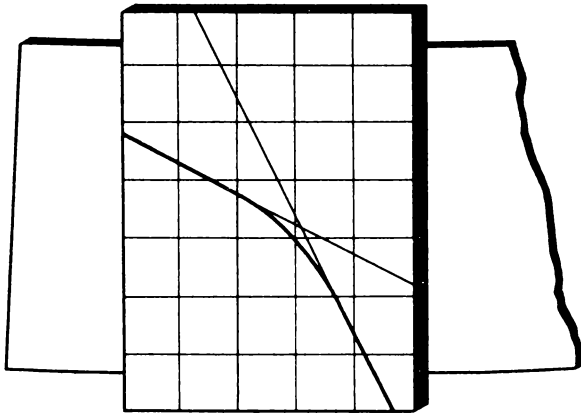# PROCEEDINGS

of the

NORTH DAKOTA
ACADEMY OF SCIENCE

ABSTRACTS

65TH ANNUAL MEETING

APRIL 27 and 28, 1973

University of North Dakota
Grand Forks, North Dakota

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ABSTRACTS

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65TH ANNUAL MEETING

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SURFACE CONTOURS OF THE SUBARACHNOID SPACE AS REVEALED BY SCAN-
NING ELECTRON MICROSCOPY. D. J. Allen, M. W. Cloyd and J. J.
Malloy. Department of Anatomy, University of North Dakota,
Grand Forks, North Dakota. (spon: F. N. Low)
    Young dogs were perfused with buffered aldehydes (Karnovsky,
JCB, 27:317A, 1965). MICROFIL (100ml) was injected to prevent
collapse of thin-walled subarachnoid veins. For scanning
electron microscopy critical point drying and gold-palladium
coating were used. Surface contours of the brain vary greatly.
Pial surfaces of cerebrum and cerebellum are smooth, showing
overlapping cells and only occasional fibrous connective tissue.
Arachnoid surfaces are rough, showing overlapping cells and are
primarily fibrous. Pial surfaces in both areas possess fenes-
trations. Other areas also possess smooth pial and rough fib-
rous arachnoid surfaces, with variations. Spinal cord pia and
nerve root sheath possess fairly smooth surfaces showing flat-
tened, overlapping cells. Many have sparse microvilli located
mostly at the cell boundaries. Fenestrations with fibrous
connective tissue underneath are present in the pia. Surface
of nerve exits which may be double or single according to level,
are very complex. Arachnoid trabeculae, fenestrations and a
lateral recess complicate observable surface contours.

BIOTIN TRANSPORT AND ACCUMULATION IN LM CELLS. Joy K. Anderson
and James R. Waller. Dept. of Microbiology, Sch. of Med., Univ.
N. Dak., Grand Forks, N. Dak.
    Biotin uptake in Lactobacillus plantarum has been shown to
occur by a process involving active transport. This study was
undertaken to determine if active transport of biotin would also
occur in a mammalian cell line. LM cells were grown in sus-
pension culture in Medium 199 containing 0.5% peptone, 10 ng
biotin/ml, and 0.12% methylcellulose. Cells were harvested from
the log phase of growth and washed once with Hank's balanced salt
solution (HBSS). All transport studies were carried out in HBSS
containing the desired level of biotin. Bound biotin levels did
not change appreciably in any experiments. Free biotin uptake at
35°C in the presence of glucose was indistinguishable from biotin
uptake at 4°C and without glucose. In both instances intra-
cellular free biotin (IFB) rose rapidly, then dropped to a level
less than 50% of the maximum, then rose again. This undulating
pattern may indicate that an exchange occurred between IFB and an
ion in HBSS. Intracellular/extracellular biotin ratios were near
2.0, and saturation kinetics could not be demonstrated. Biotin
transport was not diminished by either iodoacetate or the biotin
analogue homobiotin. These results suggest that biotin transport
in LM cells occurred by free diffusion rather than by active
transport.

PROGRESS REPORT ON BIRD LOSSES AT THE OMEGA TOWER. <u>M. L. Avery</u> <u>and J. F. Cassel</u>. Dept. of Zool., NDSU, Fargo, N.D. and <u>P. F. Springer</u>. No. Prairie Wildlife Res. Ctr., Jamestown, N.D.

The effects on migrating birds of the 1200-foot tower at the Omega Navigation Station in the James River Valley near LaMoure, N.D. have been investigated since its completion in Sept. 1971. The study is being conducted by the NPWRC in cooperation with NDSU. During the fall of 1971, searches of sampling areas at the tower site for dead and injured birds were made several times a week. Daily searches at dawn were conducted during the spring and fall of 1972. Based on the birds found on sampling areas, the total estimated losses for the three migration periods were 941, 1084, and 1037, respectively. Warblers and finches comprised the majority of the kills. Nighttime observations with a portable ceilometer during the fall of 1972 revealed that birds were attracted to the tower in significant numbers on overcast nights. Conversely, on clear nights more birds were seen 1000 feet from the tower than at the tower. Most birds observed at the tower on overcast nights milled about in a disoriented manner. The largest kills occurred on these nights and resulted from collisions with the tower and its guy wires. During the day, birds avoided the tower without difficulty. Tests of a commercial sound device to deter nocturnal migrants were made.

THE ASSOCIATION OF CHROMIUM(III) AND THIOCYANATE IONS IN ACIDIC WATER METHANOL SOLUTION. <u>R. J. Baltisberger and C. L. Knudson</u>. Dept. of Chemistry, Univ. of N. Dak., Grand Forks, N. Dak.

The rate of association of chromium(III) and various anions is accelerated in water-methanol solvents. Kinetic studies show that the acceleration is due to the reactivity of $Cr(OH_2)_5(MeOH)^{3+}$, (I). This study was initiated to determine if methanol is being preferentially displaced. Three $CrNCS^{2+}$ products of the association of $Cr(OH_2)_6^{3+}$,(II), and (I) with thiocyanate were separated and identified. One product contained no coordinated methanol, while the other two contained one methanol each and were tentatively identified as <u>cis</u>- and <u>trans</u>- isomers. The <u>cis</u>- and <u>trans</u>-species aquate at rates nearly identical to the aquation of $\overline{II}$, 1×10^{-5} sec^{-1} compared to 3.2×10^{-5} sec^{-1}. Over an initial ten hour period 70 to 80% of the association products of species I and thiocyanate retain methanol. The data indicates that there is not exclusive replacement of the coordinated methanol and that three reaction pathways are important.

THE ROLE OF SOLVATION IN ION EXCHANGE EQUILIBRIA OF CHROMIUM(III) IN WATER-METHANOL SOLUTION. Richard J. Baltisberger and Cleon M. Melsa. Chemistry Department., Univ. of North Dakota, Grand Forks.

Ion exchange selectivity of strong acid cation exchange resin is dependent on a number of factors. Two of these factors which were examined in this study are the differences, 1) in the solvation of exchanging ions in the external phase and 2) in the solvation of exchanging ions in the internal solvent phase. The equilibrium quotients, Q_e, have been determined for the exchange of pentaaquo(methanol)chromium(III), $Cr(H_2O)_5(CH_3OH)^{+3}$, with hexaaquochromium(III) ion on four different crosslinked cation exchange resins, while varying the mole fraction of the methanol-water. Q_e was found to increase with increasing crosslinkage and to go through a minimum value at 30% mole methanol. The composition of the internal solvent phase was measured for each of the exchanging ions. The greatest difference in solvation of ions occurs near the minimum in Q_e. NMR studies were used to measure solvation of ions in the external phase. In the external solvent both ions appear to be equally solvated. The results are consistent with differences in internal solvation as being the more important parameter in variation of the selectivity.

PUMPING AN ARTESIAN AQUIFER TO DRAIN THE SALINE AREA IN GRAND FORKS COUNTY, NORTH DAKOTA. L. C. Benz and E. J. Doering. Agricultural Engineers, Northern Great Plains Research Center, Mandan, N. Dak.

A 179-day, single well, pumping test on the confined aquifer was conducted to evaluate pump drainage as a means of reclaiming saline soils in eastern Grand Forks County. The high water table and salinity problems are sustained by artesian pressure in an underlying aquifer from which approximately 0.5 inch per year of saline water moves upward into the overburden. This upward flow carries about 470 lb of salt per acre per year into a surface profile that already contains 960 tons of soluble salt per acre. The pumping test reduced pressure in the aquifer to lateral distances greater than 5 miles. Assuming pressure redistribution to be analogous to linear heat flow in a slab, analysis of the pumping test data showed (1) the average hydraulic diffusivity was 1.44 ft^2 per day for the lower 40 feet of overburden and (2) continuous pumping for 7.1 years would be required to lower the water table 5 feet. From this analysis, it appears that pump drainage for the area is feasible since well, pump and operating costs would be about $0.34 per acre per year.

PISIDID CLAMS (BIVALVIA: PISIDIDAE) FROM THE TONGUE RIVER AND
SENTINEL BUTTE FORMATIONS OF NORTH DAKOTA.   David Bickel.
Dept. of Physical Sci., Minot State Coll., Minot, N. Dak.
   The family Pisididae (=Sphaeriidae) includes small fresh-water
clams commonly termed fingernail, pea, or pill clams.   Three
genera, Sphaerium, Pisidium, and Eupera range from the Cretaceous
to the present and a fourth genus, Byssanodonta, occurs in the
living fauna of South America.   Pisididae of the Tongue River and
Sentinel Butte Formations include: Sphaerium formosum (Meek and
Hayden), S. subellipticum (Meek and Hayden), S. cf. S. aequale
Russell, S. cf. S. planum Meek and Hayden, Eupera sp., Pisidium
russelli Bickel, and P. wardensis Bickel.   Pisidium russelli and
P. wardensis were recently described from one locality in the
Tongue River Formation (Bickel, 1973, Nautilus 87:14-16).
Subsequent work showed these two species to be common in the
Tongue River Formation.   Nine of 50 localities in the Tongue
River and Sentinel Butte Formations produced Pisididae.   Pisidium
russelli or P. wardensis, or both occurred in 7 Tongue River
Formation samples.   Clams belonging to Eupera were recovered from
the Tongue River and Sentinel Butte Formations.   The geologic
record of Eupera in North America is quite limited.   Yen (1946,
Notulae Nat. 166:7) referred one North American species from the
Lower Cretaceous to Eupera and mentioned records for the genus
from the Eocene of North America.

PARATHYROID HORMONE AND BLOOD FLOW TO BONES.
J. N. Boelkins, W. J. Mueller, and K. L. Hall. Dept. Physiol. &
Pharmacol., Univ. No. Dak., Grand Forks, No. Dak. and Dept.
Poultry Science, Penn. State Univ., Univ. Park., Pa.
   Labeled microspheres were used to measure the distribution of
cardiac output to 17 organs of the laying hen at 0 and 3 or 30 min
after iv injection of parathyroid hormone or its carrier.   At 3 min,
which is the time of maximum hypocalcemia in the hen, blood flow
to the femur, tibia and metatarsus was significantly reduced rela-
tive to 0 time, while at 30 min, which corresponds to the maximum
hypercalcemia, blood flow to these three bones was significantly
increased.   Femoral blood flow and the venous minus arterial cal-
cium gradients across the femur were positively correlated, irre-
spective of sampling time (0 or 30 min) and type of injection.
These results suggest that alterations in bone blood flow may be a
mechanism by which parathyroid hormone maintains calcium homeo-
stasis.   The only other organs which showed significant changes
in blood flow after PTH were the adrenals, thyroids and shell
gland; the cerebellum, parathyroids, heart, spleen, liver, pan-
creas, duodenum, colon, magnum, isthmus and kidneys were not af-
fected.   Supported by National Institutes of Health Grant AM 04362.

ALTERATION OF OFFSPRING SEX RATIOS IN MICE AFTER AIR DIVES TO AN EQUIVALENT OF 198 FEET SEAWATER. D. M. A. Brennan and S. J. Brumleve. Dept. of Physiol. and Pharmacol., Sch. of Med., Univ. No. Dak., Grand Forks, No. Dak.

It has been observed that professional divers (commercial and military) tend to have families with predominantly female children. Experiments using white mice, 30-60 days old, were designed to study the effects of hyperbaric N_2-O_2 (25% O_2) on the sex ratios in the f_1 generation offspring. All mice, female and male, were housed individually in separate cages. The male mice were divided into two groups - controls (unpressurized) and experimentals (pressurized). The experimentals were pressurized daily for 28 days in a chamber to 88.2 psig (or 7 ATA) (or 198 ft) at a rate of 0.5 ATA/min. After 1 hour of pressure they were continuously decompressed at a rate of 1 ATA/15 min. The males were each mated with one female each week during the 2nd, 3rd, and 4th weeks of pressurization. Controls were mated concurrently. Experimental results indicate a shift of nearly 10% toward an increase in female offspring of the pressurized males.

