg diet or cystine (Cys) at 12 g/kg diet. The rats were fed ad lib their respective diets for seven weeks, fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. Liver methionine synthase (EC 2.1.1.13) activity (MSy) was determined by the method of Sauer (2) and methylmalonyl mutase (EC 5.4.99.2) activity (MMM) was determined by the method of Kolhouse and Allen (3). Glucose was determined by a described procedure (4).

Table 1. Effects of Nickel, Vitamin B_{12} and Their Interaction on Body Wt., Spleen Wt./Body Wt. Ratio, Plasma Glucose, and Liver Methylmalonyl Mutase and Methionine Synthase

Treatment			Body	$\frac{\text{Spleen Wt.}}{\text{Body Wt.}} \times 100$	Glucose	Methylmalonyl Mutase	Methionine Synthase
Ni	B_{12}	AA	Wt.				
µg/g	ng/g	g/kg	g		mg/100 ml	µmoles/min/mg protein	nmoles/min/mg protein
0	0	12 Cys	229	0.251	79	0.265	0.629
0	5	12 Cys	226	0.217	87	0.324	0.579
1	0	12 Cys	232	0.223	89	0.157	0.524
1	5	12 Cys	261	0.228	95	0.385	0.597
0	0	10 Gly	190	0.221	78	0.232	0.561
0	5	10 Gly	186	0.205	82	0.228	0.542
1	0	10 Gly	203	0.204	93	0.237	0.530
1	5	10 Gly	211	0.202	99	0.282	0.524
				Analysis of Variance - P Values			
Amino Acid			0.0001	0.0001	NS	NS	.07
Nickel			0.02	0.06	0.0002	NS	NS
AA x Ni			NS	NS	NS	NS	NS
Vit B_{12}			NS	0.02	0.05	0.02	NS
AA x B_{12}			NS	NS	NS	0.07	NS
Ni x B_{12}			NS	0.01	NS	0.10	NS
AA x Ni x B_{12}			NS	NS	NS	NS	NS

Regardless of amino acid supplementation, Ni-deprived rats exhibited depressed growth. Cys supplementation enhanced growth. There was a tendency for depressed growth when Ni-supplemented rats were deprived of vit. B_{12}; this tendency was not seen in Ni-deprived rats. The spleen wt/body wt ratio (SW/BW) was lower in Gly-supplemented than Cys-supplemented rats. Vit. B_{12} deprivation elevated the SW/BW; the elevation was more marked in Ni-deprived than Ni-supplemented rats. Both Ni and vit. B_{12} deprivation depressed plasma glucose. Vit. B_{12} deprivation depressed MMM activity; the effect tended to be more marked when the rats were supplemented with Cys and with Ni. MSy activity apparently was not affected by vit. B_{12} or Ni deprivation. However Cys supplementation tended to elevate MSy activity. The findings, especially the SW/BW ratio, support the concept that there is a relationship between Ni and vit. B_{12} in the rat. MMM and MSy are two enzymes that catalyze vit. B_{12}-dependent reactions. MMM catalyzes the reaction of L-methylmalonyl-CoA to succinyl-CoA which is in the pathway that allows the carbon atoms of methionine to enter the citric acid cycle. MSy catalyzes the reaction that regenerates methionine from homocysteine. The relationship between Ni and vit. B_{12} seems to affect the MMM reaction more than the MSy reaction. That is, vit. B_{12} deprivation seems to depress MMM activity more markedly in Ni-supplemented than Ni-deprived rats. This finding suggests that Ni has a function which, when vit. B_{12} status is low, causes a metabolic change which depresses the activity of MMM. Further studies still are needed to clearly define the relationship between Ni and vit. B_{12}.

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(61) ALTERATIONS OF PHYSIOLOGICAL AND BIOCHEMICAL BLOOD CELL INDICES BY COPPER DEFICIENCY

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Numerous physiological and biochemical alterations occur as a result of copper deficiency (CuD). However, many of the alterations, which are standard indices of copper status, occur in tissues unavailable for routine study in humans or lack sensitivity to discriminate changes in copper status as seen in clinical settings. Our objective was to find a sensitive index of copper status in blood, a tissue easily accessible from humans.

Weanling, male, Sprague-Dawley rats were fed diets containing <1 µg Cu/g (CuD) or >5 µg Cu/g (CuA) for at least four weeks. Classical signs of CuD were obtained; these include depressed liver Cu, 1.4 µg/g; plasma Cu, 0.01 µg/ml; ceruloplasmin, 0.3. Value for controls were 13.9, 0.62 and 257, respectively.

Rat blood requires immediate anticoagulation with isotonic (0.134 M) disodium EDTA to maintain sample integrity. Syringes were prepared with 0.030 ml EDTA per ml of whole blood to be collected. After 40 minutes at room temperature, hematological values were measured by a blood analyzer. In a second study, two copper-dependent enzymes, cytochrome c oxidase (CCO) EC 1.9.3.1) and superoxide dismutase (SOD) (EC 1.15.1.1), were measured in several blood cell fractions. All samples for enzyme analyses were maintained at ~4°C throughout all processing steps. Platelets, (Plt) mononucleated white blood cells (MNC) and red blood cells (RBC) were isolated from the whole blood, using Percoll (1). RBCs were resuspended at a concentration of 10 g Hgb per dl. Platelets were resuspended at a concentration of 2 x 10^9 per ml. In addition to whole blood, populations of MNCs were collected from thymus (T-MNC) and spleen (S-MNC) after mincing the organs in saline. Leukocytes from bone marrow (BM) were collected by snipping off both ends from femurs, then the marrow cavity was flushed out with saline. WBCs from all sources were resuspended at 40 x 10^6 per ml.

TABLE 1
Effect of Copper Deficiency on Blood Cell Indices and Enzymes (Means ± SD, N=6)

Experiment 1:

Diet	10^6WBC/ml	10^9RBC/ml	Hgb g/dl	Hct %	MCV fl	MCH	MCHC	RDW	10^9Plt/ml	MPV fl
CuA	7.9 ± 0.9	7.0 ± 0.1	14.2 ± 0.5	41.0 ± 1.1	58.3 ± 0.4	20.3 ± 0.3	34.7 ± 0.4	12.2 ± 0.5	1106 ± 96	5.5 ± 0.1
CuD	2.8 ± 0.5	3.6 ± 0.8	6.4 ± 1.5	19.8 ± 4.6	54.8 ± 1.1	17.8 ± 0.6	32.5 ± 0.7	20.6 ± 2.4	1478 ± 104	6.3 ± 0.3

Experiment 2: SOD values are in U/10^6 MNC or U/10^9 Plts. CCO values are in U/10^9 or U/10^{12} Plts.

Diet	T-MNC SOD	T-MNC CCO	S-MNC SOD	S-MNC CCO	BM SOD	BM CCO	RBC SOD	MNC CCO	Plt SOD	Plt CCO
CuA	10.6 ± 1.1	2.4 ± 0.7	7.5 ± 0.6	1.3 ± 0.2	15.3 ± 2.5	3.1 ± 0.7	164 ± 47	3.0 ± 0.7	6.8 ± 0.3	26.7 ± 18.0
CuD	8.1 ± 0.9	1.4 ± 0.3	3.1 ± 0.7	0.4 ± 0.1	6.2 ± 0.6	1.2 ± 0.3	58 ± 16	1.2 ± 0.4	2.8 ± 0.7	1.7 ± 0.6

All the measured indices (Table 1) were significantly altered by copper deficiency. White blood cell (WBC) counts, RBC counts, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were all significantly lower ($p < 0.01$) in CuD than CuA. Red cell distribution width (RDW), a measure of the heterogeneity of RBC sizes, was greatly increased because of the smaller RBCs entering the blood. Platelet counts and mean platelet volumes (MPV) were increased by CuD. This suggests greater turnover of the platelets, requiring release of more, young, larger platelets. The increase in the number and size of the platelets suggest enhanced aggregability and clotting response might be characteristic of copper deficiency. Both SOD and CCO activities in all tissues studied were significantly ($p < 0.01$) lower in CuD than in CuA. Enzyme activity in the MNCs from thymus and spleen correlated well with the activities of peripheral blood enzymes. Of the peripheral blood enzymes measured, only RBC SOD has been measured routinely in previous copper studies. Although all were significantly depressed in CuD, the most sensitive peripheral blood component enzyme was platelet CCO, which was reduced to 18% of normal.

The reductions in CCO and SOD indicate that energy metabolism and resistance to oxidative damage are impaired in copper deficiency. Oxidative damage is a risk factor for cardiovascular disease (2). Increased platelet volumes seen in CuD are also symptomatic of ischemic heart disease (3). Taken together, this suggests copper deficiency may be a risk factor for heart disease. This is of concern because, while the RDA for copper is 1.3-2.0 mg/day, more than 50% of the population consumes less than 1 mg/day (4). The use of platelet CCO as an index of copper status may prove useful in the clinical setting.

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(62) EFFECT OF TESTOSTERONE TREATMENT ON REPRODUCTIVE ORGAN GROWTH OF ZN-DEFICIENT MALE RATS

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Zinc deficiency (ZnD) in animals and humans causes a variety of abnormal physiological responses. Of major importance is the effect on sexual maturation in the male. Previous work has shown that zinc deficiency causes testicular damage and reduces sperm count (1). In addition, it retards the growth of accessory reproductive organs including the prostate and seminal vesicles (2). Whether these changes are manifested through reduced testosterone production is controversial. Some researchers have found low serum testosterone in ZnD rats and some have not (3-5). However, serum testosterone response to luteinizing hormone was less in ZnD rats than pair-fed controls (3,5). Millar, et al. (2) observed the effects of testosterone treatment on organ development in rats deprived of zinc for 35 days. Their results were inconclusive because there were no testosterone treated or food-restricted controls. The present experiment used a more complete design and determined the effects of testosterone treatment on the growth of the testes, epididymides and other accessory reproductive organs during the early stages of zinc deficiency in the immature rat.

Twenty Sprague-Dawley rats, 4 weeks of age, were divided into 2 groups of 10 rats each. One group was fed a ZnD diet (<1 mg Zn/kg diet). Because zinc deficiency depresses food intake, the control group was fed the zinc-adequate (50 mg Zn/kg diet) diet in daily amounts equal to that eaten by rats in the ZnD group (ZnPF). One-half of the rats in each dietary group received a daily subcutaneous injection of testosterone (1 mg/kg BW) in safflower oil. The remaining rats received injections of oil only. After 2 weeks on the regimens and 6 hours after the last dose of testosterone, each rat was anesthetized with sodium pentobarbital and blood and tissues were removed for analysis. Significant differences were determined by a 2-way analysis of variance. Data are expressed as the mean ± SEM.

Table 1. Effect of Zinc Deficiency and Testosterone (TT) on Whole Body and Tissue Weights and on Serum Zn and Testosterone Concentrations

Treatment		Body	Serum		Tissue Weights				
Diet	TT	Wt.	Zn	Testos.	Testes	Epidid.	Prostate	Sem.Ves.	Vas Def.
		g	µg/dl	ng/dl	mg/100 g BW				
ZnD	-	105 ± 6	39 ± 2	27 ± 14	938 ± 127	98 ± 11	46 ± 1	110 ± 12	30 ± 5
	+	96 ± 5	48 ± 4	1077 ± 78	658 ± 69	151 ± 13	90 ± 1	151 ± 16	24 ± 2
ZnPF	-	114 ± 5	137 ± 4	23 ± 12	1059 ± 112	124 ± 10	67 ± 1	84 ± 13	45 ± 4
	+	110 ± 3	125 ± 7	760 ± 41	859 ± 90	149 ± 7	90 ± 1	156 ± 16	32 ± 4
Effects		P Values							
Diet		<0.020	<0.001	<0.009	NS	NS	<0.068	<0.001	<0.011
TT		NS	NS	<0.001	<0.030	<0.002	<0.001	<0.001	<0.023
Diet x TT		NS	NS	<0.005	NS	NS	<0.071	NS	NS

Table 1 shows that ZnD rats had significantly lower body weights and serum zinc than the control group. In addition, the deficient rats had smaller prostate glands than the control groups. The vas deferens were smaller in the ZnD rats than in pair-fed rats. Others have shown that seminal vesicle weights were lower in ZnD rats than ad libitum-fed controls (2). However, in the present experiment where differences in food intake were accounted for, seminal vesicle weights were greater in ZnD rats than in controls. Testes and epididymides weights were not affected by ZnD even though there was a trend for epididymal weights to be lower in the ZnD group. Endogenous concentrations of serum testosterone were not affected by dietary treatment. However, 6 hours after testosterone injections serum testosterone was elevated and more so in the ZnD group than controls. This suggests that the ZnD rats had a greater uptake of the hormone or that the rate of degradation was less than the controls. In general, all tissues except testes and vas deferens responded to testosterone treatment with significantly higher weights. The weights of testes and vas deferens were lower in the testosterone-treated rats than in the non-treated controls.

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(27) TRIHALOMETHANE CONTROL USING GRANULAR ACTIVATED CARBON (GAC)

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Trihalomethanes (THM's) are organic compounds containing one carbon, one hydrogen and three halogen atoms. They are likely to be found in treated surface-water supplies after disinfection using chlorine. Trichloromethane (chloroform), bromodichloromethane, chlorodibromo methane, dichloroiodomethane and tribromomethane are examples. Of these, chloroform is the most common. Recent studies have shown that THM's are both carcinogenic and mutagenic and, therefore, their presence in drinking water is of great concern (1). The United States Environmental Protection Agency (EPA) has a regulation to limit the presence of THM's in drinking water to 0.1 ppm collectively and to 0.05 ppm individually. EPA has recently proposed lowering the collective limit to .05 ppm.

Alternately, the trihalomethane formation potential (THMFP) of water can be reduced by removing organics before applying chlorine or the THM's can be removed after their formation. In conventional water treatment, pretreatment, such as alum coagulation and lime softening, removes some organics of high molecular weight. However, pretreatment is less effective in removing low molecular weight compounds that lead to the formation of THM's. Therefore, to reduce the THMFP, other treatment processes must be explored. Granular activated carbon (GAC) adsorption has been found to be the most effective method of removing natural organic carbon, synthetic organic carbon, and disinfection by-products (both low and high molecular weight compounds). A combination of conventional pretreatment and GAC adsorption may provide a cost-effective, microbially safe method of treatment for reducing THM's in treated water. This paper presents the results of a preliminary investigation on the effectiveness of using GAC adsorption with conventional pretreatment to reduce THM's in treated water from the Red River.

Samples of raw water from the entry point of the Fargo water treatment plant were collected and passed through three different treatment sequences as shown in Figure 1. A fixed bed type GAC unit, consisting of 3 columns in series with a bed depth of 0.82 m in each column, was used. Lime doses of 180, 190, 200 and 220 mg/l and chlorine doses of 5, 6, 7, 8 and 9 mg/l were applied. Residual chlorine was measured by titration, and THM's were measured using a gas chromotograph with a glass column and an electron detector.

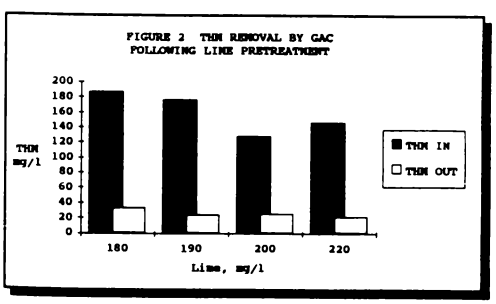
In treatment sequence one, chlorinated organics were removed in the carbon bed. A disadvantage of this approach is that during regeneration, these organics produce objectional by-products. In treatment sequence three, the GAC removes organics prior to chlorination. A disadvantage of this approach is that the filter runs may be shortened due to accumulation of biomass on the filter media. In treatment sequence two, some organic removal and chlorination are accomplished before the GAC columns. A possible disadvantage is that this could result in a loss of adsorption capacity of the carbon from the relatively high chlorine concentration in the water being filtered. Treatment sequence three consistantly produced THM concentrations less than .05 ppm. Typical results are shown in Figures 2 and 3. More elaborate studies are being conducted to investigate the ultimate capacity of GAC and to investigate the economic feasibility of GAC columns.

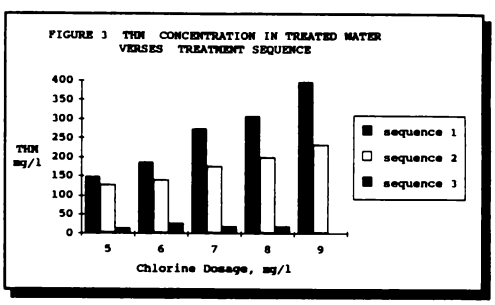
Raw water → chlorine → lime → flocc. → filtration → GAC → distribution

Raw water → lime → flocc. → filtration → chlorine → GAC → distribution

Raw water → lime → flocc. → filtration → GAC → chlorine → distribution

Fig. 1 Treatment Sequence 1, 2 and 3 (top to bottom)





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(28)

A PRELIMINARY ANALYSIS OF QUALITY OF URBAN RUNOFF FROM FARGO, NORTH DAKOTA

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Urban storm runoff can carry significant pollution loads that may impact the receiving water quality. During storm events urban runoff has been recognized as the single largest source of pollution. Heavy metals, organics and solids concentrations found in urban runoff are generally higher than secondary wastewater treatment plant effluents (1). Heavy metal concentrations found in the first flush of a storm frequently exceed values that are known to be toxic to water organisms (2). A recent nation-wide study by the U.S. Environmental Protection Agency (EPA), with assistance from the U.S. Geological Survey, detected 71 priority pollutants out of a possible 129 in urban runoff from different sites. This study also pointed out numerous exceedances of EPA water quality criteria and drinking water standards (3,4).

The Red River of the North is a major water source for Fargo, Moorhead, Grand Forks and East Grand Forks. For other communities in the Red River Valley the river is a precious natural resource to be preserved at all costs.

The potential for urban runoff from the Fargo-Moorhead area to adversely impact the water quality of the Red River is of concern due to the possible presence of toxic metals, pesticides and other contaminants in this discharge. A study to characterize the quality of this urban runoff will be beneficial to the regional agencies concerned with the environment, public health and the conservation of water resources. To conduct such a study the contaminants in the runoff must first be quantified and then their impact on receiving water quality, in terms of standards/criteria and toxicological effects, must be determined. This paper presents the results of a preliminary study on the quality of urban runoff from the city of Fargo.

Samples were collected in the spring of 1985 from a contributing drainage area of 855 hectares, approximately 12% of the total Fargo drainage area. Three rainstorms and two snowmelts were sampled from six sampling points in the drainage basin representing residential, commercial and industrial land uses. The samples were analyzed for pH, Ammonia, Nitrate, Phosphate, Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Volatile Solids (VS), Dissolved Solids (DS), Lead and Cadmium. The concentrations were compared to standards/criteria and to the river water quality (Tables 1 and 2). For all the parameters analyzed, the concentrations in the runoff exceeded the Standards/Criteria and the concentrations in the receiving water. Additional studies, including an analysis of pollutant concentrations upstream and downstream of Fargo during a series of rainstorms, should be conducted to quantify the impact of this runoff on the Red River.

TABLE 1
Comparison of Pollution Concentrations to Standards/Criteria (mg/l)

Parameter	Runoff	ND Standards	EPA Criteria
Nitrate	0.45 - 1.8	1.0	10[c]
Phosphate	0 - 2.0	0.1	---
COD	15 - 485	25[a]	---
TSS	42-1290	30[b]	---
TDS	106 -1945	---	250[c]
Cadmium	0 -.079	.01	---
pH	7.39 - 9.24	7.0-8.5	6.5-9.0[d]

TABLE 2
River Quality Compared to Average Runoff Quality (mg/l)

Parameter	April 22 River	April 24 Runoff	May 9 River	May 10 Runoff
pH	8.40	8.28	8.30	7.91
Ammonia	0.080	0.68	0.102	0.97
Nitrate	0.01	0.85	0.02	1.28
Phosphate	0.124	0.12	0.168	0.38
COD	3.2	249	---	378
TSS	62	421	---	847
TDS	---	422	345	517
Lead	0.001	0.14	---	---
Cadmium	0.0002	0.008	---	---

[a]BOD discharge standard. [b]Discharge standard. [c]For domestic water supply. [d]For protection of freshwater aquatic life.

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(2C)

THE EFFECT OF WASTEWATER OZONATION ON BIOLOGICAL ACTIVATED CARBON PROCESS

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The purpose of this study was to examine the effects of ozonation of wastewater on biological activated carbon (BAC) process. The wastewater used for this study was secondary treated effluent from Simplot potato processing plant located in Grand Forks, North Dakota.