A SIMPLE, DIRECT METHOD FOR THE ISOLATION OF CLOSTRIDIUM PERFRINGENS FROM FOOD AND WATER. P. J. Colberg and M.C. Bromel. Dept. of Bact., N. D. S. U., Fargo, N. Dak.

The purpose of this report is to introduce a rapid diagnostic method which is specific in isolating from food and water members of the clostridial family, particularly Clostridium perfringens, the causative agent of gas gangrene in man. The culture medium consisted of 0.2% neutral red dye solution combined with Liver Veal Agar (Difco). A triple pour technique was employed in order to achieve anaerobiosis. The 25 mm petri plates were incubated in a Gas Pak Jar (BBL) in an atmosphere of hydrogen and carbon dioxide at 37° C. After incubation periods of 18-24 hours, the plates were examined under natural illumination and under ultraviolet from a Mineralight. Discrete colonies of clostridial sp. were distinguished by their exhibition of two types of fluorescence. No other organisms tested or isolated from samples exhibited this unique feature. This method was found to be superior to conventional procedures. The recommended practice of heat shocking these anaerobic sporeformers was found to eliminate recovery of C. perfringens. The method is simple, rapid, inexpensive, and results in the immediate isolation of Clostridia from food and water. Supported in part by NSF.

SULFHYDRYL INVOLVEMENT IN SYNTHETIC AND HYDROLYTIC ACTIVITIES OF MULTIFUNCTIONAL GLUCOSE-6-PHOSPHATASE. William Colilla and Robert C. Nordlie. Biochem. Dept., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

Classical liver microsomal glucose-6-phosphatase (EC 3.1.3.9) has both synthetic and hydrolytic activities as Glucose-6-P + $H_2O \rightarrow$ glucose + P_i and Carbamyl-P + glucose $\rightarrow$ glucose-6-P + carbamate. Although inhibitions by sulfhydryl reagents have been reported, no detailed study has previously been undertaken. In the present study, time-dependent inhibitions of both activities are seen to be essentially parallel. Further, this is maintained with a variety of enzyme preparations (microsomes, deoxycholate treated microsomes, and partially purified preparations). The inhibition is dependent on 1) nature of the inhibitor (p-chloromercuriphenyl sulfonate>Hg^{2+}>N-ethylmaleimide>iodosobenzoate>iodoacetate), 2) inhibitor concentration, and 3) reaction pH. Cysteine, reduced glutathione, and mercaptoethanol partially reverse the inhibition; phosphate substrates (but not glucose) protect partially against inhibition. A unit change in the slope of a Dixon plot at pH 8.6 supports the involvement of a sulfhydryl group in the binding of phosphate substrates. (Supported in part by grants from the Amer. Diab. Assn. and National Institutes of Health (AM 07141).

THE HYPOLIMNETIC OXYGEN DEFICIT OF SILVER LAKE MINN. G. W. Comita, K. G. Lesteberg and T. R. Jacobsen, Dept. of Zool., NDSU, Fargo, N. Dak.

Oxygen depletion in the hypolimnion of Silver Lake began about the 7 May 1972. In the next 30 days the hypolimnion became anaerobic. The rate of depletion was about 0.05 mg oxygen cm^{-2} day^{-1}, the rate for a clearly eutrophic lake. The lake began accumulating ammonia-nitrogen on the 19 May 1972, when it was 0.03 g N m^{-2}. On the 6 June about 0.28 g N m^{-2} was recorded, which is about a 9.6 times increase. After 6 June it decreased to about 0.185 g N m^{-2} the concentration recorded on the 27 June; it then increased to a seasonal maximum of 0.417 g N m^{-2} on the 22 August. Four pulses of primary production were measured. These appear to be reciprocally related to the accumulation of ammonia nitrogen.

A COMPATIBILITY-LINKED, HAPLO-LETHAL FACTOR IN RACE 1, USTILAGO NIGRA (TAPKE). L. C. Darlington and R. L. Kiesling. Dept. of Plant Path., Col. of Agr., NDSU, Fargo, N. Dak.

After isolation, two of the four sporidia from single teliospores of race 1, U. nigra always died. Failure of these two sporidia to grow on complete media indicated lethality was not due to nutritional factors. Tetrad analysis of 180 teliospores was performed to study the inheritance pattern of this lethal factor. Sporidia of each tetrad were removed in order from their promycelium. Segregation for the lethal factor occurred in the first meiotic division in 59 teliospores. The ratio of the two possible types of sporidial arrangements was 29(1,2): 30(3,4), which fits a 1:1 ratio. Segregation for the lethal factor occurred in the second meiotic division in the remaining 121 teliospores. The ratio of the four possible types of sporidial arrangement was 29(1,3):32(1,4):31(2,3):29(2,4), which fits a 1:1:1:1 ratio. Mating tests were carried out by making Bauch tests with two known compatibility types from race 3 which showed that the lethal factor was tightly linked to one compatibility type. The linked lethal and compatibility factors were 33.6 map units from the centromere. Testing of sporidia isolated at random have shown no recombinants of the lethal and compatibility linkage.

MERCURY CONCENTRATIONS OF FOOD PRODUCTS AND OF SOILS IN NORTH DAKOTA. F. D. Deitz, J. L. Sell and M. L. Buchanan. An. Sci. Dept., NDSU, Fargo, N. Dak.   58102

Recent discovery of high levels of mercury (Hg) in waterfowl in the state prompted a Hg survey of agricultural products in North Dakota. Milk, eggs, soil, pork and beef tissue (longissimis dorsi and liver) were among the products analyzed. Tissue samples were subjected to wet-oxidation using concentrated HNO_3 and H_2SO_4 acids with vanadium pentoxide as a catalyst. Subsequently, Hg in the digests was determined by the "cold-vapor" technique, utilizing atomic absorption spectrophotometry. The milk samples analyzed averaged 0.8 ng/ml (ppb) with a range of 0.0 to 7.0 ng/ml. Beef longissimis dorsi samples average 4.5 ng/g with a range of 0.0 to 18.8 ng/g while liver samples from the same animals average 10.1 ng/g (range of 0.0 to 29.5 ng/g). Pork longissimis dorsi samples averaged 5.4 ng/g with a range of 0.0 to 12.5 ng/g while liver samples from the same animals average 12.9 ng/g (range of 0.0 to 26.2 ng/g). Eggs and soil samples had an average mercury content of 15.8 ng/g and 32.7 ng/g respectively.

RECOVERY OF VIRUS FROM VARIOUS WATER SOURCES. <u>Hugh DePaolo and</u>
<u>J. J. Kelleher</u>. Dept. of Microbiology, Sch. of Med., Univ. N.
Dak., Grand Forks, N. Dak.

A polyelectrolyte adsorption and a two-phase separation pro-
cedure were used to concentrate animal viruses from various water
sources. In applying the polyelectrolyte method to the recovery
of approximately 10^5 viral particles from lake water, various re-
covery rates were noted for five stock viruses. Quantitation of
the concentrate was done by a most probable number method (MPN)
and a plaque assay method (PFU). In laboratory studies the PFU
method seems to be more sensitive for quantitating lower numbers
of viral particles while the MPN method more applicable to larger
numbers of viral particles. Field studies conducted on lake
water and sewage samples indicate the polyelectrolyte method is
capable of recovering virus. This field study also indicated
that quantitation by MPN may be more sensitive than the PFU
method for detection of mild type virus. The effects of pH,
temperature, ion concentration and the number of viral particles
for recovery of virus on polyelectrolyte are now under investi-
gation. The two phase recovery method has been used in pre-
liminary studies on the Grand Forks lagoon system with several
viral isolates. These preliminary studies seem to indicate
aeration may have an effect on viral survival in sewage
treatment.

SALINE-SEEP DEVELOPMENT ON HILLSIDE SITES IN WESTERN NORTH
DAKOTA. <u>E. J. Doering and F. M. Sandoval</u>. Northern Great Plains
Research Center, ARS, USDA, Mandan, N. Dak.

Saline seeps and their downslope wet areas occupy several
hundred thousand acres in the northern Great Plains and about
80,000 acres in western North Dakota. New seeps have been
occurring each year. Soil borings, hydraulic head and flow
measurements, chemical analyses, and climatic data show that
seeps are caused by a combination of geologic, climatic, and
cultural conditions--geologic because seeps occur where highly
permeable, nearly horizontal, layers approach the hillside;
climatic because recharge is from precipitation that falls on
adjacent upslope land; and cultural because the prevailing
crop-fallow system of farming increases the opportunity for soil
water to percolate past the root zone. The percolating water is
salinized as it passes through the soil system. When several
highly permeable layers occur in the profile, two or more seeps
can occur at different elevations on one hillside. Discharges
from two instrumented seeps ranged from 1 to 24 gpm during 1972.
Solving the problem requires that deep percolation be eliminated
by cropping systems that use the available water in the recharge
area or that the water be intercepted above the seep and
conducted in drains to a suitable outlet.

BENZENE PHOTOCHEMISTRY---THE ALPHA CLEAVAGE

D.R. Dutton and V.I. Stenberg
Department of Chemistry
The University of North Dakota, Grand Forks

The nature of para substituents on O-phenyl-N-methyl-carbamates has been shown to influence the extent of alpha cleavage (aryl-oxygen bond scission) during irradiation. Of substituents tested, the p-phenyl and p-dimethylamino groups enhanced alpha cleavage to the greatest amount. For the separate evaluation of the inductive and resonance effects of the dimethylamino group, a comparison of O-(p-dimethylamino)-N-methylcarbamate 1 irradiation with that of O-(3,5-dimethyl-4-dimethylamino)-N-methylcarbamate 2 was done. In 2, the ortho substitution forces the dimethylamino group into a conformation which considerably restricts the lone pair electrons from resonance interaction with the aromatic ring. The partial experimental basis for the reduced resonance interaction in 2 as compared to 1 is evident from ultraviolet spectral studies of both compounds. Experimental results show that overlap of the dimethylamino group lone pair electrons with the aromatic ring, thus stabilizing the excited state, is of utmost importance in determining the extent of the alpha cleavage reaction.

METAL ATOM CHEMISTRY: ZERO-VALENT π COMPLEXES. H. F. Efner and K. J. Klabunde, Dept. of Chem., UND, Grand Forks, N. D.

The synthesis of new zero-valent complexes of hexafluoro-benzene (HFB) with first row transition metals has been accomplished in our laboratories using techniques developed by P. S. Skell and coworkers (P.S. Skell and J.J. Havel, J. Amer. Chem. Soc., 93, 6687, (1971)), in which a metal is reacted, as a free isolated atomic species, with an organic reactant. The metal atom technique has also been used for the direct synthesis of known zero-valent complexes such as bisbenzene vanadium, bis-benzene chromium and bisacrylonitrile nickel in high yields. Stable HFB complexes of special interest are those formed with vanadium, chromium and nickel. These highly reactive compounds decompose violently on heating or on contact with air or other reactive reagents at room temperature. Isolation and identification of these complexes is complicated by their reactivity and insolubility in common organic solvents. The nickel-HFB adduct appears to be a 1:1 complex which is an active catalyst and reacts rapidly with hydrogen, carbon monoxide, alkyl halides, butadiene, and other compounds under very mild conditions. More detailed studies of the reactions, structures, and bonding of these complexes are under way. Supported by Research Corp. and in part by NSF. (Grant GP-34493).

THE USE OF INSECT CELL LINES IN THE STUDY OF HORMONE ACTION.
P. E. EIDE and T. S. Adams. Metabolism & Radiation Research
Laboratory, USDA, ARS, State University Station, Fargo, ND
58102.
        The recent development of cell lines from the embryonic
tissue of houseflies, Musca domestica (now in its 57th passage),
and tobacco hornworms, Manduca sexta (now in its 19th passage),
provided us with an in vitro method for assaying the effects of
hormones and other chemicals on insect development at the
cellular level. Initial studies were conducted with the molt-
stimulating hormone ecdysterone. Both lines were different
genetically and physiologically in terms of nutritional re-
quirements, and both responded to the hormone treatment by
undergoing a dramatic morphogenetic change when compared with
the controls. The responses appear to be time- and dose-
dependent. When the cells were treated with the inactive iso-
mer 22-isoecdysone, no morphogenetic changes occurred. Auto-
radiographic studies indicated that the addition of the
hormone suppressed or stopped DNA synthesis but stimulated RNA
synthesis, a condition that occurs when cells undergo differ-
entiation.

SERUM MINERAL LEVELS IN EWES. D.O. Erickson, M.R. Light, C.N.
Haugse, and J.E. Tilton. Dept. of An. Sci., NDSU, Fargo, N. Dak.
        Serum samples from 130 ewes were analyzed for phosphorus,
calcium, magnesium, potassium and copper to study the effects of
breeds (Suffolk, Columbia and Hampshire), pasture vs. dry lot,
vitamin E and selenium treatments and bleeding at prepasture
season, prebreeding and preparturition. All of the mineral
levels were higher (P<.01) just prior to breeding than prior to
the pasture season. Potassium and copper levels were higher
(P<.01) near parturition compared to prebreeding. There were
breed differences (P<.05) for all minerals except copper with
phosphorus being higher and calcium and magnesium lower in
Hampshires. Intraruminal injections of vitamin E did not
alter blood serum mineral levels but vitamin E plus selenium
increased (P<.05) the copper levels. Pasturing ewes prior to
the breeding season did not alter the mineral levels of the
serum.

ACETATE PRODUCTION AND UTILIZATION IN AN ANAEROBIC–AEROBIC
WASTE TREATMENT SYSTEM, G. M. Fillipi* and J. W. Vennes.
Dept. of Microbiol., Univ. of N. Dak., Grand Forks.
     Acetate followed by propionate was the most abundant
volatile fatty acid produced under anaerobic conditons in an
anaerobic-aerobic waste treatment system concomitantly treat-
ing domestic sewage and potato processing wastes. Acetate
was shown to increase in the anaerobic cells at all temperatures
(1-25C) whereas propionate increased at temperatures less than
5C but decreased at temperatures greater than 10C. The two
aerated cells efficiently removed volatile fatty acids either
from raw or anaerobically treated waste. Substrate utilization
kinetics were determined following sodium acetate and ammonium
chloride additions to two aerobically-designed experimental
systems. The systems contained either combined wastes
plus the indigenous microbial population or Millipore-treated
combined wastes inoculated with a pure culture of acetate-
utilizing bacteria (Acinetobacter) isolated from the treatment
system. Nearly identical removal rates of acetate, ammonia
and BOD (5-day) were observed at temperatures of 6.5 to 8C
by both systems. However, at a temperature of 18 to 20 C
greater amounts of acetate, ammonia and BOD were shown to be
removed by the indigenous microbial population than by the
pure culture system with Acinetobacter.

A C DIELECTRIC CONSTANT IN HYDRAZINE SULFATE. Paul L. Gourley,
Dept. of Physics, Univ. N. Dak., Grand Forks, North Dakota.
          It has been reported (Journal of Magnetic Resonance,
8,311-315, 1973) that hyrdazine sulfate, $N_2H_6SO_4$, exhibits
a first order phase transition with thermal hysteresis near
$478^{\circ}K$. In an effort to further substantiate this, measurements
have been made of the real and imaginary parts of the ac
dielectric constant of $N_2H_6SO_4$ in powder samples and along the
a and c crystal axes in single crystals. The measurements were
carried out with an ac resistance-capacitance bridge employing
an oscilloscope with dual signal preamp in the subtract mode
for detection of the balance condition. Measurements were made
over the temperature range from $220^{\circ}K$ to $485^{\circ}K$ at frequencies
of 10KHz and 100KHz.The measured values for both real and
imaginary parts of the ac dielectric constant change by more
than a factor of two near $478^{\circ}K$ which suggests a phase
transition. Evidence of a thermal hysteresis was also observed.

OIL CONTENT OF SUNFLOWER SEEDS AS DETERMINED BY
WIDE-LINE NUCLEAR MAGNETIC RESONANCE (NMR). M.
Granlund and D. C. Zimmerman. Dept. of Biochem., NDSU,
Fargo, N. Dak.

Wide-line NMR is used to determine oil content by measur-
ing the hydrogen content of the oil in the seed. This study was
undertaken to establish the optimum methods of sample prep-
aration and analysis for our laboratory conditions for sunflower
seed. Two varieties of sunflower seed, Mingren and Peredo-
vik, were dried in a laboratory oven for varying times and
temperatures. Three samples of each type were weighed,
dried, and analyzed by wide-line NMR. Confectionary type and
oil type seeds dried for one hour at 130^0C or twenty-four hours
at 60^0C removed the greatest amount of moisture. It was found
that additional drying at 130^0C or 60^0C reduced the dry weight,
used in calculating the oil content, but did not significantly af-
fect the NMR reading for either the oil or confectionary type
seed. This study indicates that additional drying at 130^0C or
60^0C does not affect the ratio of NMR units per gram of seed,
nor does it increase precision of the method of analysis.

VOLATILE FATTY ACID CONCENTRATIONS OF CATTLE FED CEREAL GRAINS.
Olafur Gudmundsson, D.O. Erickson, W.E. Dinusson and C.N. Haugse.
An. Sci. Dept., NDSU, Fargo, N. Dak.   58102.

The influence of grain rations on the volatile fatty acid (VFA)
concentrations, ratios and pH in the rumen was investigated in 72
Hereford yearling steers. Six grains were tested (barley, soft
wheat, hard wheat, triticale, corn and barley with ergot) with or
without 30% oats substitution. Four of the rations contained
0.1-0.2% ergot in the grain. Rumen samples were taken by suction
strainer method approximately 3-4 hrs. after morning feeding,
every 6th week during the 147 day feeding period. The samples
were analysed for VFAs and pH was determined. Rumen concentra-
tions of acetate (C_2), propionate (C_3) and butyrate (C_4) varied
(P$<$.05) among rations as did the ratios of C_2/C_3, C_2/C_3+C_4 and
pH. The substitution of 30% oats to the various grains resulted
in significant changes in the individual acids, acid ratios and
pH of the rumen. The C_3 and C_2 levels were lower (P$<$.05), total
acids $C_2+C_3+C_4$ were lower (P$<$.05) for most rations pH was higher
(P$<$.05) and for most rations C_2/C_3 ratios were higher (P$<$.05).
The ratios of C_2/C_3 and C_2/C_3+C_4 were wider when the wheats were
fed compared to barley. When ergot was present in barley the
individual acids were lower and pH was higher indicating a
reduced microbial activity. The metabolism in the rumen varies
among feeds and combinations of feeds.

SYNTHETIC AND HYDROLYTIC ACTIVITIES OF NUCLEAR, MITOCHONDRIAL, AND MICROSOMAL GLUCOSE-6-PHOSPHATASE: A COMPARATIVE STUDY. Hans M. Gunderson and Robert C. Nordlie. Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak.

Glucose-6-phosphatase (I; EC 3.1.3.9) is generally accepted as being associated with the endoplasmic reticulum of the cell. Our studies indicate it to be also present in the nuclear fraction and strongly suggest its presence in the mitochondrial fraction isolated from avian liver homogenates. Both the hydrolytic activity, I, and the synthetic activity, carbamyl phosphate: glucose phosphotransferase (II), have been found. Activities of I and II in microsomal and mitochondrial fractions are activated by detergent treatment (deoxycholate, cholate, taurocholate, Triton X-100, cetrimide) in contrast to nuclear fractions which are activated little (<10%) or not at all. The orientation of the enzyme in the membrane could account for the contrasting affects. Km values for glucose (22mM), carbamyl phosphate (1mM), and glucose-6-phosphate (1mM), as well as pH optima, for I (6.2) and II (5.3) in detergent-free nuclei match those of detergent treated microsomes. I and II also were found in nuclei from avian kidney, and rat, rabbit, and guinea pig liver. A generalized role for I and II in glucose transport is suggested. (Supported in part by NIH (AM 07141) and Amer. Diab. Assn.)

THERMODYNAMIC STUDIES OF CHYMOTRYPSIN IN LIGHT AND HEAVY WATER. L.M. Gutnik, J.M. Linkletter and J.A. Stewart. Dept. of Chem., Univ. of N. Dak., Grand Forks, N. Dak.

Before a complete understanding of enzyme catalysis can be reached, both the mechanistic and energetics of the system must be known. The mechanistic aspect involves the structural and electronic factors, whereas the energetics concerns the thermodynamic activation parameters which are responsible for rate enhancement. If extensive thermodynamic results were available in light and heavy water, the energetics of rate enhancement could be better examined. With this in mind, the acylation kinetics of the chymotrypsin-catalyzed hydrolysis of 2,4-dinitrophenyltrimethylacetate was investigated in light and heavy water at several temperatures. The rate of aclyation was 1.4 times faster in light water than in heavy water, and as expected, there was an increase in the energy of activation(2 kcal/mole) in the case of heavy water. In both solvents the entropy of activation was negative, which shows that the system becomes more orderly when acylation occurs. In heavy water, the entropy change was less negative. These results can not be explained in terms of structural changes alone, but must be examined using solvation and solubility criteria.

PRELIMINARY STUDY OF THE CARBONATES OF THE CHERTY MEMBERS OF
THE BIWABIK FORMATION, MINNESOTA. L. R. Honeyman and
F. R. Karner. Dept. of Geol., Univ. N. Dak., Grand Forks,
N. Dak.
     Siderite (FeCO$_3$) and ankerite (ferroan CaMg (CO$_3$)$_2$) of pri-
mary, diagenetic and metamorphic origin are the principal car-
bonates in the magnetite-rich cherty members (taconite) of the
Biwabik Formation. Carbonate distribution which affects taco-
nite pellet quality, was studied in drill-core and pit samples
from the Erie Mining Co. property north of Hoyt Lakes, Minnesota.
Carbonates typically occur in irregular layers and lenses in
sequence of alternating cherty and magnetitic sedimentary beds.
Siderite typically occurs as fine-grained massive micritic
material. Ankerite occurs with siderite as rhomb-shaped crystals
up to several millimeters in length and as radial aggregates up
to several centimeters in diameter. Siderite appears to have
formed as a primary mineral during sediment deposition while
ankerite formed during diagenetic and metamorphic recrystalli-
zation. Supported in part by the Erie Mining Co.

PETROLOGY AND SOURCE ROCKS OF THE SAND OF THE PALEOCENE TONGUE
RIVER FORMATION, NORTH DAKOTA. Arthur F. Jacob. Dept. of
Geology, Univ. N. Dak., Grand Forks, N. Dak.
     The sand of the Tongue River Formation in North Dakota is
fine and angular. The matrix consists mostly of silt- and clay-
size grains of carbonate that are difficult to distinguish from
the carbonate cement. All thin sections examined are calc-
sedlitharenites according to Folk's (1968) classification.
Limestone grains are common, indicating the presence of abundant
limestone in the source area. Chert, shale, and volcanic rock
fragments are also present. Low feldspar content and quartz
grains that show mostly straight or slightly undulose extinction
indicate a scarcity of igneous and metamorphic rock in the
source area. The lack of polycrystalline quartz grains indicates
a scarcity of metamorphic rocks in the source area. The source
area probably was the uplifting Laramide ranges either in Mon-
tana, Wyoming or South Dakota where miogeosynclinal rocks were
being exposed. The basement rock in the interior of the ranges
that served as the source was not yet exposed.

AMINO ACID ABSORPTION AS INFLUENCED BY ALCOHOL. F.A. Jacobs and C.B. Fabel. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, ND   58201

Investigations were undertaken to study the influence of ethanol upon the absorption of amino acids, especially methionine, from the intestinal lumen of the rat in vivo. We have reported previously: 1) that ethanol inhibited the absorption of 3mM L-methionine, and 2) the physiological concentrations of amino acids within the intestinal lumen of rats under various feeding conditions. Rats from 3 distinct genetic backgrounds were perfused with 1mM methionine; ethanol was observed to have different effects on methionine absorption in the varied strains. With inulin (^{14}C-carboxyl) present in the perfusate as a water marker, more fluid was absorbed from the perfusate when ethanol was present. When the perfusate contained 16 amino acids as normally found in rat small intestine, ethanol was observed to have a negligible effect on the absorption of the amino acids. This suggests that ethanol may have little influence on amino acid absorption from the rat small intestine under such conditions and that the presence of the other amino acids may protect the absorption of any single amino acid from the effect of ethanol, under the conditions of our experiments. (Supported in part by NIH Research Grant No. MH 19235 ALC).

A POST-ROTATORY ELECTRONYSTAGMOGRAPHIC PROCEDURE IN THE CHRONICALLY IMPLANTED GUINEA PIG. C.B. Jensen, S.J. Brumleve, J. Hootman, and R. Peterson. Depts. of Physiol. and Pharmacol. and Elec. Engr., Univ. No. Dak., Grand Forks, No. Dak.