Preozonation can convert larger, less biodegradable organic molecules into smaller, more biodegradable organics, for example, into acetic and oleic acids. The larger, less biodegradable, organic molecules will be captured and adsorbed in the carbon pores, where the bacteria will reactivate the loaded granular activated carbon (GAC). Although aerobic bacteria are necessary to obtain the benefit from BAC, so also is the adsoptive capacity of GAC for the dissolved organic materials which will serve as food for these bacteria. This means that the surface area and pore volume of the carbon should be high. Stated another way, it is important that the organic materials present in solution be adsorbable onto the activated carbon column, since the contact times of solutions with the carbon particles in the columns or beds are normally short (15 to 30 minutes). This does not necessarily give the bacteria sufficient time to degrade larger organic molecules, ideally to carbon dioxide and water. Therefore, it is important to be able to retain the dissolved organic molecules in the carbon columns so that the bacteria then will have sufficient time to degrade them, even though the actual contact times involved are rather short.

Two sets of biological columns were used in this study. Each set consisted of two BAC columns connected in series. Ozonation of wastewater reduced 36% of COD (Chemical Oxygen Demand) in the feed water. The BAC columns treating ozonated feed water had better performance than those fed with non-ozonated feed water. The second column of each two-column system also followed the same trend. Figures 1 and 2 show the the summary performance of this study. For columns treating ozonated feed the influent COD was 26.0 and 13.3 mg/l for BAC columns 1 and 2, respectively. For columns receiving non-ozonated feed COD the feed COD was 83.4 mg/l while the effluent COD was 45.1 and 27.6 mg/l, for BAC columns 3 and 4, respectively. The TOC (Total Organic Carbon) of ozonated feed water was 31 mg/l. Effluent TOC in BAC columns were 19 and 14 mg/l, for columns 1 and 2, respectively. The percentage of COD removal for the biological columns which were fed by non-ozonated wastewater was lower than those fed by ozonated wastewater. The BAC columns produced a better quality of effluent. However, columns receiving non-ozonated feed removed higher amount of COD than those receiving ozonated feed. For the BAC columns fed with ozonated wastewater the second column performed almost as well as the first column and removed 48.8% of COD from the first column's effluent. However, the second BAC column of the series fed by non-ozonated wastewater had a poorer performance compared to the first column. The COD removal rate was 44% and 39% for the first and second column, respectively. Ozonation of secondary treated potato processing wastewaters improved biological column performance as well as operation life of BAC columns. This is attributed to the reduction in COD and TOC due to ozonation.

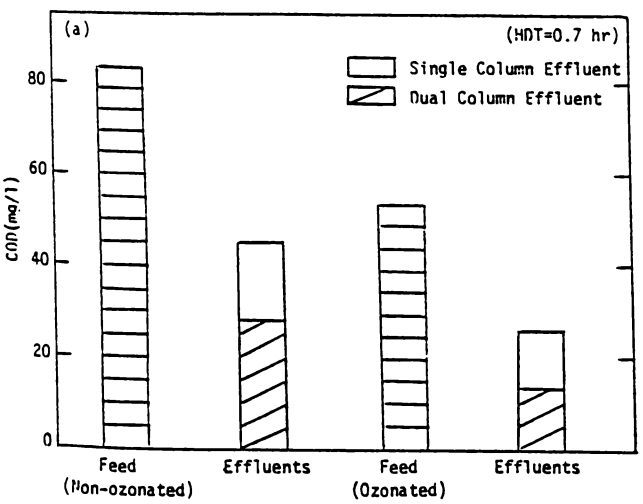

Figure 1

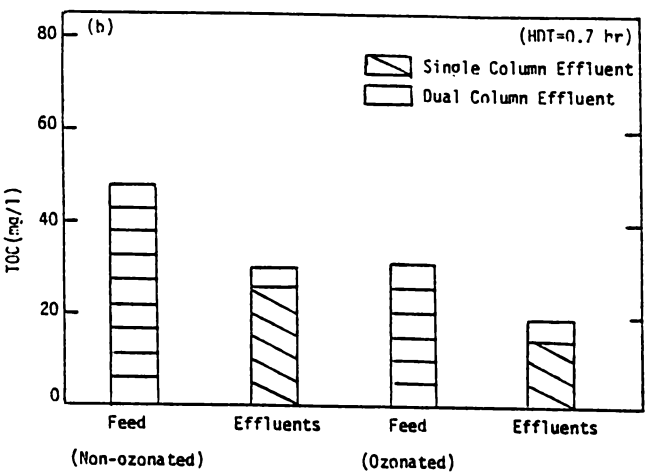

Figure 2

(105)

CHEMICAL AND PERFORMANCE RELATIONSHIPS IN ETHANOL AND NON-ETHANOL FUELS

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INTRODUCTION

The U.S. transportation fuel supply will be changing significantly in coming years because of environmental pressure to reduce carbon monoxide, ozone, hydrocarbon, and lead emissions. One of the goals of this study is to chemically characterize both ethanol and non-ethanol fuels. A database is being established with which fuel-related engine problems can be correlated. The program will provide (1) a survey of gasoline/gasohol chemical composition at the pump from ten sites throughout the U.S., (2) data on seasonal chemical changes in gasoline/gasohol supplies in "hot" and "cold" communities (in Texas and North Dakota, respectively), and (3) baseline data for tracking future changes in the chemical composition of gasoline/gasohol blends.

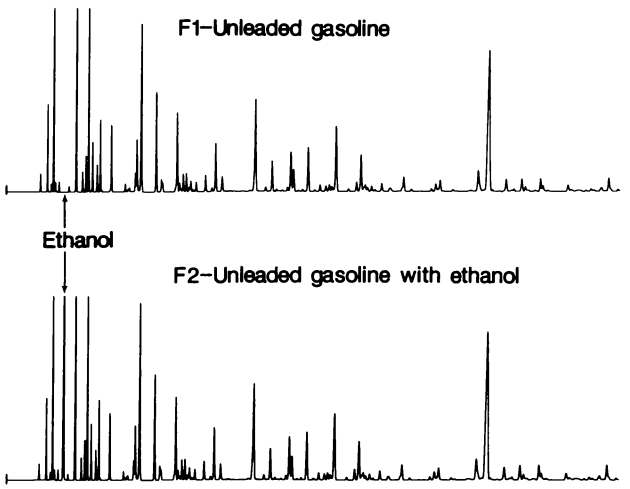

Figure 1. Gas chromatogram of unleaded gasoline compared to the same gasoline with ethanol.

ANALYTICAL WORK

Analytical techniques and results to be reported for gasoline characterization and additive identification are:

- Gas chromatography (GC) for gasoline characterization and quantitative ethanol analysis.
- Proton nuclear magnetic resonance (NMR) for unique semi-quantitative information about olefins present in the fuels. Olefins can cause fuel instability and form gums.

Work to date has included collecting samples from hot and cold regions (Texas and North Dakota) and from 10 geographic locations throughout the United States. A study of local motor fuels has demonstrated the need for "fingerprint" analyses. GC analysis of two alcohol-containing fuels from two stations indicated the presence of heavier compounds in one of the fuels that may result in the formation of engine carbon deposits. Compound identification is important. If the heavier material contains two- and three-ring aromatics, soot formation is more likely during combustion. Several GC columns have been tested to obtain optimum chromatographic separation of ethanol from other fuel components. Most recently, a Petrocol-DH column has provided the best separation of ethanol. Figure 1 shows the unleaded gasoline without ethanol (F1) and the same gasoline with ethanol (F2).

Analytical work to be reported at the presentation will include quantitation of ethanol, comparison of geographical regions, and detailed characterization of the gasoline samples by both GC and NMR.

(21)

THE MECHANISM OF REGULATION BY FRUCTOSE OF GLYCOGENESIS FROM GLUCOSE

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Glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase: EC 3.1.3.9) occupies a sensitive crossroad position in hepatic carbohydrate metabolism (1,2). An active metabolic cycling takes place between hepatic glucose and glucose-6-P (3). Regulation at this site is important in glycogenesis, glycogenolysis, gluconeogenesis, and glycolysis.

Studies by Katz et al. (4) have revealed that the presence of certain amino acids or gluconeogenic substrates are needed in addition to glucose for effective glycogen synthesis under near-physiological conditions. The mechanism of this phenomenon is unknown; however, it has been suggested that inhibition of hepatic glucose-6-phosphatase by some unidentified metabolic intermediate may be involved (4).

We examined 32 metabolic intermediates including gluconeogenic, glycolytic, tricarboxylic acid cycle, and pentose phosphate pathway intermediates, amino acids and related compounds searching for an inhibitor of glucose-6-phosphatase. The presence of such an inhibitor would explain the synergistic effects observed by Katz et al. (4). Studies were performed with intact and disrupted liver microsomal preparations from normal, fed rats. Fructose-6-P, ribose-5-P, phosphoenol pyruvate, glyceraldehyde-3-P, dihydroxyacetone-P, and fructose-1-P were shown to exhibit competitive inhibition. Values for Ki ranged from 2.5 to 75 mM with disrupted microsomal preparations and from 16 to 45 mM with intact microsomal preparations. Further kinetic analysis indicated minimal inhibition of glucose-6-phosphatase by these compounds at concentrations present in liver cells of rats under normal conditions.

Inhibitions of up to 70% were calculated for conditions involving elevated concentrations of fructose-1-P achieved in fructose fed or infused rats or in rat livers perfused with glucose and fructose (5,6,7). This inhibition of glucose-6-phosphatase favors an elevation in the concentration of glucose-6-P. This elevated glucose-6-P concentration in turn may favor the deposition of glycogen by two methods. Increased glucose-6-P concentration may i) activate glycogen synthase phosphatase promoting the conversion of glycogen synthase b to glycogen synthase a or ii) activate glycogen synthase a directly under conditions of diminished orthophosphate concentration, as seen in the presence of fructose treatment.

These effects of an increased concentration of glucose-6-P on glycogen metabolism as a result of fructose-1-P inhibition of glucose-6-phosphatase can explain the increase in glycogen deposition noted upon fructose administration. This inhibition of glucose-6-phosphatase by fructose-1-P could also explain the enhanced flux from glucose to lactate, the glucagon-insensitive hypoglycemia in aldolase B deficiency, the restoration of glycogenesis from glucose in the diabetic, and the restoration of a normal glucose tolerance curve in the glucokinase-deficient animal, all in response to fructose.

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(106) MICROWAVE ENGINEERING USING "MathCAD" SOFTWARE

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MathCAD (1,2) is a software tool for personal computers that should have a significant impact on the way microwave engineers do their work. This tool does computational algebra, linear algebra, calculus, and graphing of functions. In a previous paper (3) we showed some ways to use this package in the general area of electromagnetics. Microwave engineers will find MathCAD particularly helpful for generating radiation patterns for arrays or in studying the fields from dipole antennas. For transmission lines microwave engineers find MathCAD extremely helpful in the generation of curves giving microstrip transmission line characteristic impedance as a function of strip geometry and medium characteristics. Although computational tedium is eliminated, a careful statement of the problem is essential for obtaining useful results. Basic design studies, graphing, and technical report preparation are possible using this tool. MathCAD is an innovative package that is helpful in reducing the amount of programming required in order to profit from the capabilities of our personal computers, especially in those areas of interest to microwave engineers that make subtantial use of numerical methods.

Several examples have been developed for microwave education and engineering in the form of a series of "templates." Everything in these templates was produced by MathCAD: text, equations, numerical values, and plots. MathCAD sets up operations such as definite integrals, derivatives, and graphs using a very simple notation. Several suggestions for MathCAD programming are given in the templates themselves. One template shows how to plot a split function (Fig. 1) using MathCAD. A significant equation from microstrip circuit design (4) is used as an example. Equations and ranges are entered in a relatively natural way to produce plots or discrete values. A template can be reused for other cases by changing the original parameters.

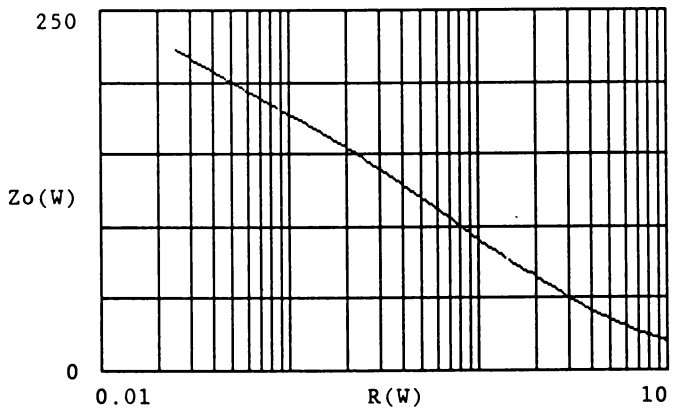

Fig. 1. Characteristic impedance as a function of R (ratio of width to height) for microstrip line over substrate with dielectric constant of 2.2.

Antenna templates give the opportunity to show the integral of a function of complex variables as well as the procedure used to obtain polar plots. Manual adjustment of polar plots is necessary to show true-size angles. These templates way be used to develop application notes and design tables as well as for preparing technical presentations, first drafts of technical reports, and material for final reports.

Control of curve resolution is a key factor in the use of MathCAD. Since plots vary in degree of complexity, the more rapidly varying functions will require more computational time to achieve satisfactory precision. The inexperienced user may overlook some important details if he or she doesn't study a given function carefully, plotting it, for example, at a high enough resolution to see those details. Any software product has its limitations. The user soon learns to make allowances for these and take advantage of the tasks that the product can perform successfully.

In this paper we show how a microwave engineer might begin to use MathCAD. Although computational tedium is reduced, a careful statement of the problem and a logical analysis is essential for obtaining useful results. This approach should soon become common in microwave engineering and education.

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(22) PURIFICATION OF ESTERASE-m FROM DIABROTICA VIRGIFERA VIRGIFERA

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Corn rootworms (Diabrotica spp.) are recognized as the most serious insect pests of corn in the United States. Crop rotation and insecticides have been used to reduce the yearly economic damage done by the rootworm, but unfortunately these have shortcomings and the continued use of insecticides is environmentally unsafe. This research is concerned with the potential for regulating the rootworm population by the manipulation of physiological or molecular processes that are involved during mating and reproduction.

Esterases are ubiquitous and highly polymorphic enzymatic proteins in insects. The esterases associated with reproduction are involved in sperm transfer, sperm activation, inhibition of female remating or other aspects of reproduction (1). A polymorphic, sex-limited esterase locus, Est-m, was discovered in the western corn rootworm Diabrotica virgifera virgifera (2). Three putative alleles of the Est-m system have been designated $Est\text{-}m^1$, $Est\text{-}m^2$ and $Est\text{-}m^{null}$. The Est-m enzymes have been localized in the male accessory gland (3). The accessory gland secretions are passed to the female during copulation and function in mating or post-mating processes. A procedure is presented which makes use of the high electroendoosmosis of the low melting point agarose, SeaPlaque (FMC BioProducts), to obtain a highly purified enzyme suitable for use in further studies on its structure and function in reproduction.

The male accessory glands were dissected from the corn rootworm. Groups of accessory glands were homogenized in 0.5 ml of 0.15 M Tris buffer, pH 7.42 at 4°C, using a variable speed homogenizer equipped with a teflon pestle. The homogenate was centrifuged at 15,000 X g for 10 min at 4°C in a Eppendorf microfuge and the supernatant was pipetted off. The pellet was rehomogenized in the Tris buffer, recentrifuged and the supernatants containing Est-m were combined. Proteins were precipitated from the combined supernatants by ammonium sulfate (80% saturation). The suspension was centrifuged at 27,000 X g for 20 min. After centrifugation, the sediment was suspended in 0.15 M Tris buffer, pH 7.42 and dialyzed overnight against the Tris buffer. The dialysate was centrifuged at 100,000 X g for 2 h at 8°C and the supernatant was decanted. The pellet was homogenized in a sintered glass homogenizer and recentrifuged at 100,000 X g for 2 h at 4°C. Proteins were precipitated a second time by ammonium sulfate (80% saturation). The sediment was suspended in a minimum amount of 0.15 M Tris buffer, pH 7.42, and dialyzed overnight against the same buffer. Electrophoresis of aliquots of the enzyme solution was performed in a horizontal electrophoresis unit with 2% SeaPlaque agarose dissolved in 0.3 M Tris-glycine buffer, pH 8.3, at 4°C for 5 h at 200 V. A portion of the gel was cut out and stained for esterase activity. The comparable areas containing esterase activity in the unstained portion of the gel were cut out and homogenized in 8 ml of the electrophoresis buffer with a sintered glass pestle. The slurry was allowed to shake overnight at 4°C. The tube was centrifuged for 15 min at 15,000 X g at 4°C and the supernatant was gently pipetted off. The supernatant was concentrated and dialyzed against 0.15 M Tris buffer, pH 7.42 in a S&S collodion ultrafiltration apparatus to a volume of 0.5 ml and stored at -20°C. The agarose pellet was resuspended in an additional 5 ml of 0.3 M Tris-glycine buffer, pH 8.3 and the homogenizing and centrifugation steps were repeated. The first concentrated fraction was added to the second fraction and concentration and dialysis were continued until the final volume was about 0.1 ml.

Electrophoresis in a 0.1% SDS gel revealed two esterase bands. An estimated molecular weight of 5.75×10^4 was determined for the top band and 4.68×10^4 for the second band. Both esterase bands reacted with a monoclonal antibody to Est-m. Isoelectric focusing of the purified Est-m in a pH gradient from pH 3-10 revealed two esterase bands, one at a pH of 9.3 and the second at a pH of 9.6. Since the source of esterase for these studies are pooled insect accessory glands, it is expected to obtain more than one Est-m allele product.

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(89) INHIBITION OF CARDIOVASCULAR EFFECTS OF COPPER DEFICIENCY WITH ANTIOXIDANTS

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Dietary copper (Cu) deficiency produces effects which include structural defects in heart and blood vessels, cardiac enlargement and anemia (1). That O_2-derived radicals may be involved in this damage is suggested by the reduction of endogenous antioxidant enzyme activity and the enhancement of tissue peroxidation occurring in Cu deficiency (2,3). We wished to further examine this relationship by determining whether feeding of exogenous antioxidants could inhibit some of the cardiovascular effects of Cu deficiency, as suggested by previous work (4).

Male, weanling, Sprague-Dawley rats were fed a purified diet (5) which was either deficient in Cu (CuD) or supplemented with 5 µg/g Cu (CuS). In Experiment 1, half of each group received deionized water and the other half deionized water containing 4.75% dimethyl sulfoxide (DMSO), a hydroxyl radical scavenger (6). In Experiment 2, all rats drank deionized water, but half of each group received a diet containing 0.02% t-butylhydroquinone (TBHQ), an inhibitor of lipid peroxidation (7). After 5 weeks, the rats were anesthetized with Na pentobarbital (65 µg/kg, i.p.) or ether, blood was drawn for hematocrit (Hct) and hemoglobin (Hb) determination and hearts and livers were collected for wet and dry weight determination and Cu analysis.

Our results indicate that, while DMSO (Table 1) had no effect on the reduced Cu status of Cu deficient rats (i.e., liver Cu), it significantly inhibited cardiac enlargement determined by wet heart weight (WHW), heart weight to body weight ratio (WHW/BW) or dry heart weight (DHW). DMSO also inhibited the cardiac edema formation (% H_2O) and anemia (Hct) of Cu deficiency. Similarly, TBHQ (Table 2) had no effect on the change in Cu status caused by Cu deficiency, but inhibited the cardiac enlargement, cardiac edema and anemia (Hct and Hb) caused by Cu deficiency.

Table 1. Experiment 1: Effect of DMSO on cardiovascular effects of Cu deficiency

Diet-treatment	Liver Cu µg/g	BW g	WHW g	WHW/BW mg/g	DHW g	%H_2O	Hct %	Hb g/dl
CuS	11.5±1.0	267±14	1.02±0.10	3.8±0.3	0.22±0.02	78.1±0.6	45±4	
CuS + DMSO	11.2±1.7	226±13	0.88±0.07	3.9±0.3	0.19±0.02	78.0±0.5	49±4	
CuD	0.6±0.1	184±16	1.88±0.16	10.3±1.2	0.39±0.03	79.3±0.6	14±4	
CuD + DMSO	0.3±0.2	169±37	1.19±0.25	7.2±1.8	0.26±0.05	77.7±0.9	25±5	
			Analysis of Variance (p values)					
Cu	.0001	.0001	.0001	.0001	.0001	.05	.0001	
DMSO	NS	.0009	.0001	.0006	.0001	.0004	.0001	
Cu x DMSO	NS	NS	.0001	.0003	.0003	.002	.01	

Table 2. Experiment 2: Effect of TBHQ on cardiovascular effects of Cu deficiency

Diet-treatment	Liver Cu	BW	WHW	WHW/BW	DHW	%H_2O	Hct	Hb
CuS	10.6±0.4	297±23	1.05±0.07	3.6±0.1	0.24±0.02	77.5±0.5	43±1	13.8±0.6
CuS + TBHQ	10.3±1.1	296±20	1.06±0.08	3.6±0.2	0.24±0.02	77.2±0.5	43+1	13.6±0.6
CuD	0.8±0.2	225±18	1.70±0.31	7.6±1.5	0.36±0.06	78.7±0.6	16±5	4.8±1.8
CuD + TBHQ	2.0±2.2	244±33	1.29±0.30	5.3±0.9	0.29±0.06	77.4±0.9	27±9	8.5±3.1
			Analysis of Variance (p values)					
Cu	.0001	.0001	.0001	.0001	.0001	.0004	.0001	.0001
TBHQ	NS	NS	.007	.0002	.02	.0004	.005	.007
Cu x TBHQ	NS	NS	.006	.0003	.009	.03	.004	.002

The results demonstrate that two different antioxidants can inhibit cardiovascular effects of dietary Cu deficiency. This further implicates free radicals as a source of damage in Cu deficiency. Because antioxidants prevent effects of Cu deficiency, our findings suggest caution in the use of antioxidants in diets of animals being studied for Cu deficiency. Finally, because TBHQ is used as a food additive, in addition to protecting food against oxidation, it may also protect the consumer against the deleterious effects of a low Cu diet.

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(23) POSSIBLE ROLE OF RNase IN THE PATHOGENICITY OF HAEMOPHILUS SOMNUS

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Haemophilus somnus is a gram-negative, pleomorphic coccobacillus. It is a bovine pathogen responsible for several diverse disease syndromes including thromboembolic meningoencephalitis, respiratory disease, septicemia, abortion, and arthritis. H. somnus has the ability to resist, phagocytic destruction and is, therefore, ineffectively attacked by the immune system. The organism has complex growth requirements and can be best grown in vitro at 37°C in brain heart infusion medium supplemented with 0.5% yeast extract and 5% calf serum (pH 7.2) (BHI-S) or in 7- to 8-day old embryonated chicken eggs. The latter is best for long-term storage of the organism. An atmosphere containing 10% CO_2 will enhance in vitro growth. H. somnus strains exhibit considerable biochemical and serological heterogeneity.

We have found that H. somnus 8025, a virulent strain supplied by Dr. James Roth, Iowa State University, secretes ribonuclease when grown in vitro. This observation led us to examine the possible role which secreted ribonuclease might have in the pathogenicity, virulence, and immunosuppressive properties of H. somnus.

We received H. somnus 8025 as a frozen culture suspended in embryonated chicken egg yolk. The culture was thawed, inoculated into BHI-S, and incubated at 37°C in 10% CO_2 for 20 hours. A 20-hour culture was used to create an inoculum source (5ml inoculum/100 ml of fresh medium) for experimental cultures. After 20 hours of growth in fresh medium, cells were removed by centrifugation at 10,000 x g for 20 min at 5°C. The cell-free supernatant was assayed for ribonuclease activity using a novel RNase plate assay system developed in our laboratory. Composition of the plating medium was 10 g Bacto-Agar (Difco), 6 g Trizma-9, 0.3 g RNA, 1.0 ug $CaCl_2$, and 10 g NaCl (all Sigma Chemicals). These ingredients were suspended in 1 l of distilled, deionized water and boiled until the RNA and agar were completely dissolved. After the solution had cooled to 50°C, 3.0 ml of Toluidine Blue (10% stock solution) was added before pouring into plastic petri plates. Control plates were made in the same way except no RNA was added or, in some plates, DNA was added. Wells (4mm) were cut into the agar plates after they were solidified and culture supernatant or purified RNase or DNase was added.

Table 1. Activity Of Ribonuclease In Plates After 22 Hours At 37°C

Volume of Supernatant	Concentration of Pure RNase (mg/ml)	Diameter of Reaction Zone RNA	Diameter of Reaction Zone DNA
--	1.0 (30 ul)	40 mm	None
--	0.1 (30 ul)	34 mm	None
--	0.01 (30 ul)	25 mm	None
--	0.001 (30 ul)	20 mm	None
--	0.0001 (30 ul)	None	None
30 ul	--	15 mm	None
25 ul	--	13 mm	None
20 ul	--	10 mm	None
Uninoculated medium control		None	None

The data indicate that RNase is present in culture supernatant of H. somnus. Chiang et al. (1) reported that some nucleic acid components, possibly associated with the membrane of H. somnus, could inhibit the phagocytic activity of bovine neutrophils. Our observation indicates a possible mechanism by which this phenomenon might occur. We are currently developing nucleotide- and endotoxin-coated latex beads (i.e. synthetic bacteria) to further evaluate the findings of Chiang et al. (1). If we conclusively show that the presence of surface nucleotides interferes with phagocytic activity, we will pursue novel strategies for new vaccine development against H. somnus.

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(63) EFFECTS OF COPPER, SULFUR AMINO ACIDS, SEX AND THEIR INTERACTION ON THE TRACE ELEMENT CONTENT OF LIVER AND KIDNEY IN RATS

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Findings from our laboratory (1) demonstrated that sulfur amino acid (SAA) nutriture can markedly affect the nature and severity of the signs of copper (Cu) deficiency. Changes in dietary SAA generally affected the signs of Cu deficiency more markedly in males than females. We have also found that Cu and cystine (CYS) and methionine (MET) significantly interact to affect the concentration of Cu in the liver (2). Therefore, a study was performed to ascertain whether changes in dietary SAA would affect the changes in organ trace element concentrations in the response to Cu deficiency by both male and female rats.

Male and female weanling Sprague-Dawley rats were assigned to groups of six in two fully crossed, two-way 2x7 factorially arranged experiments, and were fed a diet based on acid-washed ground corn and casein. The dietary variables were supplements of 0 and 6 μg Cu/g diet and amino acid supplements (in g/kg diet), arginine (ARG) 16; MET, 2.0, 6.3, and 12.6; CYS, 6.3 and 12.6; and CYS, 6.3 plus MET, 6.3. Environmental conditions have been described (1). The rats were fed their respective diets and deionized water for 6 weeks, then fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination. The liver and one kidney were removed, blotted dry, weighed, placed in a plastic bag and frozen for later analysis. Our usual methods were used to prepare the organs for trace element analysis by inductively coupled argon plasma spectrometry. The concentrations of selected elements are presented in Table 1.

Table 1. Effects of Cu, SAA and Their Interaction on Selected Elements in the Livers and Kidneys of Male and Female Rats

TREATMENT Cu μg/g	SAA g/Kg	KIDNEY (DRY) μg Element/g MALE Ca	MALE Cu	MALE Fe	FEMALE Ca	FEMALE Cu	FEMALE Fe	LIVER (DRY) μg Element/g MALE Ca	MALE Cu	MALE Fe	FEMALE Ca	FEMALE Cu	FEMALE Fe
0	16 ARG	204	5.4	81	387	11.9	204	131	1.8	1010	142	5.5	1301
6	16 ARG	171	9.4	97	381	18.7	259	137	14.4	546	165	17.1	1146
0	2.0 MET	170	3.8	68	454	8.4	156	123	1.8	855	133	1.7	1136
6	2.0 MET	177	9.0	90	369	17.4	240	133	13.8	452	149	16.8	1056
0	6.3 MET	217	4.2	81	390	7.9	145	128	1.6	1103	134	1.6	1309
6	6.3 MET	167	8.4	108	360	16.5	220	132	11.8	604	156	15.7	915
0	12.6 MET	206	3.6	68	355	8.3	169	120	1.3	1327	151	1.2	1603
6	12.6 MET	132	10.5	105	299	18.8	256	144	13.9	579	152	17.2	1152
0	6.3 MET/6.3CYS	199	3.4	62	445	8.3	147	123	0.9	1005	126	1.4	1631
6	6.3 MET/6.3CYS	160	9.5	92	346	22.5	274	136	13.7	616	144	16.5	1524
0	6.3 CYS	215	3.6	61	390	11.2	220	130	1.1	897	146	4.2	1726
6	6.3 CYS	182	8.7	96	333	19.1	273	139	14.6	538	144	15.9	1484
0	12.6 CYS	458	3.6	66	692	7.6	155	127	1.1	1118	132	2.5	1467
6	12.6 CYS	182	10.4	101	296	22.4	271	144	15.3	437	151	17.1	1873
ANALYSIS OF VARIANCE- P VALUES													
Cu		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	.0.0001	0.0001	0.0001	0.0003	0.0001	0.006
SAA		0.0001	NS	0.01	0.004	0.005	0.0001	NS	NS	0.0001	NS	0.01	0.0001
Cu x SAA		0.001	0.04	NS	0.0004	0.002	0.02	NS	NS	0.007	NS	0.02	0.008

The concentration of the trace elements presented were higher in both liver and kidney of females than males. Cu deficiency elevated the concentration of iron (Fe) in the liver; the most marked elevation for both males and females occurred when 12.6 MET was fed. In males the least marked elevation occurred in rats fed 6.3 CYS. Cu deficiency depressed the concentration of Fe in the liver of female rats fed 12.6 CYS. Liver Cu was depressed by Cu deficiency in both male and female rats; however, in the female rats the decrease was less marked when the diet was low in MET (16 ARG, 6.3 CYS). Regardless of sex and dietary SAA, liver calcium (Ca) was depressed by Cu deficiency. On the other hand, both male and female Cu deficient rats exhibited elevated kidney Ca concentrations. The most marked elevation occurred when rats were fed 12.6 CYS. Cu deficiency depressed the concentration of both Cu and Fe in the kidney of both the male and female rats. Dietary SAA modified the effect in females. Like liver Cu, kidney Cu and Fe were depressed least by Cu deficiency in females fed 16 ARG and 6.3 CYS. The results show that Cu, SAA, sex and their interaction affect the concentration of trace elements in the liver and kidney of rats. Moreover, the findings confirm that SAA nutriture affects the nature and severity of the signs of Cu deficiency.

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(90) SOMATOTYPE AND SEX BIAS IN THE USE OF BODY MASS INDEX TO CLASSIFY OVERWEIGHT AND OBESITY

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Measurement of human body composition is an important factor in assessing nutritional status and in estimating fat loss during weight reduction. Although many diverse methods of measurement are available (1), they suffer limitations that restrict their use outside the laboratory. Traditional laboratory methods are expensive and require skilled technicians, and field methods can be unreliable. An alternate approach is the use of an index of body weight relative to standing height (2). Body mass index, or BMI, is calculated as the ratio of body weight in kilograms to the square of standing height in meters to provide a practical and convenient approximation of body composition. We demonstrated earlier that BMI per se was a relatively insensitive indicator of body composition in a large sample of healthy men and women (3). However, because it is known that average height and weight are different among men and women and that these differences are related to body structure or somatotype (4), we sought to determine whether the sensitivity of BMI to predict obesity and overweight could be improved by including information about somatotype and sex.

A sample of 337 women and 285 men aged 18-73 years underwent determinations of percent body fat using hydrodensitometry (1) and assessment of somatotype using standard anthropometric methods (5). Individuals were classified as normal, overweight, or obese using the criteria of Bray (2) which defines overweight as a BMI exceeding the upper limit of an age-adjusted normal by 5 units and obese as exceeding the upper limit of normal by more than 5 units. Overweight is determined as percent body fat, by densitometry, between 30 to 35% in women and between 25 to 30% in men. Obese is determined as percent body fat, by densitometry, in excess of 35% in women and 30% in men.

Table 1
Percentage Distribution of Overweight and Obese by Sex and Somatotype

		Overweight		Obese	
		BMI	%Body Fat	BMI	%Body Fat
Total	women	20	18	4	16
	men	44	9	9	7
Endomorphs	women	28	28	8	29
	men	45	30	18	22
Mesomorphs	women	34	14	0	9
	men	55	9	13	6
Ectomorphs	women	0	12	0	0
	men	0	0	0	0

Table 1 shows the frequency distribution of the sample for overweight and obese by BMI and percent body fat. BMI and percent body fat predicted about the same percentage of overweight women (20% and 18% respectively) and about the same percentage of obese men (9% and 7%, respectively). However, the BMI underpredicted as obese 12% of the women and over predicted as overweight 35% of the men.

Endomorphs are the rotund somatotype and have the highest average body weight of the three types. The BMI and percent body fat predicted about the same percentage of overweight women and obese men. But the BMI over predicted as overweight 15% of endomorphic men and under predicted as obese 21% of endomorphic females. Mesomorphy is the somatotype of relative muscular development. Mesomorphs are the shortest of the three types. The BMI under predicted as obese 9% of mesomorphic women and over predicted as obese 7% of mesomorphic men. The BMI over predicted overweight in both mesomorphic women (by 20%) and men (by 46%). Ectomorphy is the linear somatotype. Ectomorphs are the tallest and lightest of the three types. Neither the BMI nor percent body fat predicted obesity in ectomorphic women or men, nor overweight in ectomorphic men. The BMI did under predict overweight in 12% of ectomorphic females.

Between the sexes, the BMI under predicted obese in the shorter and lighter women and over predicted overweight in the taller and heavier men. Among the somatotypes, the BMI did not predict overweight in ectomorphic women and obese in endomophic and mesomorphic women, thus rendering a false normal prediction. The BMI over predicted overweight in mesomorphic women and endomorphic and mesomorphic men and obese in mesomorphic men. If the BMI is to be used to predict overweight and obesity in individuals, a correction will be needed for sex and somatotype of the individual.

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(64) DISTRIBUTION OF COPPER AND MANGANESE IN HUMAN BLOOD AND BLOOD FRACTIONS

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Recommendations for safe and adequate dietary intakes of copper and manganese have been made. Copper and possibly manganese are essential for human growth and body development, but are found in very small concentrations in most biological systems. They are components of proteins and enzymes associated with cellular oxidation and connective tissue metabolism. As a result there is a need for accurate and precise assessment of these trace elements found in low concentrations in human tissue, including blood components (1). These trace amounts are often difficult to analyze by ordinary or routine laboratory methods. Here, we describe and evaluate a method for the isolation of white blood cells from whole blood and for sensitive determinations of trace amounts of copper and manganese in human blood fractions.

We measured the copper and manganese content in blood and blood fractions by ICAPES (inductively coupled argon plasma emission spectroscopy) and by Zeeman EAAS (electrothermal atomic absorption spectroscopy). Other methods, which include discrete nebulization of 50-100 µl aliquots of undiluted blood sample into the flame or furnace, possess matrix interferences caused by the presence of large concentrations of sodium chloride and protein. Matrix interferences may be minimized by the use of Zeeman effect background correction, STPF (stabilized temperature platform furnace), magnesium nitrate as a matrix modifier, integrated absorbance signals and pyrolytically coated graphite furnace tubes with L'vov platforms.

White blood cells, platelets, erythrocytes and plasma were separated from whole blood by use of a discontinuous gradient called "Percoll" (colloidal polyvinylpyrrolidone-coated silica) (2). Cell counts of other blood samples were made using the Coulter Counter, then they were weighed to the nearest one-tenth of a milligram in conical teflon tubes. A dri-block tube heater with drilled porcelain blocks was used to digest the samples with ultrapure nitric acid and 30% hydrogen peroxide. The samples were adjusted to volume with 2% nitric acid prior to analysis. This method gave manganese values of 10.3 ± 0.6 µg/g and 10.1 ± 0.5 µg/g for National Bureau of Standards (NBS) reference materials (SRM 1577a and 1567a); the values compare well with the certified values of 9.9 ± 0.8 and 9.4 ± 0.9 µg/g, respectively. Copper values also compared favorably with the NBS certified values, 155.8 ± 3.6 and 2.06 ± 0.20 µg/g vs 158 ± 7 and 2.1 ± 0.2 µg/g, respectively. Recoveries averaged 105 ± 7%. Table 1 summarizes copper and manganese determinations in blood and blood fractions.

Table 1
Concentrations of Copper and Manganese in Pooled Human Blood and Blood Fractions

	Whole Blood	Plasma	WBC & PLT	RBC
Cu				
Mean (µg/ml)	0.870	0.847	0.013	0.918
S.D.	± 0.035	± 0.036	± 0.002	± 0.087
% of W.B.		52%	2%	46%
Reference Values (3)	0.64 - 1.28	0.61 - 1.41	-	0.75 - 1.31
Mn				
Mean (ng/ml)	10.89	0.885	3.304	16.61
S.D.	± 0.61	± 0.092	± 1.55	± 1.23
% of W.B.		5%	29%	66%
Reference Values (3)	8.4 - 12.2	0.8 - 1.5	-	11.5 - 25.0

Mean Hct = 44.5% N = 48-70

Copper was almost evenly divided between plasma and cellular components, while only 5% of the manganese was in the plasma. Nearly 30% of the manganese was found in the platelet and white blood cell fractions.

Using the sensitive techniques of ICAPES and Zeeman EAAS we were able to detect the low amounts of copper and manganese in the small volume of platelets and white blood cells in human blood, which contribute significantly to the total blood concentrations. Our findings indicate that current state-of-the-art analytical methods may yield concentration values for these elements lower than some previously reported because of less contamination, greater specificity and enhanced sensitivity.

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(93) PRODUCTION AND DECOMPOSITION OF NATIVE PRAIRIE VEGETATION IN WESTERN NORTH DAKOTA

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Native prairie soils are inherently low in plant available nutrients. Large amounts of nutrients are used for plant growth. Dead organic matter (litter layer) contains unavailable plant nutrients and provides food for heterotrophic organisms which convert the unavailable nutrients to an available form for the plants to utilize (1). Native vegetation begins growth in the spring and continues growth until seed development occurs. At this time older leaves begin to senesce and dry up. Standing dead material eventually breaks off and becomes part of the litter layer. At this point decomposition begins.

Production and turnover of prairie vegetation, the breakdown of litter and how grazing may affect this process were studied at the Ranch Headquarters of the Dickinson Experiment Station for 1987 and 1988. During the study a drought occurred in 1988 which resulted in less herbage production. Average precipitation for the Dickinson area is 40.6 cm and 1987 had 36.0 cm while 1988 had 21.5 cm.

Productivity data were taken on a monthly basis for a silty range site on two replicated nongrazed sites and two replicated 80 acre pastures which are grazed seasonlong. Ungrazed native vegetation was clipped, using three 1/4 meter square quadrats, to ground level and separated into live and standing dead categories. After the vegetation was removed the litter layer was raked up and placed in a paper bag. The samples were oven dried at 60 C for 48 hours and weighed. Decomposition rates were determined by the litter bag technique (1). This technique involves the use of a fiberglass mesh material with one millimeter openings. Eight grams of oven dried Bouteloua gracilis litter collected in 1986 was placed in 15 x 15 cm litter bags. Fifteen bags were placed under cages on the same sites on 14 May 1987. Five litter bags were collected on 6 Oct 1987, 12 May 1988 and 7 Oct 1988, dried at 60 C and weighed to determine weight loss for that specified time.

Total above-ground herbage production peaked in July for the nongrazed site and in August for the grazed site from June to September in 1987 (Table 1). Standing dead material made up a higher percentage of the total production on the nongrazed site. A nongrazed site has more carryover of standing dead material from the previous year than a grazed site. There was a thicker litter layer on the nongrazed site. From June to September it was reduced by 6% when compared to the grazed site which was 42% less. When these comparisons were made in 1988, a drought year, total above-ground herbage production gradually increased through the growing season for the grazed site. The nongrazed site started out with more production, decreased through the year and picked up again in September. Standing dead material again made up a higher percentage of total production for the nongrazed. The litter layer for the nongrazed was still thicker and was reduced by 3% through the year while the grazed site increased by 13%. Weather conditions may have inhibited the decomposition process. The litter bag study showed that most of the decomposition occurred in 1987 (Table 1) and tapered off in 1988. The grazed site had a slightly higher decomposition rate.

A grazed site has less litter accumulation than a nongrazed site. Plant nutrient cycling may be faster, as it seems the decomposition process is faster. However, a drought year seemed to inhibit decomposition to a certain extent.

Table 1. Herbage production and litter decomposition of a silty range site for a nongrazed site and a grazed site in grams per meter square, Dickinson Experiment Station, 1987-1988.