Periorbital, subdermal electrodes were chronically implanted in adult male guinea pigs. The animals were individually confined in restraining cages constructed for this investigation and secured to a turntable. Electrical contact from the animal to a polygraph was constantly maintained through a three-channel mercury commutator attached to the turntable. Post-rotatory nystagmus was induced by rapidly stopping the turntable after 10 rotations at 40 rpms. All rotatory and post-rotatory events were performed in darkness to prevent visual fixation by enclosing the turntable and guinea pig in a metal chamber. The chamber also provided a Faraday shielding for the preparation. Electronystagmus was monitored on a CRT, recorded on polygraph paper and stored on electromagnetic tape for subsequent analogue to digital conversion and computer analysis. Evaluation of the graphical representation of vestibular eye movements is discussed.
Supported in part by ONR Contract No. N00014-68-A-0499.

GLUCONEOGENESIS IN INTACT RABBITS AND IN ISOLATED PERFUSED LIVERS.
D.C. Johnson, C.A. Huibregtse and P.D. Ray, Dept. of Biochemistry,
Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak., 58201.
    Our studies of gluconeogenesis in rabbits have been extended
to isolated livers which are perfused for about 2 hours with rab-
bit red blood cells resuspended in oxygenated Krebs-Ringer HCO_3^-
buffer. That these livers maintain physiological viability in
this system is demonstrated by maintenance of normal color, con-
stant rate of perfusate flow, constant rate of bile production
and oxygen uptake. In this system, rabbit livers convert lactate
to glucose at a rate of about 0.8 μmoles/min/g liver. This rate
for glucose formation is very close to that found for rats and
pigeons and suggests somewhat similar requirements among species.
Development of this system now offers an opportunity to pursue
more detailed studies of glucose formation in rabbits. In a con-
tinued search for factors capable of altering activities of en-
zymes involved in gluconeogenesis in rabbit liver, we find that
mannoheptulose rapidly and extensively increases the activity of
cytosolic phosphoenolpyruvate carboxykinase(PEPCK) without ex-
tensively altering the activities of fructose 1,6-diphosphatase
or glucose 6-phosphatase. While large doses of tryptophan given
to rats increase extensively the activity of PEPCK and simulta-
neously inhibit hydrocortisone-induced gluconeogenesis, no such ef-
fects are observed in rabbits. NIH AM 12705; GRS NIH 5 S01 RR 05407.

THE STATISTICAL ESTIMATION AND COMPARISON OF GROWTH RATES.
Douglas H. Johnson. Northern Prairie Wildlife Research Center,
Jamestown, N. D. 58401
    Many biological experiments require the comparison of
growth rates of different organisms. Although several
measures of growth can be defined, the fact that growth
curves generally are sigmoid in shape imparts some arbitrariness
to the choice because the rate of growth itself changes with
time, so the value used to measure growth depends upon the
time at which it was taken. However, if the data are adequately
described by one of the usual growth curves, such as the
logistic, the von Bertalanffy or the Gompertz, then a unique
parameter defining growth rate can be identified. For body
weights of two mallard (Anas platyrhynchos) strains, the
logistic curve was shown to provide the most satisfactory
fit. Parameters were estimated both by non-linear least
squares, a technique with some optimal statistical properties
but requiring fairly large amounts of computer time, and by
linear least squares applied to transformed variables, a less
exact but faster technique. Finally, the comparison of
average growth rates for the two mallard strains is discussed.

Analysis of chromosome abnormalities in three species of
Agropyron: G. A. Johnson and S. M. Jalal

Microsporocytes of three native polyploid species of
Agropyron (A. repens, A. trachycaulum, and A. dasystachum) were
analyzed for chromosomal abnormalities. Preliminary investiga-
tions indicate that the most common chromosomal irregularity
arises from the lack of homology, resulting in unoriented
chromosomes at metaphase I and II, lagging chromosomes at
anaphase-telophase I and II and micronuclei at dyad and quar-
tets. A relatively high frequency of these aberrations sug-
gests that the three species are actively evolving to achieve
complete diploidization. Physiological abnormalities of
chromosomes encountered in a few plants were, pycnosis, stick-
iness, coenocyte formation and genomic nondisjunction. These
forms of aberrations apparently arise from and act as a screen-
ing mechanism against non-harmonious genomic combinations in
these complex polyploid species.

THE SPECIFICITY OF CHEMICALLY MODIFIED CHYMOTRYPSIN.
P. E. Johnson, M. C. Low, and J. A. Stewart. Department
of Chemistry, UND, Grand Forks, N. Dak.
The 15 exposed carboxyl groups of α-chymotrypsin were
blocked with glycine ethyl ester. The modified enzyme
had no activity toward the neutral substrate N-benzoyl-
L-tyrosine ethyl ester, but slight activity toward the
N-acetyl ester. However, it was found to be nearly as
reactive as the native enzyme over the pH range 3.7 to
8 with L-phenylalanine benzyl ester which has a free
α-amino group. Therefore, it was decided to investigate
the kinetics of hydrolysis by the modified enzyme of
two other charged substrates, L-tryptophan benzyl and
ethyl esters (TBE and TEE). The specificity of the
modified enzyme for TBE and TEE was studied from pH
4 to 8. The results were analyzed in terms of a
general mechanism based on acid-base equilibria which
permitted the rate constants for catalysis by the
different protonated forms of the enzyme to be determined.

USL OF BROMIDE ION ELECTRODE TO DETERMINE REACTION KINETICS-
AN UNDERGRADUATE EXPERIMENT. J. Knoblich. Dept. of Chem.,
Jamestown Col., Jamestown, N. Dak.
     Most undergraduate organic chemistry laboratory manuals
contain at least one experiment related to reaction kinetics
but few incorporate modern instrumentation. Typically the
rate is determine by titration of either a reactant or product.
The objective of this project was to develope an undergraduate
organic syntheis-kinetic project type experiment in which the
reaction rate can be followed with a specific ion electrode.
Bryan and Olafsson (J. Chem. Ed., 42, 248, 1969) reported that
the decomposition of N-bromobenzamide in base to aniline and
bromide ion is an excellent undergraduate experiment for the
deetermination of rate constants and activation energy. They
studied the system by removing aliquots into glacial acetic
acid/potassium iodide solution and titrating with thiosulfate.
Their experimental procedure was modified by measuring the
bromide ion as it is formed during the decomposition with a
bromide ion electrode. Rate constants were determined at four
different temperatures and activation energy calculated. The
results agreed well with those reported in the literature.

DDT INDUCED MUTATIONS IN MICE. K. D. Larsen and S. M. Jalal.
Dept. of Biol., University of N. Dak. Grand Forks, N. Dak.
     DDT induced mutations were investigated in laboratory mice.
Twenty-four brown and Balb/c strain albino mice were intraperi-
toneally injected with 25, 50, 100, or 250 ppm (mg per kg body
weight) of DDT in peanut oil. Controls received either no
injection or peanut oil only. Karyotypes from bone marrow cells
of long bones were analyzed for gaps, deletions, and stickiness.
Data for chromosome aberrations were taken from 200 cells per
animal, and mitotic indices were calculated from 1000 cells per
animal. ANOVA and Dunnett's multiple comparisons test were used
to test the affect of DDT treatment. Gaps, stickiness, and
mitotic indices were not significantly affected. Deletions and
gaps plus deletions were significantly greater than controls in
treatment groups with 50 ppm and higher concentrations. DDT
appears to be a potential mutagen in concentrations found in
secondary consumers.

STUDENT PROJECT IN RENAL SURGERY: PHASE I, DEVELOPMENT OF THE
RESEARCH TEAM. G. Leidahl, P. Montgomery, G. Pilcher,
D. Shabert, and J. Ward. Division of Science, Minot State Col.,
Minot, N. Dak.
   The overall goal of the project is the study of the effect of
artificial perfusion solutions on the kidney, and the develop-
ment of a solution suitable for preservation. The scope of the
project requires that it be a team effort and that it be de-
veloped in several phases. Phase I represents organization and
development of basic techniques. Phase II will encompass the
gathering of data on the effects of autografts and homografts.
Phase III will concern the development of a closed circuit per-
fusion technique. In Phase I, which has been completed, teams
were developed in anesthesiology, lab, nursing, and surgery.
Each team analyzed and developed the skills needed in support
of the other teams and the study as a whole. Data was gathered
on the effects of nephrectomy on urine and blood in rats and
dogs.

DRY COOLING TOWERS: THE "DRY LOOK" FOR POWER PLANTS IN NORTH
DAKOTA. K. W. Li and J. R. Ward. Dept. of Mech. Eng., NDSU
Fargo, N. Dak.
   The dry cooling tower is gaining attention as a method of
rejecting waste heat from power plants. With concern over
environment pollution increasing, the dry cooling tower system
of heat rejection offers an alternative method which uses
little water, creates no thermal pollution of rivers, lakes,
or oceans, and gives much more latitude in power plant siting.
A dry cooling tower consists of a series of heat exchangers
through which heat is rejected directly to the atmosphere.
The performance of a dry tower is usually expressed as function
of "initial temperature difference"- The temperature difference
between the condensing steam and of the air entering the heat
exchangers. Economic analysis of dry towers versus conven-
tionally cooled systems. Dry towers have about a 7% greater
capital cost and a 9% greater generation cost for similar
plants. However, siting and water cost can make dry towers
more desirable. The impact of dry towers on the environment
is favorable compared to other heat rejection systems. With
this in mind, the dry cooling tower can be an important
factor in the utilization of North Dakota lignite.

THE MOE SITE---A NORTH DAKOTA PALEO INDIAN CAMPSITE. <u>L.L.</u>
<u>Loendorf</u>. Dept. of Soc./Anthro., Univ. N. Dak., Grand Forks,
N. Dak.
     The Moe Site is located along the east bank of Lake
Sakakawea near New Town, North Dakota. The site is presently
being exposed by erosion into the bank of the lake; this ero-
sion is leaving artifacts, bone, and remnants of hearths scat-
tered along the beach. It is the oldest archaeological site
known in the state of North Dakota. Although there are no
absolute dates, the artifacts found at the site indicate con-
siderable antiquity. They include Clovis specimens, Folsom
specimens, and Plano specimens of the Paleo-Indian Period.
Clovis assemblages are dated 13,000 years ago to 10,000 years
ago; Folsom assemblages are dated around 10,000 years ago and
Plano specimens are dated 9,000 years ago to 6,000 years ago.
Besides the Paleo-Indian specimens at the Moe Site, there are
artifacts representing the full Meso Indian Period. These in-
clude McKean, Duncan, Hanna and corner-notched varieties of
projectile points dated 4500 years ago to 1500 years ago. Other
specimens in the collection include projectile points repre-
senting the Neo-Indian Period dated 1500 years ago to 200 years
ago. The Moe Site, then, includes artifacts representing the
total prehistoric period on the northwestern plains.

OXIDATIVE ADDITION OF PALLADIUM ATOMS TO PENTAFLUOROBROMOBEN-
ZENE. J. Y. F. Low and K. J. Klabunde. Chemistry Dept., Univ.
of North Dakota, Grand Forks, North Dakota.
     A new low temperature reaction technique, first introduced
by Skell for reacting free metal atoms with organic compounds
(P.S. Skell and J.J. Havel, <u>J. Amer. Chem. Soc.</u>, <u>93</u>, 6687,
1971), is being studied. This method allows the direct syn-
thesis of many new and useful organometallic compounds. Using
this technique, in our laboratory we have synthesized many
organometallic intermediates for the first time in the absence
of solvent and normal ligand supports. Of particular interest
is the compound pentafluorophenylpalladium bromide (I) produced
by oxidative addition of palladium atoms to C_6F_5Br. This
species has been isolated and characterized, and is soluble in
common organic solvents. In benzene I decomposes over a four
hour period to yield equal amounts of perfluorobiphenyl, pal-
ladium, and palladium dibromide. I is very reactive with
phosphines, and several stable phosphine adducts have been
prepared and characterized. In particular their geometry has
been determined by NMR spectrometry. Other coordination
chemistry of I and other similar intermediates has been invest-
igated and will be discussed. Supported by Research Corp.
and, in part by NSF. (Grant GP-34493).

REMOTE SENSING OF BARLEY FOLIAR DISEASES. <u>Carlos Martin and
V. D. Pederson</u>. Dept. of Plant Path, Col. of Agr., NDSU, Fargo,
N. Dak.