	Herbage Production								% Decomposition		
	1987				1988						
NONGRAZED	01 Jun	01 Jul	01 Aug	01 Sep	01 Jun	01 Jul	01 Aug	01 Sep	6 Oct 1987	12 May 1988	7 Oct 1988
Live	127±22	145±34	156±45	166±45	119±45	98±35	56±23	109±45			
St. dead	119±71	178±211	48±53	55±46	119±107	109±57	114±70	121±45			
Total	246±85	323±232	204±68	221±83	238±126	207±84	170±70	230±78			
Litter	663±395	713±434	803±345	621±266	486±384	218±147	273±136	471±177	36±3.6	33±7.2	42±5.6
GRAZED											
Live	125±38	126±38	211±56	249±89	62±19	51±23	53±24	98±51			
St. dead	32±40	24±10	78±61	23±34	30±29	42±28	51±40	38±20			
Total	157±66	150±22	289±79	272±91	92±30	93±42	104±47	136±57			
Litter	302±133	228±127	393±166	175±169	137±118	139±137	100±38	155±54	40±5.8	40±2.3	46±4.1

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(94) UNEXPLAINED NONCONCURRENT DISTRIBUTION OF LEAFY SPURGE AND ALFALFA IN NONCROPPED AREAS OF EASTERN NORTH DAKOTA

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Association or nonassociation of plant species can indicate underlying effects of site, habitat, soil characteristics, common parasites, allelopathy or previous disturbance. Methods to quantify such associations are available (1). Leafy spurge (Euphorbia esula L.) is naturalized widely throughout eastern North Dakota, particularly in noncultivated areas, including grassy roadsides (2). Alfalfa (Medicago sativa L.) is also naturalized throughout the state, growing in similar sites. In casual observation in previous years, the authors noted that alfalfa and leafy spurge appeared not often found close to each other, even in areas where both were common. The present study was undertaken to see if this seeming nonassociation was real and to see if clues to its underlying cause might be evident.

During May and June 1987, three surveys were made in seven counties in eastern North Dakota where leafy spurge was abundant. At this time leafy spurge plants were in flower and were recognized easily. The areas sampled were noncultivated grassy roadsides along secondary roads. Environmental extremes which might affect occurrence of either plant species were avoided: low, wet or poorly drained areas, very dry hilltops, areas showing evidence of soil disturbance or cultivation and areas where present or past herbicide spraying was evident. On chosen sites, a series of 10 to 20 contiguous sample plots extending continuously for several kilometers were evaluated for presence of alfalfa and/or leafy spurge. In scoring individual plots, a single plant of alfalfa or leafy spurge anywhere within that plot was recorded as a positive occurrence, an extremely conservative measure. There were three sites: a) 299 plots about 8 by 90 m in adjacent parts of Cass, Ransom and Richland counties, observed May 8, 1987; b) 235 plots about 8 by 40 m in adjacent parts of Barnes and Griggs counties, observed June 3, 1987; and c) 210 plots about 8 by 8 m in adjacent parts of Dickey and Sargent counties, observed June 29, 1987. Alfalfa plants were found in 39% of plots and leafy spurge in 45%. Results were analyzed as contingency tables using the chi-square statistic and by Cole's coefficient of interspecific association (C7) (1).

Alfalfa and leafy spurge occurred together in only 8% of plots, significantly less than expected based on their overall frequencies (Table 1). Cole's C7 statistic also indicated negative association of the two plant species. Both analyses indicated that there was a significant negative association between leafy spurge and alfalfa in each of the three surveys and in the totals (Table 1). Given the extensive sampling over a large area and the sampling pattern used, topographic or edaphic factors are not likely to be responsible for the observed results. Other explanations for the observed results may be allelopathy and common parasites. Although leafy spurge is allelopathic to some species, alfalfa is not reported among these (3). Alfalfa generally does not cause allelopathic effects, although its effect on leafy spurge has not been tested adequately. A parasitic rust fungus, Uromyces striatus Schroet. infects both leafy spurge and alfalfa as alternating hosts (4). In North Dakota, U. striatus appears on alfalfa only late in the season and, while it is widespread, its severity is very low (Stack 1985, 1986, 1987, unpublished). U. striatus is destructive on leafy spurge. It becomes systemic and causes distortion on growing shoots, impairs flowering and often causes death of the plant (4). Naturally occurring biocontrol could prove an explanation for observed nonconcurrent distribution. The purpose of this survey was to document observations and suggest possible explanations. Any causal relationship needs to be determined by experimental studies.

Table 1. CO-OCCURRENCE OF LEAFY SPURGE AND ALFALFA

Site: counties	Leafy spurge	Alfalfa Absent	Alfalfa Present	Chi-square	C^7*
		(number of plots)			
A: Cass, Ransom, Richland	absent	105	125		
	present	49	20	13.7**	-0.402
B: Barnes, Griggs	absent	34	58		
	present	107	3	34.2**	-0.371
C: Dickey, Sargent	absent	43	44		
	present	116	7	55.3**	-0.766
All counties	absent	182	227		
	present	272	63	105.6**	-0.518

*C^7 Cole's coefficient of interspecific association (1).

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(65) EFFECTS OF DIETARY ARSENIC, CHOLINE AND THEIR INTERACTION ON ARSENIC DEPRIVATION SIGNS AND PLASMA CARNITINE IN RATS

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Previous research has shown an interaction between arsenic and methionine in rats (1). Arsenic deprivation affects the metabolism of creatine, putrescine, spermidine, spermine and phosphatidylcholine, all of which utilize S-adenosylmethionine (SAM) in their biosynthesis. Because SAM is involved in the synthesis of carnitine, we designed a 2x2 factorially arranged experiment to ascertain the effect of arsenic deprivation on plasma carnitine concentrations in rats. Choline was included as a dietary variable because it can influence tissue carnitine (2) and also because it can affect methionine metabolism and thus arsenic deprivation (3). Male weanling Sprague Dawley rats were assigned to groups of nine and were fed a 70% ground corn, 16% casein based diet. The basal diet was lacking in choline and contained 12 ng arsenic per gram. Dietary supplements were arsenic as As_2O_3, 0 or 1 µg/g and choline chloride, 0 or 1.0 g/kg. The rats were fed their respective diets for 10 weeks, fasted for 16 hours, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated needle and syringe. Carnitine was measured using a radiometric method (4). Other methods and environmental conditions have been described (5).

Table 1. Effects of Dietary Arsenic, Choline and Their Interaction on Body Wt., Plasma Creatine, Urea, and Carnitine in the Rat.

Treatment		Body	Plasma	Plasma	Plasma Carnitine		
As	Choline	Wt.	Creatine	Urea	Total	Esterified	Free
µg/g	g/kg	g	mg/100 ml	mg/100 ml	µmol/l	µmol/l	µmol/l
0	0	314	3.56	14.8	107.5	76.5	29.7
1	0	312	3.32	14.3	122.1	86.6	35.5
0	1	299	3.09	16.2	107.6	77.7	29.9
1	1	333	3.56	14.4	117.8	87.9	29.9
		Analysis of Variance - P Values					
As		0.05	NS	0.02	0.06	0.1	NS
Choline		NS	NS	NS	NS	NS	NS
As x Choline		0.03	0.045	NS	NS	NS	NS
Error Mean Square		489	0.24	1.76	308.6	296.9	26.7

Arsenic deprivation decreased plasma creatine and body wt. in the rats fed supplemental choline, Table 1. However, in the rats receiving no supplemental choline, arsenic deprivation tended to increase plasma creatine and did not affect body wt. Plasma urea was higher in arsenic-deprived than arsenic-supplemented rats; the increase was exacerbated by choline supplementation. Regardless of dietary choline, total and esterified carnitine tended to be lower in arsenic-deprived than arsenic-supplemented rats. Dietary choline did not significantly affect plasma carnitine.

The results indicate that altering methyl metabolism, through the dietary manipulation of choline, affects signs of arsenic deprivation; this is consonant with previous findings which indicated that arsenic has a physiological role related to methyl metabolism. Arsenic apparently has an indirect effect on the metabolism of plasma carnitine.

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(95) INFLUENCE OF PSEUDOMONAD BACTERIAL PATHOGENS ON EMERGENCE OF PHASEOLUS BEANS

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Bacterial brown spot incited by Pseudomonas syringae pv. syringae van Hall (Pss), and halo blight incited by Pseudomonas syringae pv. phaseolicola (Burkh.) Young, Dye and Wilkie (Psp) are serious diseases of dry edible beans (Phaseolus vulgaris L.) in North Dakota. They cause foliage and pod blights and are seed-borne (1,2,3). Severely infected bean seeds often fail to emerge when planted in fields (4).

Determination of the relationship between numbers of bacteria and nonemergence was the objective of this study. Dark red kidney 'Charlevoix', pinto 'U of I 114', and navy 'Admiral' beans were surface sterilized in 0.5% NaOCl and rinsed in five changes of sterile distilled water. One hundred surface sterilized beans were germinated on absorbant pads moistened with 150 μg/ml chlorothalonil suspension at 35 C for 40 hrs at which time the radicle had emerged from the seedcoat. Germinated beans were selected for uniformity and distributed into sterile Erlenmeyer flasks, 20 seeds per flask.

Pathogenic Psp strain PSP-V was isolated from diseased field-grown plants near Fargo, ND. Pathogenic Pss strain 2771 was provided by D. Hagedorn, University of Wisconsin. The pathogens were grown in fresh nutrient dextrose broth in shake culture for 48 hr at 26 C. Bacteria were collected by centrifugation (20 min, 5000 x G, 4.5 C) then resuspended in sterile distilled water with 0.02% Tween 80 (SDWT) to a concentration of 3 x 10^8 colony forming units (cfu)/m (OD at 600 nm). This suspension and sequential 100-fold dilutions were used as inoculum.

Inoculum was poured over beans in each flask until beans were immersed. A vacuum of 34 cm Hg was applied to immersed beans for 30 sec. Vacuum was rapidly released, and beans were removed for planting. Beans infiltrated with SDWT without added pathogens and beans in SDWT without vacuum infiltration served as controls.

Infiltrated beans were planted in the greenhouse in sterile 15 cm clay pots filled with autoclaved moistened medium-course vermiculite. Pots were incubated under natural light (14 hr photoperiod) at 24 ± 5 C and moistened with sterile water as needed. Pots were observed daily for two weeks for seedling emergence. Plants were considered emerged when the cotyledons had cleared the surface of the vermiculite.

In the greenhouse, emergence began after two days. After seven days no more emergence was observed, thus emergence at seven days was chosen as the standard time for comparisons. Pss significantly reduced emergence of kidney (LD_{50} = 5.6 x 10^3 cfu/ml), pinto (LD_{50} = 1.5 x 10^4 cfu/ml) and navy (LD_{50} = 1.7 x 10^4 cfu/ml) beans. Infiltration of beans with suspensions of Psp significantly reduced emergence of pinto (LD_{50} = 2.2 x 10^6 cfu/ml), and navy (LD_{50} = 2.1 x 10^6 cfu/ml) beans.

Seeds infiltrated with the highest concentration of bacteria either failed to emerge or emerged poorly. The level of contamination that reduced germination was dependent on the cultivar of beans as well as the pathogen. LD_{50}'s were calculated from the concentration of bacteria in the infiltrating medium. An indigenous pathogenic microflora would have contributed to overall dose. Evidence for indigenous pathogens can be found in reduced emergence in beans vacuum infiltrated with water only. Other studies showed the amount of liquid taken into each bean was approximately 0.04 ml. At the highest and most inhibiting bacterial concentration, seedlings were probably inoculated with 1.2 x 10^5 cfu/seedling.

The data demonstrate that dose/response relationships vary with the particular host/pathogen combination.

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(24) SPECIES- AND TISSUE-SPECIFIC DISTRIBUTION OF MITOCHONDRIAL AND CYTOSOLIC ISOZYMES OF PHOSPHOENOLPYRUVATE CARBOXYKINASE

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Phosphoenolpyruvate Carboxykinase (PEPCK) (E.C. 4.1.1.32) catalyzes the reaction

$$\text{oxalacetate (OAA)} + \text{GTP} \rightleftharpoons \text{PEP} + CO_2 + \text{GDP}$$

PEPCK is known to be distributed in a species- and tissue-specific manner. Two isozymes of the enzyme exist, mitochondrial (M) and cytosolic (C). Whereas the isozymes have been quantitated in various tissues of various species, no comprehensive comparative study of their organ and cellular distribution has ever been done. C PEPCK is essential to gluconeogenesis in liver and kidney, and can support glycerogenesis in adipose. No essential role has been defined for M PEPCK in any tissue although we have recently shown conversion of exogenous PEP to malate by reversal of the M PEPCK reaction in isolated rabbit liver mitochondria (1), and have suggested that M PEPCK might participate in lipogenesis. We are interested in exploring and defining alternative roles for these isozymes, and thus have begun a comprehensive study of their cellular distribution in liver, kidney, brown adipose tissue (BAT), white adipose tissue (WAT), brain, heart, spleen, skeletal muscle, gastrointestinal (GI) mucosa, and lung of rat, rabbit, chicken, and pigeon.

In these studies, fed animals were killed and the organs of interest removed, rinsed free of blood, weighed, and placed on ice, with the exception of adipose which was kept at room temperature. The tissues were finely diced with a scissors and homogenized with 2 volumes cold homogenization buffer (0.30 M sucrose; 10 mM Tris, pH 8.0; 1 mM EDTA; 1 mM DTT; and 2% (w/v) fat-free BSA). Supernates were prepared by high speed centrifugation (100,000 xg) of homogenates before and after 3 cycles of freeze-thawing at -70°C/10°C. PEPCK activity was assayed in the reverse direction by coupling with malate dehydrogenase and measurng NADH disappearance at 340 nm. The difference in the quantity of PEPCK assayed before and after freeze-thawing was assumed to represent M PEPCK (total activity - cytosol activity).

Quite large amounts of PEPCK activity per gram tissue is found in liver and kidney of all species. BAT (present in rat only) as well as WAT and GI mucosa in rat and rabbit have a meaningful amount of PEPCK activity. Conversely, WAT and GI mucosa from chicken and pigeon contain no measurable PEPCK. Muscle contains measurable activity, whereas heart, lung, brain, and spleen appear to contain no measurable amount of PEPCK in any of the species examined.

The essential absence of PEPCK activity from GI mucosa and WAT of chicken and pigeon compared to rat and rabbit suggests a phenomena which may be generalized to all birds. Another interesting observation currently being pursued is the decrease in PEPCK activity in rat samples subsequent to freeze-thawing. Possibilities include the release of an inhibitor from the mitochondria or cold-liability of the rat isozymes. Phenylmethylsulfonyl Fluoride (PMSF) was used to preclude the possibility of protease activity.

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(6)

Household Arthropod Pests

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Household arthropod pests have been a major concern to man. In the late 1800's fleas and other arthropods were discovered to transmit diseases and additionally assumed medical concern as well as being household pests. Many arthropods enter homes every day through hidden cracks in foundations, holes in screening, open windows, even boxes and bags brought into the home. Why do arthropod pests enter our homes? Basically there are three reasons: (i) they are seeking shelter from either very hot or very cold conditions outdoors, (ii) they are searching for a supply of food, whether it be the occupant of the home or apartment or his stored food products, or (iii) they arrive in the home by accident. Household arthropod pests are organisms which have successfully adapted to take advantage of the interior habitats created by humans.

In order to aid persons who have problems with household arthropods I have written a paper which illustrates and describes the most common pests occurring in North Dakota and surrounding states. My paper also; (1) describes habits, habitats, etc. of these pests, and (2) offers non-chemical control suggestions. Some of the pests included in the bulletin are cockroaches, clothes moths, firebrats & silverfish, ants, fleas, lice, bed bugs, flies, and centipedes. The more common ones are briefly described below.

The **boxelder bug,** *Leptocoris trivitattus* (Say), in the fall of 1988 became one of the best known insects in North Dakota. It is approximately 1/2 inch long, brownish-black, and marked with three narrow red stripes on the pronotum. They enter homes in the autumn searching for places in which to overwinter. They are not destructive and pose no public health concern, but their presence alone make them a nuisance.

Clover mites, *Bryobia praetiosa* Koch, are tiny (1/16 inch), red or brown arthropods which can often become a nuisance in the home during the spring or fall. These mites are usually found congregating on walls, floors, furniture, and window panes. A quick way of recognizing them is by using a hand lens, noting the long front legs which at first glance appear to be antennae.

Field crickets, *Gryllus* spp., are black and about 5/8 inch long. These crickets pass the winter outdoors as eggs in the soil. During the late summer, while seeking cool, shade, and moisture they move into buildings. This often leads them through cracks and crevices in the foundation of homes and businesses. Field crickets can chew holes in furniture, clothing, and drapes, but their persistent chirping and presence in the home make them nuisances.

Millipedes are brown or black, cylindrical, worm-like arthropods with two pairs of legs on each body segment. During the summer they are found in flower beds around homes and they can migrate into the home in the autumn. They enter through cracks and crevices, and under damaged weather stripping at the base of outside doors. Once inside they move to cooler, damper areas such as basements where they are simply a nuisance.

Sowbugs are oval, 1/16 to 3/16 inch long, hard-bodied, grey and somewhat armadillo-like in appearance. They often gain entry into homes via basement window wells and cracks in the foundation. Once inside, they seldom damage food or fabric as they are scavengers, usually feeding on decaying organic material. They do not bite and are nuisances by their presence alone.

Spiders are predators and frequently stray into homes where they become nuisances. Though many people fear them, they are of major importance as predators in the ecosystem. All spiders can bite, but most do so in self defense. The two most feared spiders in the Upper Great Plains are the **black widow,** *Latrodectus mactans* (Fab.), and brown recluse, *Loxosceles reclusa* Gertsch & Mulaik. The black widow has been infrequently encountered in a number of outdoor locations west of the Missouri river, but the **brown recluse,** has not been found in North Dakota.

The most common of all spiders encountered in the home are referred to as "house spiders." These spiders are responsible for the dirty corner cobwebs. These "cobwebs" are old dust covered webs abandoned by the spiders because they did not snare enough prey. House spiders can be found anywhere in the home but they prefer basement corners. The large bulbous abdomen, light grey to tan coloring, and long, thin legs are common characteristics of house spiders. Another group of spiders that can enter homes includes the "jumping and wolf spiders." They are compact, hairy, and have relatively short legs. Many jumping and wolf spiders are brightly colored and have been confused with black widows. Spiders pose a minimal health threat, but few homeowners tolerate sharing their dwelling with them.

(7)

APPLIANCE TIMER USING DIGITAL CLOCK MODULE

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The design of this appliance timer is an innovative approach to the real time switching of power to appliances. Low power logic signals derived from a timing module are used to activate solid state switching elements which apply power to the appliance. Currently, there are various mechanical and electrical timers available on the market. Generally, electrical timers provide better resolution in setting times than mechanical timers. The design of this appliance timer uses a clock module that has an array of four, red, 0.84 inch, seven segment, light emitting diode (LED) display that provides better readability than electrical timers using liquid crystal displays (LCD).

The design of this timer includes three modes of operation, which are: 1. Preset turn-on time, 2. Delayed turn-off time, and 3. Manual override. The first mode of operation allows the user to preset the time for the appliance to be switched on. Once the timing module reaches the preset turn-on time, power is applied to the connected device. The second mode of operation allows the user to operate a device for up to one hour before the power is switched off to the device. The third mode of operation allows the user to use the appliance timer as an ordinary wall outlet overriding all timing functions. Operation flow is shown pictorially in Figure 1.

The timing of the system is done with a MA1020 Clock Module. The MA1020 uses six front panel switches to program the appliance timer. The functions of the six switches are: 1. Fast time advance, 2. Slow time advance, 3. Real time set allow, 4. Activation time set allow, 5. Deactivation delay set and allow, and 6. Activation off. This module uses the standard 60 Hz signal to keep track of time, continually updating its LED displays to show the current time. The MA1020 also has an AM/PM indicator LED and an activation ON/OFF LED for user reference on its display.

Depending on the mode of operation, the output of the MA1020 is processed by digital logic circuits. The function implemented by the logic circuits is to turn on the outlet if any one of the three required conditions of the modes of operation are met. Once one of these conditions is met, a five volt signal is applied to the input of the power switching circuitry.

The power switching is done by means of an opto-isolator and a triac. An opto-isolator uses a photo diode to turn on a photo transister to transmit a low power signal from the logic control circuitry. This transmitted low power signal is applied between the input terminal and reference terminal of the triac. Once this signal is present, a short circuit condition exists between the output terminal and the reference terminal of the triac, thus supplying power to the appliance connected to the outlet. The significance of the opto-isolator is to electrically isolate the low power control circuitry from the high power output circuitry.

The power limitations of the system are determined by the triac and related circuitry. The design as it stands now, can operate for capacitive, resistive, and slightly inductive loads. A simple addition of a snubber circuit to the triac circuitry will permit the driving of highly inductive loads. The maximum current is inherently determined by the triac, which in this design, could accommodate eight amps of current. However, for precautionary reasons, the entire system is fused at five amps.

This system has very accurate timing control, digital displays using LEDs rather than LCDs to allow reduced light viewing, and isolated solid state power switching. These features give this system advantages over other timers on the market. A demonstration of the circuits operation will be given at the time of presentation.

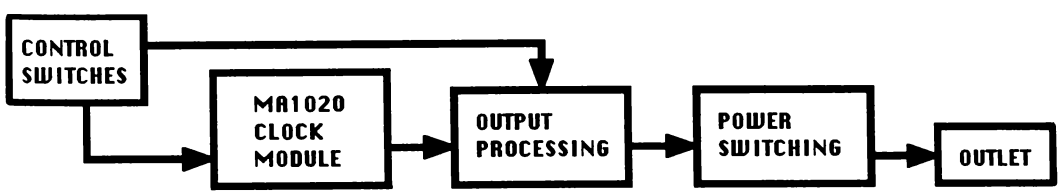

Figure 1. Operational flow of the Appliance Timer using Digital Clock Module

(8) The Effect of Solvents, Dye Concentration, pH and Temperature on the Absorbance Spectra of Nile Blue, an Oxazine Dye

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Nile blue is an organic dye which is used as a laser dye and also as a stain for fats in biological applications. It is classified as an oxazine dye. Oxazine dyes are characterized from pyronin dyes by the replacement of the central =CH- group with a =N-. Widespread use of dyes in lasers has caused much material to be published about dyes such as the rhodamines, Rose bengal, and cyanines.(1-3) Lack of information regarding absorption properties of nile blue along with its availability prompted its use for our research. The focus of our research has been to study the effects of dye concentration, solvents, temperature and pH on the absorption spectra of the organic dye nile blue. One of the interesting things that can be observed in a continuous absorption spectra is the change in the wavelength at which the monomer and dimer of a species will absorb. This change may be undetectable in some species while in others it will be quite obvious. The term monomer refers to the molecule of a species occurring singly. A dimer occurs when two identical molecules bond together in some way. In our study we attempted to obtain solutions containing only the monomeric species and solutions containing only the dimeric species. Obtaining pure monomeric and dimeric species would allow more accurate calculation of an equilibrium constant for nile blue and would also allow us to calculate an extinction coefficient for both the monomer and dimer of nile blue.

Our initial source of nile blue was obtained from the Minot State University Department of Chemistry. The concentration of the stock solution was unknown so a concentration of one molar was assigned to it and dilutions were made from this. These were labeled Nile 2, Nile 3, Nile 4, Nile 4-5, Nile 5, Nile 5-6, Nile 6, Nile 7, Nile 8, Nile 9, and Nile 10. Nile 2 represents the most concentrated solution and Nile 10 the least concentrated. More recently we received a supply of nile blue A sulfate (F.W.415.47) in powder form from Trinity Medical Center. From this powder a solution of known concentration (2.75e-4 M) was made with distilled water as the solvent. Six dilutions ranging in concentration from 8.26e-5 M to 2.20 e-6 M were made. To insure accuracy, all dilutions were mixed in volumetric flasks and graduated 5 ml and 10 ml pipets were used to deliver measured amounts of the dye in solution. All absorption spectra were run on a computer interfaced Hewlett-Packard 8452A diode array spectrophotometer. For the temperature control experiments, a Beckmann circulating waterbath was connected with tygon tubing to the special thermostatable cell holder located on the spectrophotometer.

Our study has shown that the absorption spectra of nile blue is affected by changes in dye concentration, temperature, pH and solvents. We have shown that the formation of dimers is enhanced in aqueous solutions at dye concentrations of 4.1e-5 M and above. Cold temperatures also increased the amount of dimers in solution. Organic solvents such as ethanol, acidic solvents and warm temperatures promote the monomeric species in solution. Based on our findings we think that hydrogen bonding at both ends of the dye molecule is responsible for nile blue forming dimers. The lack of dimer formation in organic solvents, even those with high dielectric constants, suggests to us that solvation interferes with aggregation.(2)

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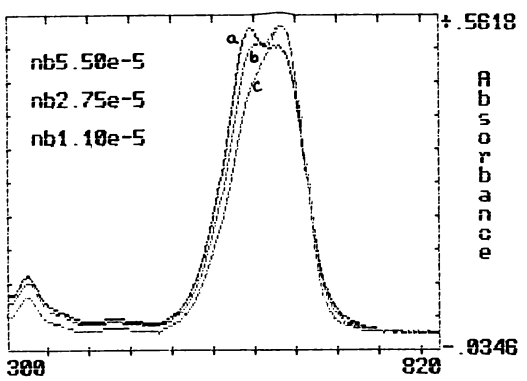

Fig. 1: Nile blue in water. a)5.50e-5 M
b)2.75e-5 M c)1.10e-5 M

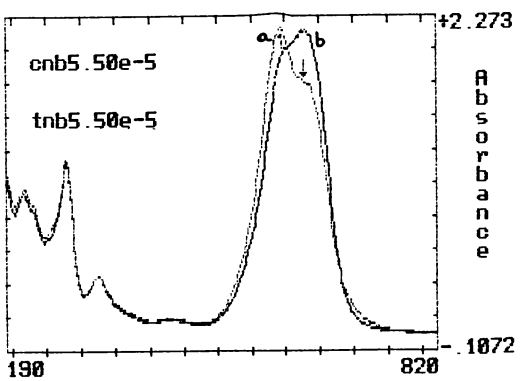

Fig.2: Nile blue in water. a)5.50e-5 M @ 2C
b)5.50e-5 M @ 47C

(9)

COPOLYMERIZATION OF DIBENZOFURAN AND DIBENZOTHIOPHENE VIA FRIEDEL-CRAFTS ALKYLATION

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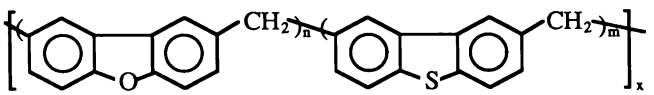
The goal of this research is to prepare polymers from aromatic nuclei and to study the properties of these products. Studies that have been done earlier show that polymers with aromatic nuclei have higher thermal stabilities and greater tensile strength than the analogous aliphatic polymers. (1)

With these ideas in mind, our research began with two different aromatic compounds called dibenzofuran and dibenzothiophene. Homopolymers prepared by Friedel-Crafts alkylation of each compound have been studied extensively. (2) Our research works with the copolymerization of these two compounds via Friedel-Crafts chemistry. From the previous studies done on the homopolymers, the expected product would have the aromatic nuclei bridged at the 2 and 8 positions by a methylene group.



Equal molar amounts (.006 moles) of dibenzofuran and dibenzothiophene were added to $AlCl_3$ (.008 moles) in nitroethane. Chloromethyl ethyl ether (0.012 moles) in nitroethane was added slowly to the solution. The reaction was left at room temperature at varying time intervals of 2, 1½, 1 and ½ hour. The reaction was quenched with cold 18% HCl after the allotted time had passed. Products were filtered through a Buchner funnel and rinsed with HCl, then water. The products obtained were yellow in color. Each product was Soxhlet extracted with THF for 24 hr. The results are based on the products that were soluble in THF.

Melting points of the copolymers were in the range of 190°C to 220°C. IR spectra were obtained from the products and the reactants. The relative intensity of a peak characteristic of dibenzothiophene (1262 cm^{-1}) was compared to the relative intensity of a peak characteristic of dibenzofuran (1195 cm^{-1}) for each copolymer obtained at different time intervals. The ratio of relative intensity of dibenzothiophene to dibenzofuran was approximately 1 to 3, respectively for each copolymer. This means the time variable doesn't have an effect on the percentages of each monomer in the product. The ratio shows there was 3 times as much dibenzofuran than dibenzothiophene in the copolymers even though we started with equal molar amounts of each. From this we can conclude that dibenzofuran is more reactive than dibenzothiophene. Future research will include varying the molar amounts of these two monomers and studying the products of these conditions.

Table 1. Characteristics of the Copolymers

Reaction Time	M.P.(°C)	IR Ratio*
2 hrs	200° - 220°	.37
1½ hrs	190° - 220°	.25
1 hr	190° - 220°	.32
½ hr	190° - 220°	.29

*Ratio of intensity of the IR peak characteristic of dibenzothiophene to the intensity of the peak characteristic of dibenzofuran.

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(10) THE SYNTHESIS AND CHARACTERIZATION OF AN AROMATIC COPOLYMER OF FLUORENE, DIBENZOFURAN AND METHYLENE CHLORIDE

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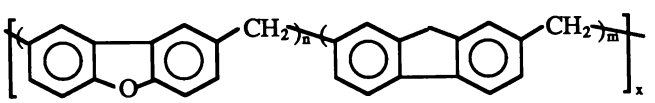
Due to their inherent thermal stability, aromatic polymers hold promise in applications where other plastics would decompose or soften. Unfortunately, as a class, because they cannot be made malleable through heating and are typically insoluble, it has proven difficult to process them into useable forms (such as fibers). My research, therefore, centers around synthesizing such polymers in the hope of finding one with suitable solubility in a convenient solvent.

The synthetic procedure consists of using the Friedel-Crafts reaction to bridge aromatic monomers with methylene groups. This was achieved by reacting the aromatic compounds with an excess of methylene chloride (CH_2Cl_2) using aluminum chloride ($AlCl_3$) as the catalyst. (1,2) A typical reaction involved dissolving 0.5 g (3mM) of fluorene ($C_{13}H_{10}$) and 0.5 g (3mM) of dibenzofuran ($C_{12}H_8O$) in 15 ml of methylene chloride and adding 1.2 g (9mM) of $AlCl_3$ slurried in another 15 ml of methylene chloride. After refluxing at 30°C for three hours, the polymer was formed, in virtually 100% yield.

This polymer is a solid, brown in color. It has a melting point higher than 400°C although it does tend to blacken at such high temperatures.

Infrared analysis using the subtraction feature of a Digi-Lab FTIR indicates that this product is not simply a mixture of two other polymers -- namely the homopolymers that would have been formed if only fluorene or dibenzofuran, and methylene chloride had been reacted. Similarly, it is doubtful that this is a "block"-type copolymer (one with long stretches of the same monomer), as such a polymer would probably show virtually the same spectrum as a mixture of the two homopolymers. This indicates some mingling of the monomeric units. A proposed structure of the copolymer is shown below.



The product does appear, however, to be a mixture of two different types, differentiated by solubility; the major component is virtually insoluble in all solvents attempted, while the other, smaller, component forms colored solutions with several solvents (toluene, acetone, methylene chloride).

Table 1. Characteristics of Polymers

Monomer(s) Employed	Mp(°C)	Color	IR Absorptions (cm^{-1})
Dibenzofuran	>400	yellow	1608, 1426, 1190, 1028, 747
Fluorene	>400	dark brown	1612, 1435, 891
Dibenzofuran and Fluorene	>400	light brown	1618, 1425, 1199, 1069, 746

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(11)

LOCAL DENSITY OF ELECTRON STATES IN FRACTAL NETWORK MODELS FOR POROUS GLASSY MATERIALS

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Certain amorphous structures including granular semiconductors and diffusion aggregates can be modeled by scaling fractal networks (1). If a geometrical object can be scaled up by some factor s in such a way that it can be decomposed into N copies of its original self it is self-similar and has a self-similarity dimension d defined by $d = \ln(N)/\ln(s)$. A self-similar geometrical figure having a non-integral self-similarity dimension is a fractal (2). The fractal we consider has a self-similarity dimension $d = \ln(7)/\ln(2+\sqrt{3}) \cong 1.5$ and is the limit graph in the sequence defined recursively by the graphs in the figures on the right where each line segment is replaced by Fig. 2 to construct the next graph in the sequence.

Fig. 1 Fig. 2

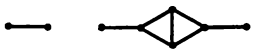
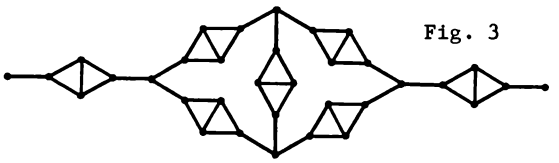


Fig. 3



We represent a molecule by a graph where the vertices represent the atoms and the connecting line segments represent the bonds. An element of the graph adjacency matrix is +1 if its indices correspond to sites which share a bond and is 0 if the sites corresponding to the indices are not bonded together. Diagonal elements are set equal to 0.

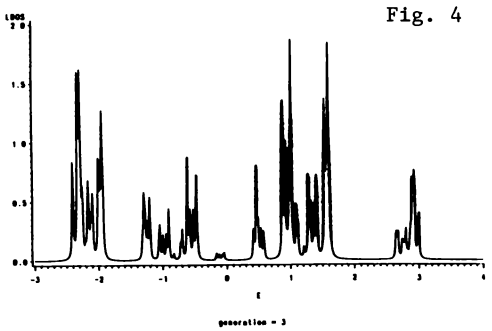
The stationary states of a molecule (those for which the energy has a definite, fixed value) and the energies of these states comprise the set of all solutions $\{(u,E)\}$ to the matrix eigenvalue equation $Hu = Eu$ in which **H** is the (Hermitian) Hamiltonian matrix, the eigenvector u gives probability amplitudes and E is the energy. We assume a one-electron model in which there is one atomic orbital centered on each site. The square of component i of the eigenvector u gives the probability of the electron in state u with energy E being found on site i (3). To distinguish the essential features, we assume tight-binding so each element of the **H** matrix is either a +1 or 0. By a judicious choice of the reference energy, the diagonal elements become 0 and the **H** matrix becomes identical to the graph adjacency matrix.

The local density of states (LDOS) $D_i(E)$ is the density of allowed electron energy levels at energy E weighted according to the time spent at site i (4). The LDOS for site i can be expressed in terms of the ii element of the matrix $\mathbf{G}(z) = [z\mathbf{I} - \mathbf{H}]^{-1}$ (5). Making use of the self similarity of the graph we construct recursion relations for the elements of G(z) (Green functions) (6). Green functions are then computed for the sequence of Hamiltonians corresponding to the graphs shown above. The LDOS are then plotted for particular sites. The LDOS spectrum is self similar in the neighborhood of certain energies because of the self similarity of the original graph.

The transmission probability for an electron moving from corner to corner on the lattice has also been calculated. These results have application in the theory of amorphous solids (1).

LDOS for fractal lattice

Fig. 4



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(12) BRAIN GLYCOLYTIC ENZYME INTERACTIONS WITH TUBULIN

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In the brain, several glycolytic enzymes are not in the soluble fraction after centrifugation of brain homogenates but rather are found associated with particles. The enzymes are especially enriched in fractions containing the synaptic plasma membrane (1). The subcellular components responsible for compartmenting the enzymes are currently unknown. Because tubulin, a structural protein, is enriched at the synaptic membrane, it has been projected as a protein which may anchor the glycolytic enzymes to the synaptic membrane. In this communication a new method was developed to examine interactions between enzymes and tubulin using an affinity chromatography approach. The method was used to examine interactions with brain glycolytic enzymes.

METHODS: Tubulin was prepared from bovine brain according to Keates (2). The tubulin was then crosslinked to either cyanogen-bromide activated Agarose or Sepharose, according to (3), with an estimated amount of 11-13 mg of protein bound per gram of agarose. Brain synaptosomes were prepared according to (4), and pure rabbit muscle enzymes were obtained from Sigma Chemical Co., St. Louis, MO.

Approximately 1 mg of each purified enzyme was applied to the column. Brain extracts were applied at level of 1.2 mg. The column was eluted with 16 ml of buffer A (0.05M MES, 0.5mM EGTA, 0.63mM Mg Acetate). A 32 ml linear gradient from buffer A to buffer B (0.05M MES, 0.05mM EGTA, 0.63 mM Mg Acetate, and 250 mM KCl) was created, and 2 ml fractions were collected and assayed.

RESULTS: The data indicates that the purified rabbit muscle enzymes including lactate dehydrogenase (LDH-m), pyruvate kinase (PK), aldolase, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) bind with tubulin that is covalently bound to Cn-Br Agarose. Phosphoglycerate kinase (PGK) was also shown to convincingly bind to the tubulin column. Other enzymes including the heart form of LDH did not bind under these conditions.

Enzymes isolated from brain synaptic membranes bound similarly, with the following exceptions: purified LDH-m bound to the tubulin in the column but the enzyme from the synaptic membranes did not appear to bind. PGK was not tested because of the small amounts located in the brain synaptic membrane fraction. The remaining glycolytic enzymes, PK, aldolase, and GAPDH bound, and triosephosphate isomerase (TPI) did not bind to either the tubulin or control column. The relative affinity of the enzymes for tubulin as determined by the relative amount bound, and ionic strength at which the enzymes eluted is as follows: aldolase $>$ GAPDH $>$ PK $\geq$ LDH $>$ TPI.

DISCUSSION: A column prepared by crosslinking tubulin to Agarose was shown to be an effective means for determining interactions of tubulin, with enzymes both in the purified state and in mixtures. Some of the enzymes are suspected of binding to the same or similar sites on the tubulin because LDH-m binds in the absence of but not in the presence of other enzymes as occurred in synaptic membrane preparation. Finally the results of the present study indicate that tubulin may anchor the glycolytic enzymes at the synaptic membrane. The significance of this binding is not established. However because the glycolytic enzymes are suspected components of the microtrabecular lattice and because some of the enzymes bind to tubulin (tubulin is a component of the cytoskeletal system) may indicate that the cytoskeleton and the microtrabecular network are interactive.

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(13)

POLYMERIZATION OF 2,8-DIBENZOFURANDICARBOXYLIC ACID

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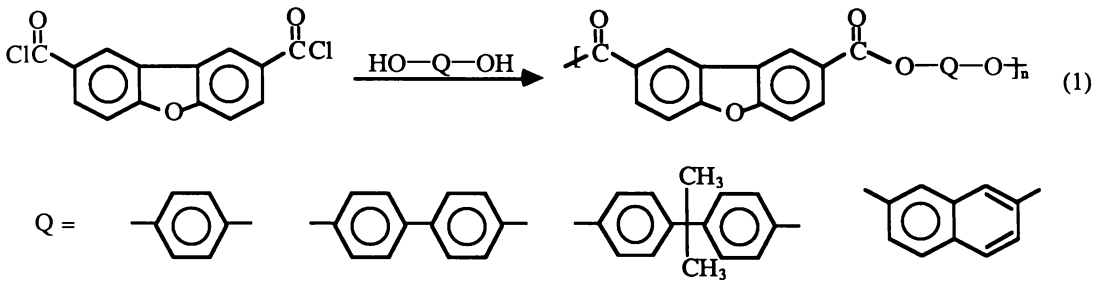
Introduction:

The main objective of this research was to prepare fully aromatic polyesters using dibenzofurandicarboxylic acid as the monomer. (eq 1) The linkages between these dibenzofuran monomers were provided by an array of aromatic bisphenols, e.g., hydroquinone, bisphenol A and 4,4'-biphenol. These polyesters were studied for their solubility, molecular weight and thermal stability.



(1)

Experimental:

Dibenzofuran was acetylated by using a 2.5:2.5:1.0 mole ratio of $AlCl_3$ to acetyl chloride to dibenzofuran in a simple Friedel-Crafts reaction. The reaction produced a 94% yield of diacetyl derivative with a melting point of 158-160°C (lit. 162-163).(1) The 2,8-diacetyldibenzofuran was characterized by IR and ^{1}H-NMR.

The diacetyl derivative was then converted to the diacid in 99% yield via oxidation with NaOCl. The 2,8-dibenzofurandicarboxylic acid had a melting point above 400°C and was characterized by IR and ^{1}H-NMR. At this point the diacid was converted to the diacid chloride by refluxing the diacid in a mixture of $SOCl_2$ and pyridine. After recrystallization in toluene a 38% yield of 2,8-dibenzofurandicarboxylic acid chloride with a melting point of 225-229°C (lit. 232°C)(1) was recovered.

The most successful polymerization reactions were carried out using the isolated diacid chloride in a three-necked flask equipped with a condenser and a nitrogen inlet. The diacid chloride was placed in the flask along with approximately 100 ml of dried o-dichlorobenzene. Under a steady stream of nitrogen the preferred bisphenol was added and the reaction was allowed to proceed for 48 hrs at a temperature around 180°C. The polyesters that were recovered all had melting points above 400°C and were characterized by IR and ^{1}H-NMR when possible.

Discussion:

Fully aromatic polyesters using 2,8-dibenzofuran dicarboxylic acid chloride as the monomer and a variety of bisphenols have been successfully prepared. The polyesters produced were generally light colored powders. These polymers were identifiable by the characteristic carbonyl and carbon-oxygen stretches at approximately 1720 cm^{-1} and 1200 cm^{-1} respectively, typical for aromatic esters. The thermogravimetric analysis of the polymers is pending.

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(30) THE SYNTHESIS, CHARACTERIZATION AND REACTIVITY OF POLYBENZYLCHROMIUMTRICARBONYL

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Introduction:

The enhancement of benzylic hydrogen acidity in π-(alkyl arene) tricarbonylchromium complexes has been clearly demonstrated in the past 17 years.[1] However, relatively few examples of organo-metal carbonyl polymers have been reported, and no studies of benzylic proton activation by arene chromiumtricarbonyl polymer complexes have been published. By the direct reaction of soluble polybenzyl with chromium hexacarbonyl in a mixed dibutyl ether, THF solvent system, a polybenzylchromiumtricarbonyl copolymer was generated. The presence and amount of chromiumtricarbonyl in the polybenzyl was confirmed by NMR, IR, GPC and elemental analysis. Selective multiple benzylic deprotonation was achieved by applying the same condition used for benzylic deprotonation in $Cr(CO)_3$-diphenylmethane .[2] Quenching the anions in D_2O and MeI confirmed this. Future work will apply these reactions to insoluble polybenzyl and coal.