Infrared Ektachrome film was used as a remote sensor to record
foliar disease reactions of barley varieties Larker, Dickson and
Nordic protected or unprotected with Dithane M45 fungicide at
Fargo and Langdon, N.Dak. in 1971. Spectral reflectance was ana-
lyzed by determining the percent transmission of specific light
wavelengths through individual plot images. At Fargo, where spot
blotch was the most prevalent foliar disease, differences in
yield, 1000 kernel weight, percentage of plump kernels, test
weight and disease ratings between sprayed and nonsprayed plots
were positively correlated with differences in 675 nm light trans-
mission through plot images. At Langdon, where Septoria leaf
blotch was the most prevalent foliar disease, differences in
disease ratings and differences in light transmission were highly
and positively correlated, but correlations between differences
in yield or kernel characteristics and differences in light tran-
smission were low and erratic. This study demonstrated that in-
frared color film correctly assessed the severity of foliar
diseases of barley, but assessment of the effect of disease on
yield or kernel weight and size depended on the nature of the
foliar diseases and reactions of the varieties to them.

EFFECT OF PLANT EXTRACTS ON GERMINATION OF WILD OAT SEED, <u>AVENA
FATUA</u> L. <u>Marvin Mattson, G. E. Schultz, and L. J. Schermeister</u>.
Dept. of Agron., N. Dak. State Univ., Fargo, N. D.

Water extracts of wild North Dakota plants were evaluated
for physiological activity on the germination of wild oat seed.
Extract derived from one gm dry weight of plant tissue in five
ml distilled water was added to a filter paper in petri dishes.
Each extract treatment included 10 seeds placed in a petri dish
with three replications. Treatments were maintained in a seed
germinator at 70 F and evaluated for germination and seedling
development over a 14-day period.

Water extracts of <u>Asclepias</u> <u>ovalifolia</u> leaves, stems, roots
and flowers, <u>A</u>. <u>syriaca</u> stems, <u>A</u>. <u>incarnata</u> leaves and flowers,
and <u>A</u>. <u>verticillata</u> flowers suppressed normal germination up
through five days, after which some showed coleoptile elongation.
By 13 days branched adventitious roots were evident but normal
radicle growth was inhibited. Water extracts of <u>A</u>. <u>verticillata</u>
leaves, stems and roots, <u>A</u>. <u>incarnata</u> stems and roots, <u>A</u>. <u>pumila</u>
leaves, <u>Delphinium</u> <u>virescens</u> leaves and <u>Xanthium</u> <u>italicum</u> leaves
were physiologically inactive. (Extracts were provided by NDSU
Themis Project DADA 17-69-C-9023.)

ARTIFICALLY INDUCED ESTRUS IN THE MOUNTAIN LION, FELIS CONCOLOR. C. F. Mehrer. Dept. of Biol., Grand Forks, N. Dak.

Estrogens have been used to induce estrus in various domestic animals including those in which ovulation is induced. However, this technique has not been adapted to the large felids. This experiment was designed to ascertain the effect of estrogenic compounds on estrus in the mountain lion, Felis concolor Linnaeus. A twelve year old mountain lion was given intramuscular injections of 50 ug estradiol benzoate for eight consecutive days; 100 ug and 500 ug were injected on days 9 and 10, respectively. Vaginal smears were taken before the first inoculation and on sucessive alternate days. The vaginal epithelium changed from a basel to superficial cornified type beginning on day 5. The animal showed behavioral patterns suggestive of heat on day 10. Copulation was observed twice on each of days 2, 3, and 4 after the last inoculation, day 10.

IMPORTANCE OF GEOLOGY IN SELECTION OF SANITARY LANDFILL SITES IN NORTH DAKOTA. S. R. Moran and B. M. Arndt. North Dakota Geological Survey, Grand Forks, North Dakota.

It is possible to develop a sanitary landfill nearly everywhere in North Dakota. However, in many areas the engineering modification or control measures that must be utilized to prevent the migration of leachate are so extensive that development of a site is not practical. Geology is one of the most important factors influencing the extent of engineering modification necessary to assure a safe disposal project. The occurrence and movement of groundwater is the single most important geologic factor affecting the suitability of a potential landfill site. Groundwater is the medium by which leachate is generated and transported away from the landfill site. Groundwater provides the water supply in many areas and must be protected from pollution by leachate. Therefore selection of a landfill site should include consideration of the groundwater flow system in the area. Because gravel and sand rapidly transmit large quantities of groundwater, they commonly are the source of both large and small water supplies. In most instances, sanitary landfills located in abandoned gravel pits will cause pollution of groundwater.

STUDIES ON CORN LEAF SURFACE BACTERIAL FLORA. J. S. Mullins and D. G. Davis, USDA, ARS, Metabolism and Radiation Research Laboratory, State University Station, Fargo, N. Dak.

Experimental studies were designed to examine the population size, species diversity and seasonal variation of the resident bacterial microflora on the leaves of North Dakota grown corn. Furthermore, to determine the influence of a herbicide on this flora, foliar application of dalapon (2,2-dichloropropionic acid) was investigated. Leaf surfaces were sprayed with dalapon at a rate of 1.5 lb/A. Upper leaf surface cultures were made before and after dalapon treatment by the Rodac plate method. Samples were taken using three bacteriological media; Trypticase-soy, a nitrogen-free medium, and a dalapon enrichment medium. Aerobic, heterotrophic bacteria were found to be unevenly distributed on the leaf surfaces, following a modified Poisson distribution. A variation of 0-1000 colonies/cm^2 was observed on the Rodac plates, with an overall summer average of 38 colonies/cm^2. Qualitative and seasonal changes in the bacterial flora were evident throughout the summer and the predominating flora consisted of chromogenic gram positive rods. Dalapon treatment did not significantly alter the numbers of organisms present on the leaf surfaces.

FEASIBILITY OF GROWING RAINBOW TROUT (SALMO GAIRDNERII RICHARDSON) IN PRAIRIE POTHOLE LAKES OF N. DAK. G. L. Myers and J. J. Peterka. Dept. of Zoo., NDSU, Fargo, N. Dak.

Three lakes were selected in 1971 to represent extremes in sizes of pothole lakes found in the vicinity of the study area, near Jamestown, N. Dak. Lake I was 30.0 acres (maximum depth, 5.5 feet); Lake II, 57.2 acres (maximum depth, 8.0 feet); Lake III, 10.1 acres (maximum depth, 4.0 feet). Each lake was stocked in May with 200 rainbow trout (300 fish per lb.) per surface acre. The percent recovery in October was 0.0, 4.4 (1.7 fish per lb.), and 0.8 (3.5 fish per lb.); estimated percent survival from May to October was 0.0, 5.1, and 4.5 respectively. Lakes II and IV (surface area of 25.7 acres and maximum depth of 7.2 feet) were stocked in 1972 with 200 trout (71 fish per lb.) per surface acre. The percent recovery in October was 0.1 (2.1 fish per lb.) and 0.0; estimated percent survival to October was 0.5 and 0.0 respectively. Summerkill resulting from decomposition of a large bloom of Aphanizomenon killed all trout in lakes I and IV in August. Mortality from May through June ranged from 23% to 95%.

Beaver influences on stream channels, bottoms, algae, and macro-
benthos.   Joe K. Neel, Department of Biology, University of
North Dakota, Grand Forks, North Dakota.

Beaver dams constructed just above riffles have been main-
tained only for a few months. Their abandonment (usually in
late summer) has almost invariably resulted in early failure of
a short segment, which has diverted most or all discharge to a
narrow gap. This concentration of hydraulic force has scoured
and enlarged existing riffle channels and washed out new ones,
usually building or extending rocky bars downstream. These
actions have frequently produced slack water or even dry beds
in formerly active channels, which have been restored by ice
break up twice in 7 years. Increased scouring of existing
channels has denuded them of algae and macrobenthos, which
have not been replaced until the following growing season.
Widening of active flow areas associated with some channel for-
mation has increased development of some animals and plants.
Immediate effects are usually catastrophic in new or enlarged
channels.

THE AMINO ACID AND PROTEIN CONTENT OF TWO SPECIES OF
COPRINUS, D. E. Norquist*, S. W. Khalil and L. J. Schermeister.
Dept. of Pharmacognosy, Col. of Pharm., N. Dak. State Univ., Fargo,
North Dakota.

The amino acid and total protein content of the fruiting bodies of
two species of Coprinus at different stages of maturity were deter-
mined. The amino acids were qualitatively identified using paper
chromatography, quantitatively determined as total protein by
Kjeldahl, and as individual amino acids using a "Technicon" amino
acid analyzer. The variation of total and individual amino acid con-
tent at different stages of maturity may be useful in distinguishing
between the two species chemotaxonomicaly. All protein amino acids
identified were found in both species. However, the occurrance of
non protein amino acids varied markedly. Total protein (Kjeldahl)
varied widely for the individual plant parts at different stages of
maturity (18.55 to 40.70%).

STUDIES ON BIOSYNTHESIS AND METABOLISM OF CATECHOLA-MINES. S. S. Parmar and S. J. Brumleve. Dept. of Physiol.· & Pharmacol., Sch. of Med., Univ. No. Dak., Grand Forks, N. Dak.

Changes in tissue catecholamines (CA) and their urinary metabolites suggested a biochemical basis for mental disorders. Administration of copper-tyrosine complex (Cu-T) on alternate days for one week increased CA in rat adrenals, brain and heart but failed to alter tissue weights. Noradrenaline in adrenal glands, brain and heart increased 60%, 97%, and 93% respectively, with 20 mg/kg of Cu-T. Adrenaline increase was of low order, whereas dopamine increased by 41% in brain and 43% in heart. Equivalent doses of tyrosine failed to exhibit such effects indicating stimulation of CA biosynthesis. Involvement of monoamine oxidase (MAO) and catechol-o-methyl transferase in CA metabolism prompted determination of MAO activity of rat brain, heart and liver after intracerebroventricular administration of MAO inhibitors. Enzyme inhibition, observed immediately, has indicated fast transport of inhibitors from brain to peripheral tissues which could possibly provide a basis for neural control of peripheral cardiovascular functions. (Supported in part by Sch. of Med., Gen. Res. Support, USPHS, NIH Grant No. 5 SO1 RR05407.)

INHERITANCE OF A MORPHOLOGICAL FACTOR LIMITING INFECTION IN SELFED LINES OF USTILAGO HORDEI (Pers.) Lagerh. W. Pedersen, L. Jensen, R. Kiesling, and L. Darlington. Dept. Plant Path., Col. of Agr., NDSU, Fargo, N.Dak.

Selfings of the four sporidia from single teliospores of races 8 and 10, U. hordei sometimes failed to infect Odessa barley (C.I. 934) which is susceptible to all known races of this fungus. When the infection failed on Odessa, no infection was found on any other barley variety tested. Jensen in 1971 attributed this infection failure to poor development of infection-hyphae. Further microscopic examinations showed that these matings develop sparse, atypical infection-hyphae. Selfings of sporidia from single teliospores which were derived from a single sporidial selfing of race 8 wild type resulted in a ratio of three virulent to one avirulent dikaryon. Teliospores were selected from each virulent line, and their sporidia were selfed. Tetrads from two of these F3 selfed lines segregated 3:1 and the third in a 4:0 ratio with virulence dominant. This agreed with the hypothesis of a single recessive gene conditioning poor infection-hypha development.

SHORELINE DEVELOPMENT ON RESERVOIRS. D. T. Pederson. Experimental Col., Minot State Col., Minot, N. Dak.

To provide guidelines for controlling shoreline erosion an Army Corps of Engineers reservoir, Lake Ashtabula, located in Southeastern North Dakota was studied from 1969-1971. The reservoir was formed when the Baldhill Dam blocked the flow of the Sheyenne River in 1950. Shoreline configuration, erosion processes, and conditioning factors were noted for stations located at 100-foot intervals along the reservoir shoreline. Specially written computer programs were used to calculate erosion at each station and to find means, standard deviations, and correlations for the various categories considered. Erosion to 1970 at each station was also compared to projected erosion based on the stable shelf concept. The major observed erosion processes were slumping, frost weathering, block separation, and collapse of overhangs. The most important conditioning factors were groundwater regime, shoreline use, shoreline orientation, and organic activity. Shoreline erosion was found to be a major source of sediment filling the reservoir. The shoreline in 1970 had eroded to approximately six percent of the stable shelf projection with the greatest development on the headlands. Supported by Natl. Sci. Fndn. and Water Resources Res. Inst. Grant (P. L. 88-379).

REMOTE SENSING OF WHEAT FOLIAR DISEASES. Vernyl D. Pederson and Carlos Martin. Dept. of Plant Path., Col. of Agr., NDSU, Fargo, N. Dak.

Infrared Ektachrome film was used as a remote sensor to record foliar disease reactions of hard red spring wheat varieties Manitou, Thatcher, Marquis, Waldron and Justin protected or unprotected with Dithane M45 or RH124 fungicides at Langdon, N.D. in 1971. Spectral reflectance from individual plots was analyzed by determining the absorbance of specific light wavelength bands by the plot images on an aerial photograph taken 11 days before harvest. In plots where only leaf rust was controlled by RH124, differences in yield, 1000 kernel weight, and test weight between sprayed and unsprayed plots were highly and positively correlated with differences in absorbance of 675 nm light. In plots where all foliar diseases were controlled, including stem rust on the suscept Marquis, correlations were lower and non-significant. This study demonstrates that infrared films correctly predicted the relative effects of various severities of leaf rust on yield, 1000 kernel weight and test weight, but underestimated the effects of stem rust on Marquis.

COMPARATIVE MOSQUITO DENSITY AND INTRA-SPECIFIC VARIATIONS IN THE MOST COMMON SPECIES IN FARGO-MOORHEAD AREA. R. D. Peterson II and M. S. Quraishi. Dept. Ent., N. D. State Univ., Fargo, N. D.

During the summer of 1968 various mosquito collection techniques were employed to better evaluate Red River Valley mosquito populations. Light traps, artificial box shelters, pit shelters, and human bait were used in an attempt to further the knowledge of mosquito bionomics in the areas studied. Light trap studies accounted for the majority of mosquitoes collected. Artificial box shelters and human bait contributed moderate numbers. The pit shelters yielded negligible numbers of mosquitoes. Of particular interest was the presence of uncommon abdominal scale patterns in female and male Aedes vexans which will be discussed in detail. Supported by the U. S. Army Medical Research and Development Command, Contract No. DADA 17-69-C-9023 under Project Themis.

EFFECT OF FASTING ON AMINOTRANSFERASES IN FROG. A.S. Reddi, Dept. of Biochem., Sch. Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

Effects of fasting on the levels of aspartate and alanine aminotransferases was investigated in frog tissues. Activity of aspartate aminotransferase was found to be greater than alanine aminotransferase in all of the tissues and thus provided support to our earlier observations of other animal tissues. Activity of both the aminotransferases increased significantly on fasting. Such an increase was maximum in brain, which was followed by muscle and liver. Protein content in brain remained unaltered whereas a decrease was observed in muscle and liver during the entire period of fasting. These results have indicated that the increased aminotransferase activity is presumably associated with the increased proteolysis and consequently increased associated metabolic pathways by the process of fasting.

INHIBITION OF AMINOTRANSFERASES BY ASCORBIC ACID. A.S. Reddi
and P. Satyam. Dept. Biochem., Sch. Med., Univ. N. Dak.,
Grand Forks, N. Dak. and Dept. Zool., S.V.A. Coll., Tirupati,
A.P., India.
    Ascorbic acid is known to promote several enzymatic
reactions. However, its mechanism of action is not clearly
understood. Effects of different concentrations of ascorbic
acid on aspartate and alanine aminotransferase activity was
investigated in various tissues of frog. Aspartate amino-
transferase activity was found to be greater than alanine
aminotransferase activity in all of the tissues studied.
Both the aminotransferases were inhibited by ascorbic acid
where the degree of inhibition was found to be dependent
on the source of the enzyme. Furthermore, inhibition of
these aminotransferases by ascorbic acid was found to be
concentration dependent. Such an inhibition presumably
accounts for the conformational changes in enzyme
molecules by ascorbic acid.

PRELIMINARY DESCRIPTION OF PRECAMBRIAN IRON FORMATION, PEMBINA
COUNTY, NORTH DAKOTA. R. E. Richardson and F. R. Karner.
Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dak.
        Precambrian Iron Formation has been obtained by drilling
of the 7500-gamma magnetic anomaly centered one mile east of
Akra in northeastern North Dakota. Preliminary investigation
of a 348-foot interval of NX diamond drill core shows that the
rock consists predominantly of magnetite-bearing quartz-biotite
schist. The schist occurs in an inclined sequence with alter-
nating cherty and magnetitic microlayers. Lithologic layering
and schistosity are parallel and inclined at angles of 20 de-
grees or less to the core axis. Schist layers are typically
a few inches to several feet in thickness and consist of fine-
grained quartz with scattered biotite, epidote and minor
magnetite and chlorite. The interlayered zones are typically
several inches thick and consist of alternating cherty and
magnetitic microlayers typically one-half to one centimeter
in thickness. These rocks are lithologically similar to the
oxide facies of the Lake Superior-type Precambrian Iron For-
mation. Supported in part by University of North Dakota
Faculty Research Grant.

Studies on Helminths of North Dakota. VI. Centrovarium lobotes (MacCallum, 1895) in the flesh of common shiner minnows. John M. Saethre and Omer R. Larson. Dept. of Biol., Univ. N. D., Grand Forks, N. Dak.

Metacercariae of C. lobotes commonly encyst in the flesh of cyprinid minnows, with adults occurring in the intestine of pike, bass and bullheads. This study was an attempt to characterize the host/parasite tissue relationships, and the incidence of the fluke in common shiners, Notropis cornutus (Mitchill). Sectioned material showed that metacercariae possessed an inner hyaline cyst and an outer fibrous connective tissue wall. The latter was intimately associated with the connective tissue of the host's intermuscular septa. A granular degeneration of the outer wall was often seen. Thirty-nine of 40 common shiners taken from the Forest River near Inkster, N. D., possessed C. lobotes (ranging from 1 to 269 worms/infected fish). The longest and heaviest weight class of fish harbored the greatest number of encysted worms, thus suggesting an accumulation of parasites with time. Fifteen of 1,228 (=1.22%) metacercariae examined were progenetic (i.e., possessed eggs although encysted in the flesh).

GLACIAL STRATIGRAPHY OF NORTHEASTERN NORTH DAKOTA. Salomon, Nena L. Univ. of N. Dak., Grand Forks, N.D.

The glacial drift of northeastern North Dakota consists of seven formations that are differentiated by outcrop characteristics, grain size, and the lithology of the coarse-sand fraction. The formations are described here in descending stratigraphic order. The Falconer Formation consists of silty till that contains many fragments of shale and igneous rock. It was deposited by a minor readvance of the latest Wisconsinan glacier. The southwestern extent of this unit is marked by the Edinburg Moraine. The Dahlen Formation consists of silty till that contains many fragments of shale and igneous rock. It was deposited by a Late Wisconsinan glacier that advanced from the northwest. The Gardar Formation consists of silty till that contains many shale fragments. It was deposited by an Early Wisconsinan (?) glacier that advanced from the north. Unit A consists of clayey till that contains many fragments of shale. Unit B consists of silty, sandy till that contains many fragments of shale and igneous rock. Unit C consists of clayey till that contains many fragments of shale and carbonate rock. Unit D consists of clayey, silty till that contains many fragments of igneous and carbonate rock. Other units consisting of till occur in the eastern part of the area studied; correlation of these units with the above named formations is not yet possible.

THE IMPLICATIONS OF COSMIC DUST STUDIES. R. Scattolini.
Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak.
    Black magnetic spherules are present in the glacial and sea
ice of Fletcher's Ice Island (T-3) in the Arctic Ocean. These
opaque, spherical, magnetically susceptible, highly reflective
particles were measured and counted to determine their size
distribution and average diameter. The diameters of spherules
from the T-3 glacial ice range from 5 to 160 microns and average
21 microns. The diameters of spherules from the sea ice range
from 5 to 145 microns and average 23 microns.
    These spherules can be derived by ablationary processes from
meteoritic and perhaps cometary sources. A meteoritic origin has
been generally accepted but it cannot account for the total num-
ber of spherules present in most collections. The hypothesis
that some black magnetic spherules are cometary in origin may
have some important implications for the explanation of geomag-
netic field reversals.
    If cometary bodies have caused geomagnetic field reversals,
cometary debris (including black magnetic spherules) should be
more abundant in those deep-sea sediments deposited when polar-
ity reversals occur. To test this hypothesis it is proposed that
a study be made of black magnetic spherule abundance in deep-sea
sediments for which paleomagnetic information is already known.
Such information may provide additional independent support for
the Heezen-Glass hypothesis that comet-earth interactions produc-
ing tektites have caused reversals of the earth's magnetic field.

THE PHOTO-DEOXYGENATION OF ANILINE AND NAPHTHYLAMINE-N-OXIDES.
Joseph E. Schiller and Virgil I. Stenberg. Dept. of Chem.,
Univ. N. Dak., Grand Forks, N. Dak.
    The objective of this study is to investigate amine oxides
as potential sources of atomic oxygen in solution. The N-oxides
of trimethylamine, triethylamine, N,N-dimethylaniline, N-phenyl-
piperidine, N,N-dimethyl-1-naphthylamine and N,N-dimethyl-2-
naphthylamine undergo deoxygenation during irradiation to form
the parent tertiary amine as the major type of product. The
monodemethylated parent amine is also observed as a product.
The oxygen mainly appears in the form of water during the re-
action. With benzene or cyclohexene as the solvent, products
are observed where oxygen adds to the solvent molecules. The
quantum yield for the photodeoxygenation has been found to be
strongly dependent on the orientation of the N-O bond relative
to the aromatic chromophore. For N,N-dimethylaniline-N-oxide
and N-phenylpiperidine-N-oxide, the quantum yield for disap-
pearance of starting material is 0.32 and 0.3, respectively.
In azatriptycene-N-oxide and benzoquinuclidine-N-oxide where the
N-O bond is held orthogonal to the aromatic rings, the quantum
yields are $0.00 \pm .04$ and $0.02 \pm .02$, respectively. Glpc was
used for separation and quantitative analysis. The irradiations
were done using a Rayonet reactor.

WATERSHED PROJECTS AND NORTH DAKOTA ARCHAEOLOGY

F. Schneider and J. Logan. Dept. of Soc./Anthro., Univ. N. Dak., Grand Forks, N. Dak.

The State of North Dakota faces, potentially, a great loss of information regarding man's occupation of the State in both prehistoric and historic periods. Forty-three projects are proposed for the Soil Conservation Service Small Watershed Program. The projects, encompassing portions of 26 counties, will affect 14.3% of the total land surface of the State. Archaeological investigations have been conducted in .82% of the affected area.

PERSISTENCE AND DEGRADATION OF THE FUNGICIDE 1,2-BIS(3-METHOXYCARBONYL-2-THIOUREIDO)BENZENE IN SOIL.

I. R. Schultz and J. R. Fleeker. Dept. of Biochem., NDSU, Fargo, N. Dak.

Degradation of the experimental fungicide 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene (TD-1771) was studied in loamy fine sand and silty clay soils. In both soils, the fungicide was converted to 2-methoxycarbonylaminobenzimidazole. At 10 ppm and 20^0, the conversion was complete within 10 days. The benzimidazole was degraded at a much slower rate than the parent compound. A second degradation product was detected, but its structure not determined.

RESPONSE TO ACTH BY SUPERFUSED ADRENALS OF WILD AND DOMESTIC
HOUSE MICE (MUS MUSCULUS). Robert W. Seabloom. Dept. of Biology
Univ. N. Dak., Grand Forks, N. Dak.
      Superfused adrenals of wild and laboratory strains of
house mice were stimulated with ACTH. Superfusates were assayed
fluorometrically for corticosterone. Lab males had signifi-
cantly smaller adrenals, lower resting levels and lower stimu-
lated levels than lab females. Their adrenals were smaller and
exhibited lower stimulated levels than wild males. Wild males
had smaller adrenals and lower resting levels than wild females,
but their response to ACTH stimulation was the same. There
were no significant differences between lab and wild females in
any of the parameters measured. Adrenals of wild house mice
reared from birth by lab females responded in the same manner
as their wild-caught counterparts; this indicated that the ob-
served levels were due to genetic differences between wild and
domestic stocks rather than to the stimuli of capture, cap-
tivity and handling.

VASCULAR FLORA OF RANSOM, RICHLAND, AND SARGENT COUNTIES, NORTH
DAKOTA. Gerald Seiler and W. T. Barker. Dept. of Botany, North
Dakota State University, Fargo, N.D.
    This study is based upon field work completed during the
growing seasons of 1970, 1971, and 1972 and on a study of the
specimens previously collected from Ransom, Richland, and Sar-
gent counties, North Dakota. This southeastern portion of the
state includes a portion of the Drift Prairie and the Lake
Agassiz Basin (Red River Valley). The Sheyenne delta, built
when the Sheyenne River emptied into Glacial Lake Agassiz, forms
a unique sandhills region within the study area. As a result of
this study 780 vascular plant species are recorded for this
area. One hundred eighty new NDSU herbarium records for the
area are recorded. Ninety-five plant families are represented
in the area, with the Compositae being the largest followed by
the Gramineae and the Cyperaceae.