Therefore, a Friedel-Crafts polymerization was carried out by treating α-chlorotoluene with $SnCl_4$ in CH_2Cl_2 at -62°C. A low yield, 2.24 g (26.5%) of soluble polybenzyl softening point 74°C, was obtained. Characterization was carried out using IR, GPC, NMR and elemental analysis. The GPC of the polybenzyl gave a M_n = 2030.5, using polystyrene standards, and elemental analysis gave 92.32% C and 6.70%H. The ^{1}H-NMR gave broad peaks centered at 7.0 ppm and 3.8 ppm. Integration of the peaks gave a 2:1 ratio of aromatic to methylene hydrogens.

Structural characterization by high field (75 MHz) ^{13}C-NMR was carried out by following the procedure outlined by Hasan and Tsonis. Similar results were obtained for the type of branching in the polybenzyl.[3] Of the substituted aromatic rings, 20.1% were monosubstituted, 54.5% disubstituted, 15.7% trisubstituted, and 1.3% were tetrasubstituted. However, these calculated parameters did not take into account the contribution of each of the three carbons of the trisubstituted phenyl. Therefore a series of simultaneous equations were set up to take into account the contributions of each type of substitution to the four areas integrated. Solving these equations gave 20.5% mono, 37.5% di, 39.4% tri and 3.0% tetra substitution.

The complexation of soluble polybenzyl was carried out by refluxing the reactants for four days in a (90% dibutyl ether: 10% THF) mixed solvent system[4] to give a fine bright yellow complexed polymer (softening point 80°C).

Characterization was carried out using IR, GPC, elemental analysis and NMR. The IR spectrum confirmed that chromium tricarbonyl units were complexed to some of the phenyl rings in the polymer. Strong C=O stretching frequencies were present at 1874.8 and 1959.1 cm^{-1}. GPC analysis gave a Mn = 3116.4. Elemental analysis demonstrated 14.67% Cr was present in the polymer, which indicated that 41.2% of the phenyl rings were complexed with $-Cr(CO)_3$ moieties. Final conformation of complexation was performed by ^{1}H-NMR and ^{13}C-NMR. Mono-complexed $Cr(CO)_3$-diphenylmethane proved to be an excellent model for comparison with $Cr(CO)_3$-polybenzyl. In both the ^{1}H and ^{13}C-NMR, $Cr(CO)_3$-polybenzyl gave broadened shifts very close to those for $Cr(CO)_3$-diphenylmethane.

With successful complexation achieved, attention was turned to benzylic deprotonation. Benzylic deprotonation is the thermodynamically favored process, however the powerful electron-withdrawing effect of the metal makes attack at the ring protons kinetically favored. Fortunately, one basic system, KH/18-Crown-6-ether in THF gives selective benzylic deprotonation.[5] Integration of the ^{1}H-NMR indicated 97.0% monoalkylation at the complexed benzylic positions.

With the route for alkylation established, these methods can now be applied to the benzylic alkylation of insoluble polybenzyl and coal. By alkylation of complexed insoluble polymers, solubilities can be increased by the forcing apart and resulting breakage of cross linkages. Complexation has already been achieved in both insoluble polybenzyl and coal. Characterization by IR, gave strong C=O stretches at 1967, 1890.2 cm^{-1} and 1979.0, 1886.4 cm^{-1} respectively. Future work will involve characterization by elemental analysis, solid state NMR and ultimately deprotonation and alkylation.

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(31) NON-ENZYMATIC MODIFICATION OF MYOSIN: A POSSIBLE ROLE IN DIABETIC NEUROPATHY

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Introduction

A number of reducing sugars are capable of reacting non-enzymatically with the amino groups of various extracellular and intracellular proteins (1). A significant consequence of the high blood glucose concentration in diabetes is that tissues which are insulin independent, e.g. brain, ocular lens and RBCs, have their intracellular environment bathed in abnormally high glucose levels (2). In this study the effect of incubating reducing sugars with myosin and actin on activity was determined. Further, the extent of incorporation of sugar into the intracellular proteins, actin and myosin was quantitated by HPLC following reduction with sodium borohydride.

Materials and Methods

Actin and myosin were prepared from rabbit skeletal muscle by the method of Katz (3). All myosin incubations were performed at 4 mg/ml, 27°C, in 0.05M Tris buffer, pH 8.0, and 1mM DTT. Glycerol was added at 20% for protein stabilization. Reducing sugar concentrations, 50mM, 150mM, 250mM, 350mM and 500mM were studied. Sorbitol, a non-reducing sugar, is unable to react nonenzymatically with amino groups of proteins and therefore was used in all control experiments. Glycated lysines were quantitated by HPLC (C18 ultrasphere ODS column). Actin-activated Mg^{2+}-ATPase activity of myosin was determined by following the formation of inorganic phosphate by the method of Fiske SubbaRow.

Results

Actin stimulated myosin ATPase was reduced in minutes when fluorescein isothiocyanate was reacted with either protein, suggesting that lysine modification altered the proper functioning of these proteins. The actin-activated Mg^{2+}-ATPase activity was significantly reduced in myosin, when incubated with fructose. This inhibition is dependent on both sugar concentration and incubation time. The three reducing sugars, fructose, glucose and ribose had different inhibitory effects on myosin activity, with glucose having the greatest effect on activity.

Discussion

Fructose was the primary reducing sugar used in this study. In comparing diabetic and control sciatic nerve biopsies, fructose levels were shown to be in the vicinity of 1/10 that of glucose levels (4). Fructose is known to form the Schiff base adduct at a rate seven times that of glucose (5), with this fast rate, high concentrations of fructose are not needed to have pathological effects on the nerve.

The formation of Schiff base and Amadori adducts with cytoarchitectual proteins alters activities as shown in this communication. In addition the derivatization may also interfere with the dynamic equilibrium among the respective subunits and polymers, resulting in the observed decrease in axoplasmic transport and ultimately leading to neuropathy. (Supported by Grants from the American Diabetes Association and the North Dakota Lions Foundation).

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(32) PROTEOLYTIC CHARACTERIZATION OF TUBULIN BINDING SITES FOR GLYCOLYTIC ENZYMES

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Tubulin is a noncovalent heterodimer. The two chains are highly homologous, each having a molecular weight of about 50 kDa and a carboxyl terminal rich in acidic amino acids. These acidic carboxyl terminal tails are exposed on the surface of microtubules when tubulin polymerizes (1).

Controlled treatment of tubulin or microtubules with the protease, subtilisin, results in the cleaveage of the carboxyl terminals (2). Tubulin treated in this manner is still able to polymerize to form microtubule-like structures but will no longer bind MAP_2 (2,3). Both tubulin and microtubules have been shown to bind glycolytic enzymes (4,5) and the binding is postulated to be electrostatic. Since many of the glycolytic enzymes are basic proteins (pI $\geq$ 8). It was of interest to determine if the acidic carboxyl-terminal tails are involved in the enzyme binding.

Materials and Methods: Purified rabbit muscle glycolytic enzymes and subtilisin were obtained from Sigma as lyophilized powders. Tubulin was prepared by the method of Keates for high tubulin recovery (6). Proteolysis was carried out at 35°C at 1% protease:tubulin (w/w) in buffer A (0.1M MES, 1.0mM EGTA, 1.25 mM Mg acetate, pH = 6.6). Binding of enzymes to tubulin or subtilisin-treated tubulin was measured in buffer B (buffer A diluted 1:4), either with or without KCl and PEG. The ratio of enzyme to tubulin was 1:10 unless otherwise stated. The binding mixture was brought to a volume of 100 ul with buffer B and incubated for 15 min at 15°C, then centrifuged at 85,000 xg for 11 min at 15°C. The supernatants and pellets were then separated, the pellets resuspended in 100 ul B, and both fractions were assayed for enzyme activity. Lysine residues on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were derivatized with pyridoxal phosphate as described by (7) and binding to tubulin and microtubules was determined by affinity chromatography and ultracentrifugation respectively.

Results: SDS-PAGE electrophoresis showed that MAP_2 no longer binds to subtilisin treated microtubules and both tubulin subunits were decreased in size by approximately 2kDa. Saturation levels of pyruvate kinase (PK) and lactate dehydrogenase (LDH) were both decreased when comparing binding to subtilisin treated and untreated microtubules. The binding of PK and LDH in the presence or absence of PEG with increasing KCl were identical with microtubules and subtilisin treated microtubules. The amount of GAPDH bound to subtilisin treated microtubules was increased at saturation. Modification of GAPDH with pyridoxal phosphate caused a decrease in enzyme activity and a decrease in binding to tubulin as shown by affinity chromatography.

Discussion: Because modifications of lysine groups of GAPDH reduced binding, interactions with carboxyl groups on tubulin were suspected. Binding to the glutamate rich carboxyl tails could readily be tested. However, removal of the tails did not abolish enzyme binding. Further, the binding of PK, LDH, and GAPDH to microtubules, prepared by cycling methods, is not due to the presence of MAP_2 since binding to microtubules continued to occur even after the carboxyl tails of tubulin were removed and MAP_2 binding was abolished. Removal of the carboxyl tail increased GAPDH binding but decreased net PK and LDH binding. The presence of the carboxyl tail does have some effect on the binding of these enzymes, possibly by changing the overall charge or conformation of the protein.

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(33)

VALIDATION OF THE WILLETT FOOD FREQUENCY QUESTIONNAIRE FOR ASSESSING NUTRITION INTAKE DURING PREGNANCY

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Nutrient intake during pregnancy can affect birth weight. Maternal weight gain of less than twenty lbs is correlated with low birth weight-i.e. less than 5 lbs, 8 oz (1). Low birth weight is an important influence on infant mortality and morbidity (2). The need for an accurate tool to assess nutrient intake during pregnancy becomes crucial. Therefore, this study was designed to validate a modified (2 month) food frequency questionnaire for use in assessing nutrient intake during the second and third trimesters of pregnancy and to determine if differences in total nutrient intake and food choices exist between the second and third trimesters.

The Willett Food Frequency Questionnaire (WFFQ) is a self administered questionnaire that identifies frequency of consumption of specific food in time frames such as "1 per week", "1 per day", etc. Comparison of the WFFQ with a more traditional technique of measuring nutrient intake indicated that the two methods were moderately to highly correlated (r=.60) (3). The original WFFQ was based on nutrient intake averaged for a one year period. The WFFQ has been modified to reflect intake over a two month period. This smaller time frame makes the modified version applicable to nutrient intake during pregnancy. Because previous research indicates that the 24 hour recall is a valid tool for nutrient assessment (4), data collected from the WFFQ will be compared with data collected via the 24 hour recall.

Fifty women, aged 20-35 years, in the beginning of their second trimester were recruited via poster and referral. All women were free from complications of pregnancy. Twenty-four hour recalls were taken at weeks 16 and 21 in the second trimester and weeks 30 and 35 in the third, by interview in the subjects' homes. The WFFQ was administered at weeks 21 and 35. Women were weighed at week 16 and 30, and self-reported weights were collected from the WFFQ.

The accuracy and reproducibility of reported intake on the 24 hour recall were improved by the use of food models, diagrams and examination of actual serving utensils. An experienced interviewer (LS) conducted all 200 recalls. "Computrition", a computer program which is based on the USDA Handbook 8 on nutrient composition of foods, was used to compute nutrient intakes from the 24 hour recalls.

Pearson r correlation coefficients are being used to validate the WFFQ. The mean intake of kilocalories, total fat, protein, calcium and ascorbic acid per subject for each trimester is being correlated with the WFFQ. Intakes from the WFFQ are being compared for each subject between trimesters and Student's paired "t" test is being used to assess differences in food choice between trimesters. The majority of the women gained between 25 and 35 lbs; the mean self-reported weight gain for the group at week 35 was 27.24 lbs. The mean birthweight was 7 lbs, 12 oz.

Validation of the WFFQ for use during pregnancy will provide an accurate, simple and relatively inexpensive tool for evaluating nutrient intake in future investigations. This, in turn, may provide useful information concerning the role of nutrition and positive pregnancy outcome.

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(34) REGULATION OF THE PROMOTER-SPECIFIC NUCLEOSOME-FREE REGION OF SV40 BY T-ANTIGEN INTERACTIONS AT SITE I

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Simian Virus 40 (SV40) infected cells provide a model system for studying chromatin structure-function relationships. The small, well-defined genome is present in high concentration compared to cellular genes, and SV40 nucleoprotein complexes can be easily separated from bulk cellular chromatin. In lytically infected cells, SV40 minichromosomes are complexed with cellular histones in a chromatin structure similar to cellular chromatin.

A subpopulation of chromosomes extracted from the nuclei of lytically infected cells possesses a nucleosome-free gap of approximately 400 bp [1] that encompasses the viral origin of replication, transcription promoter and enhancer elements, 5' ends of early and late mRNA, and three T-antigen binding sites. The exact function of the nucleosome-free structure in SV40 chromatin is not known, however this feature is found in promoter regions of cellular genes poised for transcription [2].

Multiple genetic elements located within the nucleosome-free promoter of SV40 chromosomes are known to contribute to the generation of a nucleosome-free region [3]. In contrast, the availability of the late structural protein VP1 appears to limit the generation of a nucleosome-free region [4], while the formation of virions from SV40 previrions results in the loss of nucleosome-free regions originally present in the chromosomes [5]. Since T-antigen Site I is located at or near the early border of the nucleosome-free region, it has been suggested that interaction of T-antigen at Site I might be involved in the regulation of chromatin structure within the nucleosome-free promoter, possibly determining the early boundary for this region.

We have examined the relationship between T-antigen interactions at Site I and the nucleosome-free promoter in SV40 chromatin by analyzing three mutant strains that are defective for T-antigen interaction at Site I, cs1085 (deleted Site I), scs111 (constructed by replacing the BstXI to SfiI fragment of wtSVS with the mutant fragment from cs1085) and tsA58 (defective T-antigen) and their wild-type parental strains 776, SVS and VA4554, respectively, for the presence of this structural feature. The proportion of chromosomes containing a nucleosome-free promoter was measured by digesting chromatin from each viral strain with restriction endonucleases that recognize unique sequences within (BglI, KpnI, MspI) or outside of (EcoRI) the nucleosome-free region. A significant increase in the amount of digestion at the BglI, KpnI, and MspI sites was observed for all 3 mutant strains compared to wild-type (Table 1), indicating a substantial increase in the proportion of SV40 chromosomes containing a nucleosome-free promoter in these mutants.

Since a nuclcosome-free region is present in SV40 chromosomes from mutants lacking T-antigen bound to Site I, the results suggest that T-antigen bound to Site I is not necessary for the generation or maintenance of the early boundary of the nucleosome-free region. Moreover, the results also suggest that the proper binding of T-antigen at Site I is involved in the regulation of chromatin structure at the SV40 promoter by determining the relative proportion of SV40 chromosomes with a nucleosome-free region.

	PER CENT MOLECULES LINEARIZED			
VIRUS	BglI	KpnI	MspI	EcoRI
776	57	42	44	31
cs1085	88	69	62	28
SVS	57	41	52	21
scs111	93	75	77	32
VA45-54	57	47	43	37
tsA58	84	65	65	34

TABLE 1.

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(35) # Chronobiology of *Hybomitra lasiopthalma* (Macquart) and *Tabanus similis* Macquart, in the North Dakota Sandhills

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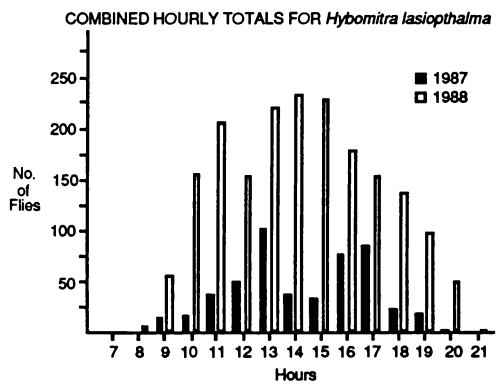
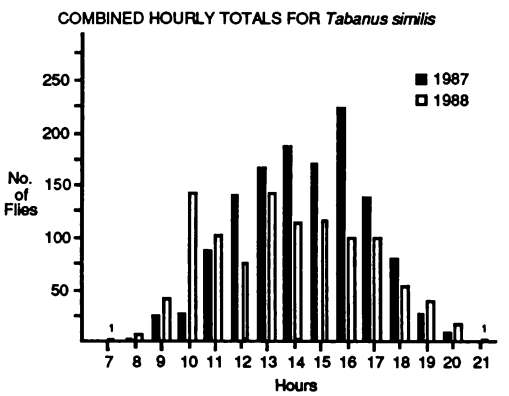
Horse flies are in the family Tabanidae (Diptera). Two species, *Hybomitra lasiopthalma* (Macquart) and *Tabanus similis* Macquart, are common in southeastern North Dakota. Tabanids are important pests of cattle (3; 4). They may cause a great deal of stress to animals (3), as well as being possible vectors of disease (1). These detriments may be realized as lower milk production (2), lower weight gains (5), and lower economic returns for the rancher. For example, in 1983 the annual loss in beef cattle production due to tabanid attacks was $40 million in the U.S.A. $30 million was attributable to reductions in weight gains (5), which are due to irritation, blood loss, and higher energy requirements.

Research was carried out during 1987 and 1988 in the northern unit of the Sheyenne National Grasslands of Custer National Forest in Ransom county, near McLeod, North Dakota. The study area consisted of approximately 320 acres (1 square half-section), which was divided into two separate sites, with four separate habitats within each site. These habitats are A) The transition between the prairie and the groveland, B) a hill surrounded by trees, C) a wooded upland, and D) a wooded lowland. The Grasslands are operated under the supervision of the National Forest Service and exist with multiple uses for recreation and livestock. About 97% of the 70,180 acres is open for livestock grazing.

The goals of this project were to (a) describe the seasonal occurrence of the dominant tabanid species within the study area, (b) describe their daily flight activity, (c) describe flight behavior variations (if any) with respect to the four different habitats at each of the two research sites and (d) locate larval habitats. Seasonal and daily activity was recorded on the basis of adult catches made with the use of unbaited Manitoba traps (6).

The dominant tabanid species within the study area are *Hybomitra lasiopthalma* (Macquart) and *Tabanus similis* Macquart. *Hybomitra lasiopthalma* emerges late in May and is present in large numbers until the second week in June. *Tabanus similis* emerges about one week later than *Hybomitra* and has populations spread throughout June, July, and into early August. Daily flight activity showed a typical monophasic diel cycle with the greatest activity occurring in early to mid-afternoon (refer to figures below) during bright sunny days with little wind. Very few flies showed matinal or crepuscular activity and none were nocturnally active. In 1987, both horse fly species showed a preference for the transition habitat at each site. Due to the extremely dry conditions in 1988, no definitive preferred adult habitat was detected. During July 1988, a suitable larval habitat for *Tabanus atratus* Fabr. was located immediately adjacent to the southernmost boundary of the study area. No immatures have been collected for either *Hybomitra lasiopthalma* or *Tabanus similis*.





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(36) INHIBITION OF RETINAL PERICYTE PROLIFERATION IN AN IN VITRO MODEL OF LOCAL TISSUE HYPOXIA

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A major clinical manifestation of insulin-dependent diabetes is the proliferation of blood vessels in the retina. Retinal neovascularization occurs in over 50% of diabetes patients; retinopathy and its complications represent the single leading cause of vision loss in the U.S. (1).

Investigations in this and other labs have shown that hypoxic conditions (e.g., 5% O_2) can induce proliferation of cultured microvascular endothelial cells. Adenosine, a low-molecular weight metabolite and potent vasodilator released by hypoxic tissues, is believed to mediate this hypoxic "neovascularization response" via agonistic purinoceptors located in the endothelial cell membrane. Blockage of these receptors with a specific inhibitor abolishes the proliferative response seen in both hypoxic and adenosine-treated cultures of endothelial cells (2).

In the normal quiescent retina, neovascularization is inhibited by the presence of pericytes in a 1:1 ratio with endothelial cells. The pericytes inhibit endothelial proliferation by cell-to-cell contacts and through the secretion of soluble factors, many which await definitive characterization (3). It is proposed that conditions such as hypoxia, which stimulate retinal angiogenesis, also function by disinhibiting the endothelial cells, possibly through mechanisms which affect the metabolism and proliferation of retinal pericytes. We report here the effects of adenosine on the in vitro proliferation of retinal microvascular pericytes.