COMPARATIVE EFFECT OF $\underline{P,P}$'-DDT ON HEPATIC MICROSOMAL ENZYMES IN YOUNG QUAIL, CHICKS AND DUCKLINGS. <u>M. Sifri, J. L. Sell and K. L. Davison</u>. An. Sci. Dept., NDSU, & Metabolism and Radiation Laboratory, USDA, ARS, Fargo, N. Dak.   58102.

Separate experiments of two weeks duration were conducted with 1 week old Japanese quail, W. L. chicks, and mallard ducklings.   The treatments were: 1) control diet; 2) control diet + 100 ppm $\underline{p,p}$'-DDT; 3) control diet + pentobarbital (25 mg/kg, i.p., daily); 4) control diet + DDT + pentobarbital.   The specific activities of microsomal aniline hydroxylase and aminopyrine N-demethylase and the concentration of P_{450} were much higher in chicks compared with quail and ducklings. Dietary DDT induced these mixed function oxidases in ducklings while in chicks no significant changes were observed.   Aniline hydroxylase and N-demethylase activities of quail treated with DDT were 40 and 70% of the controls, respectively. <u>In vitro</u> additions of $\underline{p,p}$'-DDT (0, 10^{-7}, 10^{-5}, 10^{-3}M) to the incubation mixtures decreased aniline hydroxylase activities in chicks and quail (relative activities in percent; 100, 70, 55, 53 in quail and 100, 95, 71, 67 in chicks) while there was no effect in ducklings.   It seems there are genuine differences among species of the <u>Aves</u> class concerning $\underline{p,p}$'-DDT metabolism.

EFFECT OF ETHANOL INGESTION ON LIVER MICROSOMAL ENZYMES INVOLVED IN PHOSPHATIDYL CHOLINE BIOSYNTHESIS, <u>David N. Skurdal and W. E. Cornatzer</u>. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.   58201

Female rats were fed a liquid diet for 10 weeks in which 36% of total calories was ethyl alcohol and contained vitamins, minerals, choline, and amino acids.   Control animals were pair-fed the same diet containing sucrose.   The ethanol fed animals develop a fatty liver.   Lecithin is involved in removal of triglycerides from the liver.   Liver lecithin biosynthesis is known to occur by two different major pathways in liver microsomes.   Liver microsomes were prepared and the Kennedy-Weiss pathway involving CDP-choline and α-β-diglycerides catalyzed by choline phosphotransferase activity was determined to be 1.5 times greater in the ethanol fed than sucrose controls.   The Bremer-Greenberg pathway involving the methylation of phosphatidyl ethanolamine from adenosyl-methionine by phosphatidyl methyl transferase activity of liver microsomes was found to be two times greater in the ethanol fed animals than sucrose controls.   This increase lecithin biosynthesis in liver microsomes could be a reflection of the direct effect of ethanol on the proliferation of endoplasmic reticulum which is known to contain 30% of phospholipids, of which 48% of the lipid P is lecithin, and thus less would be available to transport triglycerides out of the liver.   (MH Grant 19234).

EFFECTS OF DIURETICS ON CANINE RENAL FUNCTION AFTER
CHRONIC TREATMENT WITH LITHIUM. H. W. Spencer, T. K.
Auyong, T. W. Nielsen. Dept. of Physiology and Pharmacology,
School of Medicine, Univ. N. Dak., Grand Forks, N. Dak.

The use of diuretics was employed to further elucidate the locus
of action of lithium ion on the canine kidney. Female mongrel
dogs, weighing from 10 – 18 kg were used. The animals were
screened for normal function prior to treatment with lithium in-
cluding glomerular filtration rate (GFR) and renal plasma flow (RPF)
using standard laboratory methods. Levels of diluting ability
(C_{H_2O}) and concentrating capacity ($T^C_{H_2O}$), using pitressin and
10% mannitol respectively, were determined. After daily doses
of 60 mg/kg bwt Li_2CO_3 twice a day for 30 days, they were given
sodium ethacrinate 0.5 mg/kg bwt or chlorothiazide 7.1 mg/kg iv.
Overt changes in GFR or RPF were not evident. The animals show-
ed an altered ability to concentrate ($T^C_{H_2O}$). A general increase
over controls of free water clearance and decrease in sodium ex-
cretion was noted. The changes seen in free water clearance
produced by lithium suggest the abberation to be located in the
distal tubule of the nephron.
Supported by USPHS, NIMH Grant MH 20788-02.

THE EFFECTS OF HYPOXIA, HYPEROXIA, OR SUCCINATE ON THE
ANESTHETIC AND TOXIC DOSAGES OF NITROUS OXIDE IN MICE.
L. C. Stetzner. Dept. of Physiol. and Pharm., Sch. of Med.,
Univ. of N. D., Grand Forks, N. D., 58201

The anesthetic and toxic dosages of nitrous oxide (N_2O) under
normoxic (150-200 mm Hg) conditions were determined for 25-30
gm male Swiss-Webster mice. Values of N_2O at 24 psi for AD_{100}
(slightly time variable) and 36 psi for LD_{100} (time variable) are
characteristic, using a one psi/min compression rate. All groups
were compared to 60-min normoxic N_2O controls. Pressures were
balanced with N_2 as the diluent gas and with 86° F as the ambient
temperature. Hypoxia (70-80 mm Hg) and hyperoxia ($\sim$ 2100 mm Hg)
did not significantly alter the toxicity of N_2O, while succinate
(0.4 M $\sim$ 0.8 cc/animal I.P.) decreased N_2O toxicity. At 36 psi,
the normoxic N_2O control was approximately 85% lethal in 60 min,
averaging 6.5 min; and the succinate was approximately 65% lethal
in 60 min, averaging 18.0 min. These factors did not markedly
shift the anesthetic dosage, but in some cases they varied the on-
set. Nitrous oxide induced cholinergic stimulation (salivation,
lacrimation, urination, defecation) above the AD_{100}. (Supported by
Burroughs Wellcome Fund and ONR Contract N00014-68-A-0499)

BRAIN GABA LEVELS OF MICE FOLLOWING EXPOSURE TO SUBANES-
THETIC AND ANESTHETIC DOSES OF NITROUS OXIDE. Dept. of
Physiol. & Pharm., Sch. of Med., Univ. of No. Dak., Grand Forks,
No. Dak. G. T. Syftestad, L. C. Stetzner, and B. De Boer.
   Brain gamma-aminobutyric acid (GABA) is increased in small lab-
oratory animals subjected to high pressure helium but is decreased
when the animals are pretreated with convulsant agents. Nitrous
oxide (N_2O) at 21 psi (total pressure 36 psi) produces Stage III an-
esthesia in rats. In the present study, brain GABA levels were ob-
tained for mice after short periods of Stages I, II, or III anesthesia
using N_2O. Adult male mice, maintained at 86° F, with O_2 150-200
mm Hg, were compressed at 1 psi/min with 24 psi N_2 and/or N_2O,
to a total pressure of 39 psi maintained for 12 minutes, followed by
decompression at 1 psi/min to 1 ATA. GABA/gm of brain weight (BW)
was determined. Control mice pressurized with 24 psi N_2 had GABA
levels of 168 ug/gm BW. Animals given 16 psi N_2-8 psi N_2O (Stage
I) had GABA levels of 185 ug/gm BW; 8 psi N_2-16 psi N_2O (Stage II)
GABA 184 ug/gm BW; 24 psi N_2O (Stage III) GABA 164 ug/gm BW.
Control animals pretreated with a stressing dose of epinephrine
(2 mg/kg I.P.) had reduced GABA levels of 151 ug/gm BW. Supported
by ONR Contract N00014-68-A-0499.

FLUORESCENCE CHARACTERISTICS OF COALS. F. T. C. Ting and
H. B. Lo. Dept. of Geol., Univ. N. Dak., Grand Forks,
N. Dak.
       Exinites in low rank coals exhibit strong fluorescence
under UV excitation. High intensity xenon or mercury lamps
are used as light source. The fluorescence color and inten-
sity of different coals ranging from lignite to anthracite
have been observed. The results show that the fluorescence
colors of exinites change from yellow to those of longer
wavelength as the coal rank increases. The characteristics
of fluorescence spectra can be used as a complementary rank
parameter for lower-ranked coals. The fluorometric studies
of coal and coaly materials in sedimentary rocks may have
several important applications: (1) age determination of
deposits of unknown age, (2) determination of coal rank, and
(3) determination of the potential of presence of petroleum.
Supported by NSF (grant GA-31950).

FORMCOKE FROM LOW-RANK COALS: EXPERIMENTS WITH NORTH DAKOTA
LIGNITE. <u>F. T. C. Ting and B. L. Ramsey</u>. Dept. of Geol.,
Univ. N. Dak., Grand Forks, N. Dak.
    Experiments were carried out to convert North Dakota lignite
into metallurgical coke. Lignites are first charred, mixed with
a suitable binder, pressed in a mold, and then further carbon-
ized to produce a formcoke. The strength of the formcoke is
determined by: (1) binder type and percentage used, (2) bri-
quetting pressure, and (3) conditions of final carbonization.
Preliminary studies indicate that best results were obtained
by using 10-20% asphalt with a melting point of 75-95° C as a
binder, briquetted at 600-800 Kg/cm^2, and carbonized at a low
heating rate (5-8° C/min.) to 600° C, followed by a more rapid
heating rate (16-20° C/min.) to 900° C. Supported by AISA
(grant no. 23-275).

DIRECT IDENTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM
CARBON BLACK. <u>Robert G. Todd, R. N. Thompson, G. Taylor, and
Carl Nau</u>. Dept. of Environ. Health, Univ. of Okla. Med. Ctr.,
Oklahoma City, Okla.
    Carbon black has been known to contain polycyclic aromatic
hydrocarbons for about 20 years. At least 4 of these hydrocar-
bons have been shown to be carcinogenic to animals, and benzo(a)-
pyrene has been shown to cause cancer in man. Most of the pre-
vious methods of identification of the polycyclic aromatic hydro-
carbons involved long and tedious procedures. Gas chromatography
has been suggested for improved separations and analysis time.
In this study the gas chromatogram of a carbon black extract in
carbon disulfide was compared to that from the desorption of the
polycyclic aromatic hydrocarbons from the same carbon black with
a pyrolyzer unit. Ten eluted compounds (from the injected carbon
black extract) were tentatively identified by retention times and
confirmed by comparing their excitation and fluorescence spectra
with those of known standard solutions in isooctane. Six of the
same polycyclic aromatic hydrocarbons from the collected chroma-
tographic peaks in the desorption experiment were similarly
confirmed. It was concluded that a shorter, direct qualitative
method for the identification of polycyclic aromatic hydro-
carbons from carbon black had been developed.

HERPESVIRUS HOMINIS STUDIES IN CELL CULTURES. J. Varani, J. J. Kelleher and W. W. Nelson. Dept. of Microbiology, Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

Cultures of primary rabbit kidney cells were infected with a Type II strain of Herpesvirus hominis at 37C, at 40C and at 41C. Growth of the virus at 37C resulted in rapid destruction of cells with production of high titres of virus. Virus growth was slowed at 40C. Virus-induced cell destruction proceeded more slowly and yields of virus were reduced. Virus growth was completely inhibited at 41C. However, virus was not completely destroyed. After returning virus-infected cultures to 37C, virus was recovered from 80-100% of the cultures. From 1-34 days elapsed between incubation at 37C and onset of observable virus growth. During the period of incubation at 37C before observable virus growth occurred, virus could not be recovered from cells using standard isolation techniques. The effects of 17 β-estradiol and progesterone on virus infection at 37C and 41C were investigated. Estradiol at concentrations of 0.5-5 µg/ml had no appreciable effect on the amount of virus produced at 37C. Virus was recovered at 37C from about 80% of the cultures treated with 2 µg/ml estradiol and preincubated at 41C. Treatment of cultures with 0.5-5 µg/ml progesterone at 37C resulted in a 1-3 log decrease in virus production. Virus was recovered at 37C from only 50% of the cultures treated with 2 µg/ml progesterone and preincubated at 41C.