Bovine retinal microvascular pericytes were isolated as described previously (4). Briefly, retinas were aseptically removed, washed, homogenized and sieved through a nylon mesh (88 μ) screen. The vessels were collected from the screen and placed in 0.1% collagenase for 90 minutes at 37°C. The suspension was pelleted, washed several times in Dubecco's Modified Essential Medium (DMEM), and placed into 35mm tissue culture dishes. After reaching confluence, the pericytes were passaged onto culture dishes in standard medium (DMEM + 20% fetal bovine serum, 2.4 g/L HEPES, 1.1 g/L sodium bicarbonate, 50 mg/L gentimycin, 50 mg/L fungizone, and 50 mg/L ascorbic acid) with or without the addition of 50 μM adenosine. Cells were harvested in 0.1% trypsin, and triturated into a monodispersion. Triplicate dishes were counted every other day using an electronic cell counter.

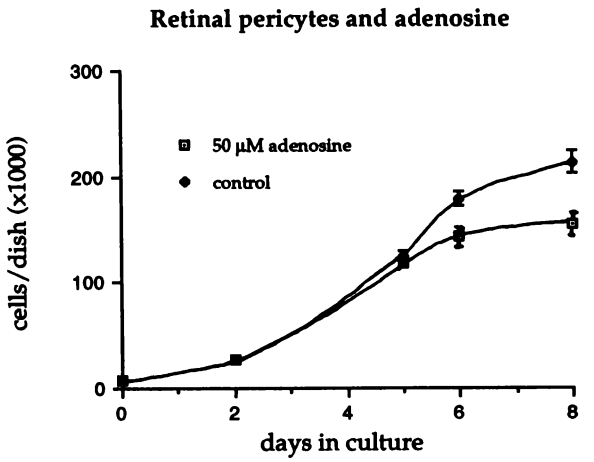

Fig. 1. Retinal pericytes were plated at an initial density of 8,000 cells/dish and were cultured for the time period indicated. The number of cells per dish was recorded as mean ± standard error.

These results demonstrate that adenosine, which has a stimulatory effect on the growth of endothelial cells in vitro, also acts to suppress the proliferation of retinal pericytes. The mechanism for this inhibition is currently under investigation in our laboratory. Since the eye is markedly affected by hypoxic conditions during the proliferative stage of diabetic retinopathy, understanding the exact nature of the effects of hypoxia and adenosine on retinal pericytes may aid in the management of this disease.

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(37) EVIDENCE FOR NUCLEOSIDE KINASE ACTIVITY IN RABBIT HEART AND LIVER MITOCHONDRIA

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Nucleoside diphosphate kinase (NDPK) (EC 2.7.4.6) has been proposed to catalyze the trans-phosphorylation of adenine and guanine nucleotides within the matrix of mitochondria. While often assumed to catalyze conversion of GTP formed by the succinate thiokinase (STK) reaction in the Krebs cycle to ATP, reported measurements of NDPK in the matrix of mitochondria show its assayable activity to be extremely low or absent (1). Reports also exist of an apparent mitochondrial ATP-linked STK activity in addition to a GTP-STK activity from varied tissue sources in several species (2). We have investigated these activities in rabbit heart and liver mitochondria.

Methods: Mitochondria were isolated and treated with digitonin essentially as described by Greenawalt (3). Mitoplasts were then sonicated and the high-speed supernate therefrom was subjected to gel filtration chromatography on Sephadex G-25-300. Fractions were collected and assayed spectrophotometrically for NDPK activity using TDP as substrate (4). NDPK activity was also measured following separation of nucleotide reactants and products via reverse phase HPLC and quantitating the formation of GDP. STK activity was measured by following succinyl-CoA formation at 235 nm with the addition of 0.1 mM GTP or ATP (5). Glycerol gradients (10-40%) in 10 mM Tris-HCl, pH 7.4 and 1 mM EGTA, were centrifuged at 274,000 xg for 16 hours. Fractions were collected and assayed for NDPK, STK, as well as for appropriate enzymes as molecular weight markers.

Results: Direct measurement of NDPK activity from liver and heart matrix fractions obtained from mitoplasts revealed enzyme levels to be <7 nmol/min/mg matrix protein. ATP-STK/GTP-STK activity ratios prior to chromatography ranged from 0.12-0.17. Fractions obtained via gel filtration showed only trace amounts of apparent ATP-linked STK activity. However, an apparent ATP-linked STK activity could be recovered upon addition of μM concentrations of GDP as exemplified by studies of heart matrix (fig. 1). Analysis of the glycerol gradient revealed a peak of GTP-STK activity with a higher molecular weight than purified GTP-STK which exhibited an apparent ATP-linked STK activity upon addition of 8 μM GDP as well as trace amounts of NDPK activity (fig. 2).

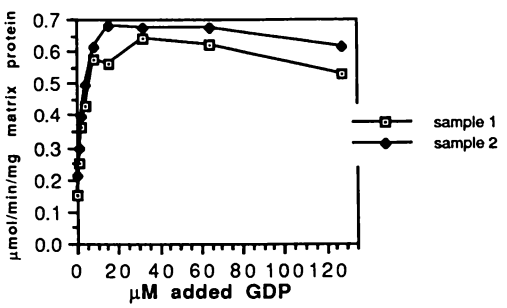

Fig. 1: Apparent ATP-linked STK Activities (Heart Matrix)

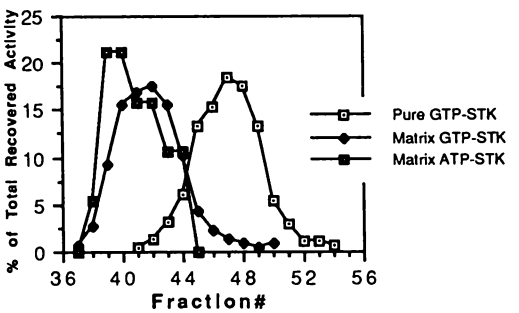

Fig. 2: Representative glycerol gradient (Heart Matrix)

Discussion: Data indicate that mitochondrial NDPK activity in rabbit liver and heart is notably small compared to STK activity. Data are also consistent with coupled STK/NDPK activities acting in concerted fashion. The apparent flux through the coupled system is greater than what can be accounted for by isolated NDPK activity -- hence the possibility exists for potential substrate channeling. Physical interaction between the enzymes is also implied by the cosedimentation observed in the glycerol gradient studies. Moreover, the apparent ATP-linked STK activity in rabbit liver and heart may be due to the combination of coexisting GTP-linked STK and NDPK activities. Similar work with mitochondrial phosphoenol-pyruvate carboxykinase may suggest an analogous relationship with a NDPK activity.

(Supported by National Science Foundation ASEND grant RII8610675).

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(38) MAXIMIZING PRODUCTION OF PHYTOTOXIC METABOLITES IN CULTURES OF COCHLIOBOLUS SATIVUS

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Cochliobolus sativus (Ito & Kurib.) Drechs. produces phytotoxic metabolites when grown in liquid culture (1). Two toxic components have been characterized and have been shown to cause root stunting on wheat and barley (2,3). Screening for resistance to cereal root rot caused by C. sativus may be possible using these phytotoxic extracts. Large scale screening of wheat and barley genotypes for root rot resistance requires that large quantities of the toxic components be produced. In previous studies production was limited to small volume cultures (50 ml) (1,3). The present study was done to establish a method to identify the time of optimum toxin production in larger volume cultures.

C. sativus (strain #R02, originally recovered from an infected spring wheat plant) was grown in liquid culture in 600 ml of glucose-peptone mineral salts broth (1) in 2.8 l culture flasks on an orbital shaker at 25 C. Each flask was inoculated with a suspension containing 10^7 conidia of C. sativus. Samples (10 ml) were removed under sterile conditions every 24 hr for 120 hr, and thereafter every 12 hr up to 192 hr. Mycelium was removed from the samples by filtration immediately after withdrawal and relative viscocity was determined using an Ostwaldt Viscosimeter (Model 300); the pH was also noted at this time. Samples were then frozen until bioassayed. Each sample was thawed, filtered by mild vacuum through Whatman #42 filter paper and then through a 0.45 μm membrane filter. Each sample was bioassayed using germinated lettuce seedlings. In this test relative phytotoxicity is indicated by root growth inhibition (1). Four replicate plates were used for each sample time. To each plate was added 2 ml of test solution. Ten lettuce seedlings with roots 5 $\pm$ 1 mm long were placed in each plate. Sterile distilled water and sterile culture medium were used as controls. Culture broths were diluted 1:3 with sterile distilled water to lower their activity to a measurable range (1). Plates with seedlings and solutions were incubated in the dark at 25 C for 30 hr after which root length of each seedling was measured. The pH, relative viscocity and root length means for each sampling are shown in Figure 1. Standard deviations in root length measurements are indicated by vertical bars.

Significant root growth inhibition was first observed at 48 hr. Inhibition increased at subsequent sampling times, reaching a maximum at 144 hr, after which it began to disappear. By 192 hr this activity had almost disappeared from the broth. The pH dropped slowly over the entire time but did not show behavior which would make it a useful indicator of maximum phytotoxicity. Relative viscocity increased only slightly until 96 hr; after 96 hr it increased sharply from 1.6 to 5.9 at 132 hr, and thereafter leveled off. The time at which the relative viscocity was determined to have leveled off (144 hr), was close to the time of maximum root inhibition. This experiment was repeated twice with similar results; in each case the time at which relative viscocity leveled off after a peak corresponded closely to the time of maximum root growth inhibition.

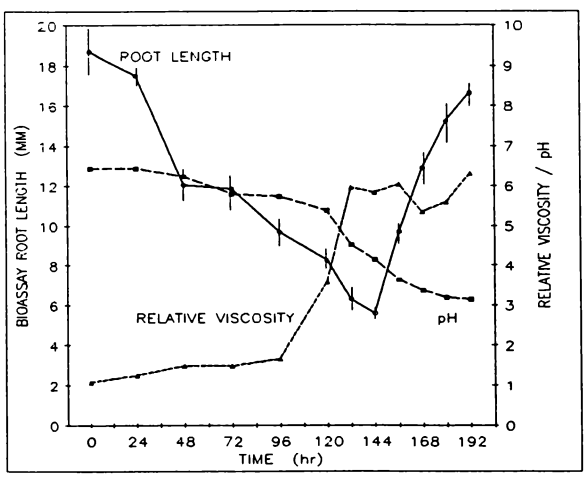

Figure 1. Phytotoxicity, pH and relative viscosity in cultures of C. sativus over time. Vertical bars indicate standard deviations.

From four subsequent production runs 21 cultures (600 ml each) were harvested at the time of peak relative viscocity. Of these cultures 71% contained broths of high toxic activity. This is a great improvement over the previously used method of harvesting cultures at specific times where only 16% of the cultures contained high activity. The bioassay accurately indicates level of activity in the culture broths but cannot be used for testing during growth because it takes too long (30 hr).

Relative viscocity can be used to predict harvest time for maximum toxin activity. This test can be performed very easily and results obtained within one hour. Rapidity is important since nearly 1/3 of the toxicity was lost within 12 hr after peak activity was reached. Use of this rapid test makes possible production of large batches of highly active toxin for mass screening of wheat and barley lines for disease resistance.

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(39) SYNTHESIS OF OLIGO(ρ-PHENYLENEHYDROXYMETHYLENE)

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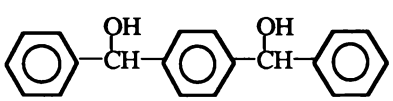
Previous work in our group has focused on Friedel Crafts alkylation reactions of homocyclic and heterocyclic aromatic nuclei. This procedure has proved useful in the case of soluble low molecular weight polymers, which can be obtained by careful control of stoichiometry and reaction conditions.[1] Usually, the products obtained are not linear, but contain segments of crosslinking and chain branching, due to the nonregiospecific nature of this reaction. Crosslinking of the chains hinders the solubility of the polymer, rendering the solution or melt processing of these materials difficult. Our current aim is to utilize a more regiospecific reaction to produce soluble, linear polymers, while maintaining desirable physical and mechanical properties. The method we have chosen involves condensation of a dilithioaromatic with an aromatic dialdehyde. This affords regiospecific polymerization to give materials with hydroxymethyl bridges between the aromatic nuclei. As a model reaction, 1,4-dibromobenzene was treated with τ-butyllithium to form a 1,4-benzene dianion. Subsequent condensation of the dianion with benzaldehyde produced 1,4-bis(α-hydroxybenzyl)benzene (1).[2] The fixed substitution of the starting material is carried over to the product, by this method.

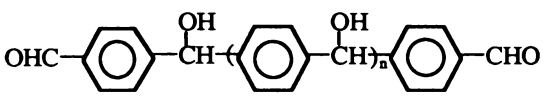
Herein we describe the ongoing investigation of the step growth polymerization of 1,4-dibromobenzene and terephthaldicarboxaldehyde to produce oligo(ρ-phenylenehydroxymethylene) (2). Synthesis of the oligomer was achieved by addition of dibromobenzene (1.18g, 0.005 mol) to a solution of τ-butyllithium (11.8 ml, 0.02 mol) in dry ether, which had been cooled to -20°C. The reaction was run in a three-necked flask equipped with an addition funnel, a drying tube, a magnetic stirrer and a slow flow of N_2. After the addition of dibromobenzene the reaction mixture was allowed to warm to room temperature and stirred for 3 hours. Terephthaldicarboxyaldehyde (0.61 g, 0.005 mol) was added to the reaction mixture. A further 90 minutes of reaction time was employed before quenching the reaction with 60 ml of NH_4Cl. The light brown solid produced was separated from the solution and washed with ethanol. The reaction produced 0.08g of a light brown solid having a melting point of 110-114°C. Initial work has produced low yields, but the reaction conditions are currently being optimized.

The structure of the oligomer was deduced through NMR and FT-IR (Diffuse Reflectance Infrared Transmittance Spectra DRIFTS) spectroscopy and the correlation of these spectra with those of the model compound 1. Although the IR bands of the oligomer are slightly broader, there is good agreement with the data obtained for 1. Table I illustrates this correlation. As expected, the oligomer displays an additional peak at 1695 cm^{-1} due to the presence of the aryl aldehyde group. The ^{1}H-NMR resonances of oligomer 2 correspond well with those of model compound 1. These values are listed in Table II along with the terminal aldehydic chemical shift present in 2. Similarly, ^{13}C-NMR data of 1 and 2 agree well, with the exception of the carbonyl resonance inherent in 2. The presence of terminal aldehyde bands in the IR spectrum of the oligomer suggests the proposed structure 2. The length of the product can be determined using proton integration values. The relative integration values for our compound are 8:1 for the aromatic to aldehydic protons. This ratio is believed to be a result of equal molar mixtures of 2, with n=1 and 3 whose integration ratios are 6:1 and 10:1 respectively.

Work is currently underway to increase the chain length of the oligomer to produce poly(ρ-phenylenehydroxymethylene). The initial results of this investigation are promising and lead us to believe that the halogen exchange reaction will produce linear, regiospecific polymers.



1



2 n = 1, 3

Table 1 FT-IR Spectral Results

Compound	IR bands (cm^{-1})
1	3265, 1599, 1508, 1280, 1014, 835
2	3356, 1695, 1605, 1508, 1278, 1014, 842

Table II ^{1}H-NMR Spectral Results

Compound	Proton Chemical Shift (ppm)
1	7.33, 5.77, 4.76
2	9.97, 7.30, 5.73, 4.71

ACKNOWLEDGEMENT: The authors gratefully acknowledge financial support from the National Science Foundation for the purchase of the Varian (VXR-300)NMR and the Digilab FTS 40 FT-IR spectrometers.

1. Glenn, J.E. and Jones, M.B., ACS Polym. Prepr. in press.
2. Livesay, M.T. and Jones, M.B., to be presented at 19th Nat. ACS Meeting, April 1989.

(40) BENZYLIC FUNCTIONALIZATION OF π-ARENE CHROMIUM TRICARBONYL COMPLEXES

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Our research group is investigating the use of π-arene chromium tricarbonyl ($Cr(CO)_3$) complexes in the development of selective and quantitative degradation techniques for coal structure elucidation through benzylic functionalization and rearrangement. The enhanced benzylic acidity of π-arene chromium tricarbonyl complexes should lead to facile derivatization of the benzylic position resulting in the potential for degradation via one of several rearrangement schemes (e.g. benzylic hydroperoxide and Beckmann rearrangements). We report here several examples of benzylic functionalization in the π-arene chromium tricarbonyl complexes of coal model compounds.

All reactions were carried out in Schlenk glassware under an inert atmosphere of argon. The π-arene chromium tricarbonyl complexes were prepared by heating the arene with $Cr(CO)_6$ in either a tetrahydrofuran (THF)/dioxane or a n-butyl ether/THF mixture. The solvents were either used as purchased or dried and distilled from suitable drying agents. Reaction time, temperature and base used are as indicated below. Gas chromatography (GC) and actual weights were used to quantitate yields. Nuclear magnetic resonance (NMR), infrared spectrometry and mass spectral data were used to determine reaction products. Hexamethylbenzene, 1,2,3,4-tetrahydronaphthalene (tetralin), and diphenylmethane were used as coal model compounds.

Deprotonation of hexamethylbenzene chromium tricarbonyl with nBuLi (2 eq.) in THF at -23°C followed by quenching with excess methanol-O-d, resulted in > 97% mono-deuterium incorporation. In an attempt to form the benzylic alcohol necessary for the benzylic hydroperoxide rearrangement, the anion was treated with two known oxygen transfer reagents; 2-(p-toluenesulfonyl)3-phenyloxaziridine[2] and chromium pentoxide-pyridine complex.[3] In both cases low mass recoveries were encountered with little or no conversion to the benzylic alcohol. It was found, however, that reaction of the anion under the above conditions with tributyl borate followed by hydrolysis of the resulting boronic ester with acidic H_2O_2 resulted in a 79% conversion to pentamethylbenzyl alcohol. When treated under benzylic hydroperoxide rearrangement conditions (concentrated H_2O_2 in acetic acid) this primary alcohol yielded only 5% of the rearranged product, pentamethylphenol. Future attempts will be made at optimizing the yield of the phenol.

Reaction of the anion of tetralin chromium tricarbonyl (formed as above) with tributylborate resulted in 60% recovered yield of the ring hydroxylated products 5,6,7,8-tetrahydro-2-naphthol and 5,6,7,8-tetrahydro-1-naphthol in a 9:1 ratio. Attempts at using weaker bases such as lithium hexamethyldisilazide, sodium hexamethyldisilazide, and t-BuOK under many reaction conditions has proven unsatisfactory. These results suggest that a different base is needed if successful benzylic deprotonation is to compete with aromatic ring deprotonation.

The chromium tricarbonyl complex of diphenylmethane ($DPMCr(CO)_3$) offered a greater benzylic proton acidity due to the additional attached phenyl ring. Treatment of $DPMCr(CO)_3$ with 2.1 equivalents of lithium diisopropylamine (LDA) at 0°C in THF followed by quenching with excess D_2O led to significant deuterium incorporation into the benzylic position as seen by ^{13}C NMR (J_{C-D} = 19.6 Hz). The corresponding reaction with tributyl borate resulted in only 7% conversion to the benzylic alcohol, benzhydrol, along with 9% conversion to ring hydroxylated product(s). The remainder of the reaction mixture was starting material. These results suggested that anion formation was equilibrating between the ring and benzylic position. In the hopes of selectively trapping one anion or the other, an in-situ reaction of the borate with the anion formed above from LDA was attempted. This scheme resulted in a 57% yield of only ring hydroxylated products. This reaction did, however, provide valuable information about the position of ring deprotonation. It was found by ^{13}C NMR to consist of 66% meta and 34% para hydroxylation. The chemical shifts of the meta isomer were confirmed by independent synthesis and compared to those of the commercially available para isomer.[4]

Recent reports[1] in the literature of the use of potassium hydride (KH) and 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) in THF to deprotonate π-arene complexes in the benzylic position was also investigated. Treatment of the anion formed from KH/18-crown-6/THF system separately with excess D_2O and with methyl iodide confirmed benzylic anion formation. In the case of the D_2O quench only a di-deuterated product was found (as confirmed by ^1H, ^2H, ^{13}C NMR and GC-mass spectrum). The methyl iodide quench resulted in 90% conversion to 1,1-diphenylethane with the remainder of the reaction mixture containing starting material. Further work will be done on this base/solvent system to determine its applicability to π-arene complexes in general.

1. Semmelhack, M.F., et al. (1981) Tetrahedron, 37, 3957.
2. Davis, D.A. et al. (1979) Journal of American Chemical Society, 4, 1044.
3. Regen, S.L.; Whitesides, G.M. (1973) Journal of Organometallic Chemistry, 59, 293.
4. Ceccon, A. et al. (1983) Journal of Organometallic Chemistry, 254, 199.

(41)

A NEW ROLE FOR IRON(III) CITRATE AND MALATE COMPLEXES IN IRON HEMOSTATIS

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The mechanism or mechanisms by which the body controls non-heme iron absorption are unknown at the present time. Nutritional studies have identified ascorbic acid (Vit. C) and citric, malic and tartaric acids as ligands (iron complexers) promoting non-heme iron absorption. The role of Vitamin C in enhancing iron absorption has been attributed to its ability to a) complex iron and b) reduce iron(III) to iron(II), the bioavailable form of iron. The alpha-hydroxypolycarboxylic acids (citrate, malate and tartrate) are thought to function by complexing iron and keeping it in a soluble form for duodenal absorption.

The iron(III) complex of citrate undergoes photoreduction at acid pH's forming iron(II) and eliminating CO_2; and the tetravalent anion of citric acid, present at pH's 11 and higher, also undergoes oxidative decarboxylation. The behavior of malic and tartaric acids is similar to citric acid.

Recent studies in our laboratories show that the iron(III)-citrate complex, $[Fe_2(H_{-1}cit)_2]^{2-}$ (H_{-1} cit = tetravalent anion of citric acid) undergoes a slow, thermal oxidative decarboxylative reaction at pH's 1.8 and 3.0 producing iron(II). The reduction reaction is rapid in solutions exposed to room light but is slow in the dark. The rate of iron(III) reduction in the dark is significantly enhanced by adding iron(II) trapping reagents such as 1,10-phenanthroline, or 2,2-'bipyridyl. In the absence of citric acid, the reduction of iron(III) to iron(II) by either trapping reagent alone is negligible. The rate of reduction of iron(III) in solutions containing a large excess of citric acid (i.e., 10:1 or greater) depends on the concentration of the trapping reagent and the total iron(III) concentration as well as pH. The large excess of citric acid avoids extensive complexation of iron(III) by the trapping reagent and converts the iron(III) to the dimeric species.

The slow oxidation of alpha-hydroxypolycarboxylic acids complexed to iron(III) may be an important reaction because the body could chose, on demand, to catalyze this reaction to increase the concentration of bioavailable iron(II). Hence, the redox reaction of alpha-hydroxypolycarboxylic acid complexed iron(III) play an important role in controlling iron hemostatis.

(42) DATA ACQUISITION SYSTEM FOR GOES SERIES SATELLITES

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With the advent of environmental satellites, there have been continuing advances made in their mechanics and communication formats. The most advanced and recent of the satellites to go into orbit are the Geostationary Operational Environmental Satellite (GOES) series.

The latest of these satellites are the GOES D-H satellites which transmit data using mode AAA format. This format has been used for many years and has been proven to be quite useful. One problem with the mode AAA format, however, is that it is quite expensive to transmit the actual data. This is because each block of data must occupy 50ms of time. If there is not enough data to fill the time, then the block is filled with data zeros. One other problem is that there is one block out of every twelve that contains nothing but zeros. These problems made mode AAA format rigid and limited the types and amounts of data that could be transmitted.

One of the latest advances in satellites is three-axis stabilization. This allows for data to be transmitted continually without interruption and also allows for varying periods of block lengths. This permits a greater flexibility in the transmission of the data for a much lower cost. When the transmission of data in a particular block is complete, the next block of data can be transmitted immediately, instead of transmitting zeros. This quickens the transmission of blocks and allows for more blocks to be transmitted in the same amount of time. This new technology is the main reason for creating the latest transmission format, GVAR (GOES I-M Variable), which will be the format for the GOES I-M series satellites planned to be launched in the near future.

In the fall of 1988 the Atmospheric Sciences Department at the University of North Dakota came to the Electrical Engineering Department for help. They wanted a system that could not only receive data from the existing GOES D-H satellites transmitting with mode AAA format, but also wanted a system that, with a flip of a switch, could receive data from the GOES I-M satellites transmitting with GVAR format.

Separate commercial systems, known as frame synchronizers, are available to receive both types of formats, but none that can receive both. The cost of the individual systems range anywhere from $10,000 to $30,000 each. What the Atmospheric Sciences Department wanted was one system to do both for under $15,000. A system to accomplish this has been designed at a cost of about $500 for parts.

The frame synchronizer was designed to accept data in logic form from either an optical fiber link or direct wiring. The optical fiber link option is added to enable the frame synchronizer to be located large distances away from the receiving dish. Besides just the data stream, there must also be an accompanying clock signal at 2.11 MHz that is in synchronization with the data stream.

The output of the system has five channels for the five types of data, infrared one and two, visual, documentation, and sounder/auxiliary data. The different types of data are sent in a specific sequence according to the format of the data received. They are separated and directed to the appropriate output channels by the system. Each of the channels outputs the data in parallel form and has its own handshaking signals to communicate to the receiving computer.

In the case where the receiving computer is not able to input the data as fast as the frame synchronizer can output, there is also some on-board memory that temporarily stores the data until the computer has time to input the data.

The only external controls to the frame synchronizer are a system clear button and a format switch. The system is automatically cleared on start-up, but the external button was added in case the system gets out of synchronization with the data. The format switch has two positions, AAA and GVAR, representing mode AAA and GVAR formats, respectively.

At this time, the frame synchronizer has not been put into actual use. This is due to the lack of the receiving equipment needed. Nevertheless, the system has been extensively tested using simulated data streams.

FRANZ H. RATHMANN
8 April 1904 - 18 September 1988

Franz Rathmann was born in Gotha, Florida, and raised in St. Paul, Minnesota. He received his B.A. in Mathematics and Astronomy and his M.A. in Organic Chemistry from the University of Minnesota. His doctorate (Natural Science), from the University of Goettingen, Germany, was awarded in 1941 on the basis of his work on the synthesis of vitamins B1 and E.

During World War II Rathmann achieved the rank of Lieutenant Commander in the U.S. Navy. After the war he worked for the Navy on rocket propellants at the University of Minnesota. He also taught at the University of Illinois, James Millikin University, the University of Omaha and the University of Saigon, Vietnam, before joining the faculty in the Department of Chemistry at North Dakota Agricultural College (NDSU) in 1955. He was promoted to Professor of Organic Chemistry in 1959, retiring from that position in 1974.

Rathmann published some 70 papers in various American, German, British, French, Vietnamese, and Russian journals. He also served as an abstractor for Chemical Abstracts for some 40 years. He was the North Dakota Academy of Science's delegate to the Council of the American Association for the Advancement of Science for 18 years.

Upon retirement Rathmann returned to his early hobby and followed the paths of solar eclipses around the world, reporting on the events every year at the annual meetings of the Academy.

Rathmann was a member of the Academy from 1955 until his death.

JOYCE (LAVANCHY) LABORDE
7 August 1930 - 25 October 1988

Joyce Lavanchy was born in Sussex, England and received her Nursing diploma in Eastbourne, Sussex. She immigrated to the United States where she earned her master's and doctoral degrees in Nursing from the University of Illinois.

She came to the University of North Dakota in 1981 where she became Professor of Nursing and taught in the graduate program in the College of Nursing. Her research focused on the relationships of osteoarthritis and multiple sclerosis to climate. Laborde received several major grants to fund her research and shared the results of her research though numerous publications and presentations.

Laborde was nationally and internationally recognized for her professional accomplishments. She was designated a Bristol-Myer Fund Scholar by the American Nurses Foundation. She was an officer and a reviewer for the Midwest Nursing Research Society, a reviewer for Nursing Research and Health, the Sigma Theta Tau International Research Grants Program, and a delegate on the Geriatric Medicine and Rehabilitation Specialist Team at the United States-China Conference in Beijing.

Laborde was a member of the Academy from 1983 until her death.

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

(Founded 1908; Official State Academy 1959)

ARTICLE I - Name and Purpose

1. This association shall be called the North Dakota Academy of Science.

2. The purposes of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

ARTICLE II - Membership

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

2. Annual dues for the various categories of membership shall be determined by the members present at the Annual Meeting.

ARTICLE III - Officers

1. The officers of the Academy of Science shall be a President, President-Elect, and the Secretary-Treasurer who shall perform the duties usually pertaining to these offices. The President-Elect shall be chosen by ballot at the Annual Meeting and will hold the office for one year and then assume the office of President for one year. The Secretary-Treasurer shall be appointed for a three-year term by the Executive Committee.

2. The Executive Committee, consisting of the above-named officers, the retiring President, and three members-at-large, shall have charge of the ordinary executive duties. The members-at-large shall be elected for a three-year term on a rotation basis.

ARTICLE IV - Meetings

1. There shall be an Annual Meeting each year held at such time and place as the Executive Committee may determine.

2. Special meetings shall be called by the President upon the request of ten percent of the active members. Only matters specified in the call can be transacted at a special meeting.

3. Ten percent of the active members shall constitute a quorum at the Annual Meeting. Special meetings require twenty percent of the active members for a quorum.

ARTICLE V - Miscellaneous

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

2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in, or intervene in, any political campaign on behalf of any candidate for public office.

3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

ARTICLE VI - Amendments

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

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

NORTH DAKOTA ACADEMY OF SCIENCE

BY-LAWS

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

2. The annual dues shall be determined by a two-thirds vote at an Annual Meeting. These dues are payable January 1 of each year. (1965 Rev.)

3. Members shall be dropped from the active list on December 31 following the nonpayment of dues during the membership year commencing the previous January 1. A member may return to the active list by paying the current year dues and a membership renewal charge of $5.00. (1975 Rev.)

4. Every member in good standing shall receive a copy of the annual Proceedings of the North Dakota Academy of Science. (1965 Rev.)

5. Special offices such as Historian may be created by the unanimous vote of the members at the Annual Meeting. (1965 Rev.)

6. The Executive Committee shall annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science. (1979 Rev.)

7. The Committee structure of the Academy shall be as follows, the President appointing the members and chairpersons for all except the Executive Committee:

   a. Executive Committee

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

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

   b. Editorial Committee

      Membership: Three members, three-year terms.

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

   c. Education Committee

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

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

   d. Denison Awards Committee

      Membership: Six members, three-year terms.

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

   e. Necrology Committee

      Membership: Three members, three-year terms.

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

f. Nomination Committee

Membership: The five most recent past-presidents.

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

g. Resolution Committee

Membership: Three members, three-year terms.

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

h. Membership Committee

Membership: Unlimited number, appointed annually.

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

8. The Nominating Committee shall bring in two nominations for each office. Other nominations may be made from the floor. The officers shall be elected by ballot at the Annual Meeting. (1965 Rev.)

9. Categories of membership:
   a. Active members shall be persons interested or actively participating in some scientific endeavor. Active members may participate in all activities of the Academy.
   b. Student members shall be graduate or undergraduate college students in some field of science. Student members may participate in all activities of the Academy, with the exception of holding office.
   c. Sustaining members are persons or organizations interested in the activities of the Academy. Sustaining members may participate in all activities of the Academy, with the exception of voting or holding office. Sustaining members may be of three types: Individual, Corporate, or Institutional. (1965 Rev.) This bylaw is implemented by the following action of the Executive Committee (10-25-85):

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

      Benefits accruing to Corporate Sustaining Members include:

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

      Until action is taken otherwise, the Corporate Sustaining Membership fees shall be placed in the North Dakota Science Research Foundation for the support of scientific research.
   d. Emeritus membership. Any member in good standing upon formal retirement is eligible for emeritus membership. Nominations may be forwarded to the Membership Committee by any member, and it shall be the responsibility of the Membership Committee to review the membership list for possible candidates. The Executive Committee shall approve nominations. Emeritus members shall retain all rights of active members but will be exempt from payment of dues. (1973 Rev.)
   e. Honorary Membership. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to

science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. Any member may submit nominations along with supporting data to the Membership Committee. Approval of nominations shall be by a two-thirds majority of those attending the annual meeting. (1973 Rev.)

10. The President, with the approval of the Executive Committee, shall appoint members to serve on ad hoc committees. Reports of ad hoc committees shall be presented to the Executive Committee or to the annual meeting. Ad hoc committees serve only during the tenure of the president who appointed them. (1965 Rev.)

11. The Executive Committee shall appoint an Editor who shall edit the Proceedings. The Editor shall be appointed for a three-year term. The salary of the Editor shall be set by the Executive Committee. (1975 Rev.)

12. The annual dues shall be $12.00 per year for professional members, with $2.00 designated for the North Dakota Science Research Foundation, and $5.00 per year for student members. (1985 Rev.)

13. The Executive Committee is empowered to charge a publication fee of authors of up to $10.00 per page. (1965 Rev.)

14. All student research participants shall receive a properly inscribed certificate and be invited to the dinner as the guests of the Academy. (1965 Rev.)

15. All activities of the Academy, including grant applications, are to be handled through the Academy offices from now on. (1966 Rev.)

16. The Executive Committee of the North Dakota Academy of Science be instructed to establish a J. Donald Henderson Memorial Fund and that the committee administer this fund and that the proceeds from this fund be used to promote science in North Dakota. (1967 Rev.)

17. The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be January 1 to December 31. (1973 Rev.)

18. The Academy establishes the NDAS Achievement Award, to be awarded periodically to an Academy member, in recognition of excellence in one or more of the following:

    a) Nationally recognized scientific research.
    b) Science education.
    c) Service to the Academy in advancing its goals.

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

Revised May 1988

OFFICERS AND STANDING COMMITTEES FOR 1988-89

EXECUTIVE COMMITTEE

Bonnie Heidel, Past-President
ND Parks and Recreation Department

Forrest H. Nielsen, President
Human Nutrition Research Center

David Davis, President-Elect
Metabolism and Radiation Laboratory

A. William Johnson, Sec.-Treasurer (1986-89)
University of North Dakota

Clark Markell, Member-at-Large (1986-89)
Minot State University

William Dando, Member-at-Large (1987-90)
University of North Dakota

Carolyn Godfread, Member-at-Large (1988-91)
Bismarck

EDITORIAL COMMITTEE

Claude Schmidt (1988-91), Chairman
Metabolism and Radiation Laboratory

Duane Erickson (1986-89)
North Dakota State University

Douglas Johnson (1987-90)
NPWRC, Jamestown

RESOLUTIONS COMMITTEE

Richard Baltisberger (1988-91), Chairman
University of North Dakota

Allen Kihm (1986-89)
Minot State University

Lee Manske (1987-90)
North Dakota State University

NOMINATING COMMITTEE

Elliot Shubert (1986-91), Chairman
University of North Dakota

Michael Thompson (1985-90)
Minot State University

Gary Clambey (1984-89)
North Dakota State University

William Barker (1987-92)
North Dakota State University

Bonnie Heidel (1988-93)
ND Parks and Recreation Department

SCIENCE EDUCATION COMMITTEE

Clark Markell (1987-92)
Minot State University
(Executive Liason, Chairman)

Mike Burton (1984-89), Agassiz Junior
High School (Science Olympiad)

Jerome Knoblich (1986-90), Jamestown
College (Minigrants Coordinator)

Michael Stoy (1986-91), Bismarck State
College (Junior Academy)

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

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

Ron Royer (1988-92), Minot State University
(Newsletter)

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ORING, LEWIS W.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
ORR, PAUL H.	1010 RIVER DRIVE SOUTHEAST	EAST GRAND FORKS	MN 56721
OWEN, ALICE K.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
OWEN, JOHN B.	1118 REEVES DRIVE	GRAND FORKS	ND 58201
OWEN, SHUBEL D.	210 SOUTH 12TH STREET	GUTHRIE CENTER	IA 50115
O'CONNELL, JAMES W.	535 EIGHTH AVENUE SOUTHWEST	VALLEY CITY	ND 58072
PADMANABHAN, G.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
PARK, CHUNG S.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
PARMAR, SURENDRA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
PATTERSON, DONALD D.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
PATTON, BOB	RR 1, BOX 13E	STREETER	ND 58483
PECKHAM, RICHARD A.	1016 2ND AVENUE SW	MINOT	ND 58701
PEDERSON, A. ROBERT	414 20TH AVENUE NORTH	FARGO	ND 58102
PEEP, ELVIE	RR 1, BOX 76	GLENBURN	ND 58740
PENLAND, JAMES G.	1804 SOUTH 36TH STREET	GRAND FORKS	ND 58201
PETERKA, JOHN J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
PFISTER, PHILIP C.	30 MEADOWLARK LANE	FARGO	ND 58102
POELLOT, RHONDA LEE	3816 SIMONVIEW COURT	GRAND FORKS	ND 58201
POLITI, EILEEN E.	109 CHEVY CHASE	MINOT AFB	ND 58704
POWERS, BRIAN	P.O. BOX 1727	FARGO	ND 58107
POWER, GREG	106 3RD STREET	RIVERDALE	ND 58565
PRATT, GEORGE L.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
PRUNTY, LYLE	318 23RD AVENUE NORTH	FARGO	ND 58102
RABINDRAN, EVELYN A.	RURAL ROUTE 1, BOX 285	JAMESTOWN	ND 58401
RADA, KEVIN	814 NORTHWESTERN DRIVE	GRAND FORKS	ND 58201
RADONOVICH, LEWIS	1522 CHERRY	GRAND FORKS	ND 58201
RALSTON, NICK V.C.	4859 FIFTH AVENUE NORTH	GRAND FORKS	ND 58201
RALSTON, ROBERT	MAYVILLE STATE UNIVERSITY	MAYVILLE	ND 58257
RAND, ROGER W.	542 FIFTH AVENUE SOUTHWEST	VALLEY CITY	ND 58072
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RAWAT, BANMALI	UNIVERSITY OF NEVADA, RENO	RENO	NV 89557
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REED, LYNN	315 HAMLINE STREET, #1	GRAND FORKS	ND 58201
REEVES, PHILIP G.	812 NORTH 25TH STREET	GRAND FORKS	ND 58201
REICHMAN, GEORGE A.	306 SIXTH AVENUE NORTHWEST	MANDAN	ND 58554
REID, JOHN R.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
REINKE, ROBERT	BOX 391	RAY	ND 58849
REZANIA, SHAHIN	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
REZVANI, AHMAD	BOX 8073	GRAND FORKS	ND 58201
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RICHARDSON, JOLENE	BOX 31	VELVA	ND 58790
RIES, RONALD E.	908 SECOND AVENUE NORTHWEST	MANDAN	ND 58554
RIGLEY, LOUIS	DICKINSON STATE UNIVERSITY	DICKINSON	ND 58601
RINDT, DIANE	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
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ROBERTS, KRIS	1414 SPAULDING AVENUE	BISMARCK	ND 58501
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SARGEANT, ALAN B.	N PRAIRIE WILDLIFE RES. CENTER	JAMESTOWN	ND 58401
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SCHLICHTING, JAMES D.	2222 BIRCHMONT DRIVE	BEMIDJI	MN 56601
SCHLOSSER, ISAAC	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
SCHMIDT, CHERYL K.	815 DUKE DRIVE, #102	GRAND FORKS	ND 58201
SCHMIDT, CLAUDE H.	STATE UNIVERSITY STATION	FARGO	ND 58105
SCHNEIDER, FREDERICK	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
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SCHULZ, JOHN T.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
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SCOBY, DONALD R.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND 58105
SEABLOOM, ROBERT W.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
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SEVERSON, ROLAND G.	705 CHESTNUT STREET	GRAND FORKS	ND 58201
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SHUKLA, SANJAY	114 24TH AVE. N.	FARGO	ND 58102
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SIDERS, WILLIAM A.	1105 SOUTH 22ND STREET	GRAND FORKS	ND 58201
SILVERMAN, LOUIS B.	2524 OLSON DRIVE	GRAND FORKS	ND 58201
SIMONS, PAUL B.	ANG COAL GASIFICATION	BEULAH	ND 58523
SIMS, RODGER L.	718 25TH STREET NORTH	GRAND FORKS	ND 58201
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SMITH, GLENN S.	1115 NORTH 14TH STREET	FARGO	ND 58102
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STOAKS, RALPH D.	8047 EL CAPITAN DRIVE	LE MESA	CA 92041
STORBAKKEN, LISA	1019 4TH AVE. S.	FARGO	ND 58103
STOY, W. MICHAEL	1826 NORTH BELL STREET	BISMARCK	ND 58501
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SWANSON, RICHARD J.	507 THIRD STREET COURT	WEST FARGO	ND 58078
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THOMPSON, MICHAEL B.	2208 CRESCENT DRIVE	MINOT	ND 58701
THRASHER, LAWRENCE C.	104 KUCHENSKI DRIVE	DICKINSON	ND 58601
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TIMPE, RONALD C.	604 STANFORD ROAD	GRAND FORKS	ND 58201
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TRIEBOLD, BEVERLY	1729 NORTH 4TH STREET	FARGO	ND 58102

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WYCKOFF, JOHN W.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND 58202
WYMORE, ROBERT W.	350 FIRST STREET NORTHWEST	MAYVILLE	ND 58257
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