THE VERTICAL DISTRIBUTION OF CHANNEL CATFISH (ICTALURUS PUNCTATUS) IN THE LITTLE MISSOURI ARM OF LAKE SAKAKAWEA, NORTH DAKOTA, 1968. C. H. Wahtola Jr. and J. B. Owen. Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

In the summer of 1968, 313 channel catfish (Ictalurus punctatus) were captured in gill nets at four sampling stations in the Little Missouri arm of Lake Sakakawea, North Dakota. These stations spanned the gradual transition from near river (station 4) to reservoir (station 1) conditions. Vertical gill nets 50 and 25 feet in depth and six foot deep experimental gill nets fished one above another were used to sample fish from different depths from top to bottom. Catfish at the stations in deeper water did not show depth preference. Catfish from the shallow station nearer the river-reservoir confluence showed a slight preference for the bottom earlier in the summer which became more pronounced later in the summer. Supported by the North Dakota Game and Fish Department and the U.S. Bureau of Commercial Fisheries (N.M.F.S.) under P.L. 88-309.

ENVIRONMENTAL AND PRODUCTIVITY RELATIONS OF A
POTAMOGETON PECTINATUS COMMUNITY. *M. K. Wali* and *A. L. Kollman,* Dept. of Biol., Univ. of N. D., Grand Forks, N. D.

Potamogeton pectinatus L., a marl former, grows as a natural monospecies culture in Fox Lake, 16 km northeast of Devils Lake, N. D. The water, sediment, dissolved oxygen, temperature and plankton relationships were studied in this small (0.4 x 1.2 km) and shallow (uniform depth 65-75 cm) lake. The macrophyte was harvested in five replicates at each 2-week interval during the growing seasons for determinations of biomass production and energy content. The water, sediment and biomass samples were analyzed chemically for major and trace elements.

The mean pH was 8.0, specific conductivity was 9.97 Mmhos/cm and Na concentration 7220 ppm for the unvegetated sediments. Samples from below the macrophyte community had a lower pH value 7.8, lower sp. conductivity, 9.56 Mmhos/cm and 300 ppm less of Na. Mean Ca values were 1651 ppm greater below the plants than in unvegetated areas. While there were no significant differences for Cu, Li, Sr and Zn, Fe and Mn were greater in vegetated sites. The peak biomass production of *Potamogeton* was 293 gm/m^2 (8.5 x 10^5 cal/m^2) in mid-August. The rate of community production from initiation to maxima was approximately 3.75 gm/m^2/day (1.1 x 10^4 cal/m^2/day). *Supported by OWRR Grant No. A-031-NDAK.*

THE PHYLOGENETIC DISTRIBUTION OF ENZYMES INVOLVED IN THE METABO-
LISM OF S-ADENOSYL-L-HOMOCYSTEINE AND DERIVATIVES. <u>R.D. Walker
and John A. Duerre.</u> Dept. of Microbiology, Sch. of Med., Univ. of N. Dak., Grand Forks, ND.

Bacteria, yeasts, plant leaves, and the major organs from fish, frogs, snakes and mammals were assayed for the presence of S-adenosylhomocysteine nucleosidase, S-ribosylhomocysteine cleavage enzyme and S-adenosylhomocysteine hydrolase. S-Adenosylhomo-cysteine nucleosidase activity was determined by measuring the appearance of reducing sugars from S-AH. Ribosylhomocysteine cleavage enzyme was measured by the appearance of free homo-cysteine (S-H) from RH. Adenosylhomocysteine hydrolase activity was measured by the method of Cantoni et al. (J. Biol. Chem, 234, 603, 1959). All gram negative bacteria tested except <u>Alcalige-nes faecalis</u> were found to contain AH nucleosidase and RH cleav-age enzyme. All of these microorganisms were negative for AH hydrolase. Adenosylhomocysteine hydrolase was found in most of the other systems tested including yeast, higher plants, fishes, frogs, snakes and mammals. None of these systems were found to contain AH nucleosidase or RH cleavage enzyme. In the chordates the AH hydrolase was found predominantly in the liver with no activity being detected in any of the brains, hearts, lungs or muscles. Gram positive bacilli and all organs from <u>Polydon spatula</u> were found to be negative for all enzymes tested.

REGULATION OF BIOTIN TRANSPORT IN <u>LACTOBACILLUS</u> <u>PLANTARUM</u>. James R. Waller, Gail R. Hendrickson and Joy K. Anderson. Dept. of Microbiology, Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

During studies on free biotin (FB) transport in <u>Lactobacillus</u> <u>plantarum</u>, a marked decrease was observed in the amount of biotin accumulated by cells grown in Wright-Skeggs medium containing excess biotin. Levels of biotin in the growth medium from 0.5 ng/ml (deficient) to 5 ng/ml (excess) resulted in progressive decreases in internal FB (IFB) from 180 to 13.5 ng/mg cells (dry wt.). Also correlated with the increasing biotin content in the growth medium were increasing levels of endogenous bound and free biotin. At 4 to 5 ng biotin/ml of growth medium, the cells were saturated with bound biotin, endogenous IFB exceeded 0.1 ng/mg cells, measurable amounts of biotin were present in the growth medium from which the cells had been harvested, and biotin transport was 89 to 92 per cent inhibited. Cells transferred from low biotin growth medium to high biotin medium for 1 to 3 hours exhibited a marked increase in endogenous bound and free biotin, but no significant reduction in FB transport or accumulation. It appears that external biotin may control the biotin transport system by a repression mechanism in which IFB levels in excess of 0.1 ng/mg cells acts to trigger the repressive effect.

TEACHING PHYSICAL GEOLOGY BY THE KELLER PLAN AT MINOT STATE COLLEGE. R. G. <u>Walsh.</u> Dept. of Phy. Sci., Minot State College., Minot, N. <u>Dak.</u>

The Keller Plan is a self-paced, tutorial, and competency based instructional method. It has gained favor in physical science teaching, but has seen limited use in physical geology. This project was designed to test the plan in terms of student and staff attitudes, and to determine if resources were sufficient to implement this type of course as a viable alternative to the traditional lecture-lab. approach. A class of 38 students served as the pilot group. Evaluation instruments included pre- and post-attitude surveys on method, written student, faculty, and tutor comments, plus daily diary accounts. Preliminary evaluation indicated favorable student reaction. Staff reaction was mixed. Lab. materials were in short supply for several areas. Instructor resoursefullness was necessary to sustain individual learning. Most students readily took responsibility for their own learning. Feasability of offering this option each year was considered excellent.

VIRULENCE PATTERNS OF THE NATURAL LEAF RUST POPULATION AND SOME
EPIDEMIOLOGICAL FACTORS THAT INFLUENCE RUST SEVERITY IN NORTH
DAKOTA.   J. E. Watkins and G. D. Statler. Dept. of Plant Path.,
Col. of Agr., NDSU, Fargo, N. Dak.
    The distribution of virulence of Puccinia recondita was
studied using leaf rust cultures collected from wheat cultivars
throughout N.D. Virulence was identified on eight near isogenic
wheat lines. Major changes in the number of virulence combina-
tions along with the number of isolates virulent to host genes
Lr1, Lr2, Lr2D, Lr10, Lr16 and Lr18 and Agent were found between
the 1970, 1971 and 1972 leaf rust collections. The appearance
of leaf rust in 1972 was approximately two weeks earlier than in
1971; however, only trace amounts were present during June and
early July. By mid July the rust readings for 1972 were lower
than those of the previous year. These lower readings and the
slow initial development may be attributed to cold periods in
mid June in which the temperatures dropped below $8^{\circ}C$. The
potential destructiveness of leaf rust was demonstrated by
measuring yield and protein losses as a result of the rate of
rust build-up and rust severity. Significant increases in both
yield and protein were obtained by controlling leaf rust on
Manitou with Rohm and Haas RH-124 (4-n-Butyl-1,2,4-triazole).
The severity of leaf rust was well correlated with yield loss.

THE CHEMISTRY OF CARBONMONOSULFIDE. C. M. White and K. J.
Klabunde, Dept. of Chem., U.N.D., Grand Forks, N. Dak. 58201
        The purpose of this study is to ascertain some of the pro-
perties of, and the chemistry of carbonmonosulfide, CS, a moiety
analogous to CO. There is little known about this reactive
molecule even though it is an intermediate in the photolysis and
combustion of organosulfur compounds. In our laboratory, the CS
entity is generated in an electric discharge at a pressure of 1
to 10 microns, by dissociation of CS_2 into atomic sulfur and CS.
The CS is swept into a 1 1 reactor, immersed in liquid N_2, where
it is codeposited with a substrate. Various reactants have been
studied, but under the conditions used, only the hydrogen hal-
ides and the halogens reacted. The products of the reactions
were crudely separated by a vacuum distillation train, and then
fully separated by gas chromatography. The resulting products
were characterized by NMR, mass spec., and IR. The following is
a summary of those reactions: $CS + Cl_2 \rightarrow CSCl_4$; $CS + Br_2 \rightarrow$
$CSBr_4$; $CS + BrCl \rightarrow CSCl_4 + CSBr_4 + CSCl_3Br$; $CS + I_2 \rightarrow N.R.$; $CS +$
$ICl \rightarrow CSCl_4 + I_2$; $CS + HCl \rightarrow C_4H_4Cl_4S_4$; $CS + HBr \rightarrow C_4H_4Br_4S_4$.
In addition, we have reacted CS with BCl_3 and obtained an air
sensitive compound, which we believe is Cl_3BCS. From the above
results, we conclude that CS, unlike other carbenes, reacts as
a Lewis base, and not as an electron deficient Lewis acid. Sup-
ported by NSF (Grant 34493), PRF and NDEA.

RANGE PRODUCTION IN RELATION TO PRECIPITATION. W. C. Whitman and C. N. Haugse. Depts. of Botany and Animal Sci., North Dakota State Univ., Fargo, N. Dak.

Range vegetation yield data obtained from cage clippings on grazed range at the Dickinson Experiment Station over the 25-year period, 1946-70, have been analyzed in relation to amounts and distribution of precipitation recorded at the Station. Correlation coefficients of 0.84, significant at the 1% level, were obtained for effective precipitation (Sept., Oct. ppt. from previous year plus Apr., May, June, July ppt. of current year) and for Apr.-May-June-July precipitation and range forage yields. Precipitation obtained in April and in May also showed fairly good relation to range yield, but predictability from these values would be low. For the period of the trial annual precipitation averaged 15.99 in, effective precipitation 11.83 in, and forage production 1009 lbs/acre. Forage production per inch of effective precipitation has ranged from a low of 44 lbs to a high of 125 lbs with an average of 84 lbs per acre. Regression values indicate a production of about 100 lbs forage (dry-weight) per inch of effective or seasonal precipitation. The use of these relations to predict the possible effect of rainfall modification procedures on range production in southwestern North Dakota seems justified.

BROWSE STUDY IN SOUTHWESTERN NORTH DAKOTA BADLANDS. D. E. Williams and H. Goetz. Dept. of Botany, NDSU, Fargo, N. Dak.

Serviceberry (Amelanchier alnifolia Nutt.) was studied in the Little Missouri River Badlands of southwestern North Dakota from 1971 to present and will be continued through 1973. Data reported are for 1971. The study includes analysis of annual twig growth, annual production, utilization as browse, community structure and development, and growth habits. Average annual twig growth was 2.84 cm. Average annual growth was greatest in seedlings (6.60 cm) and least in young shrubs (2.38 cm). Total annual yield was 93.84 kg/hect. (172.31 lbs/acre) for all sites, age and form classes combined. Leaves constituted 82% of the total annual production. Mature shrubs contributed 78% of the total production. Shrubs in form classes 4 (mostly available, little or no hedging) and 7 (mostly unavailable) contributed 74% of total production. Less than 20% of the total production was available as deer browse. Average utilization for all sites was 11%. Clones of this species appear to be deteriorating due to environmental influences, such as snow breakage, rust fungus, and damage from domestic livestock. During the 1972 season, twig growth, production, vegetation analyses, and utilization studies were carried out on all sites. These data are in the process of analysis and interpretation.

STEREOSELECTIVE REACTIONS OF N-METHYL-S-ALANINE, <u>Larry A. Wingert* and John G. Brushmiller</u>. Dept. of Chemistry, Univ. of N. Dak., Grand Forks, North Dakota 58201

The neutral coordination compound 2, 4-Pentanedienonatobis-(N-Methyl-S-alaninato)cobalt(III) $[Co(N-Me-S-alan)_2(acac)]$ was synthesized by ligand exchange reactions from $[Co(acac)_3]$. Although 16 isomeric forms of the complex are possible only three are found experimentally in agreement with the prediction on the stereoselectivity of N-Methyl-S-alanine. The isomers isolated are all optically active and are assigned as Δ -<u>trans</u>-N-C$_2$, Λ -<u>trans</u>-N-C$_2$ and Λ -<u>cis</u>-N-C$_2$ the absolute configurations being assigned by pmr (<u>trans</u> isomers) and stereospecificity (<u>cis</u>-N-C$_2$). The hydrogens of the methyl groups of coordinated acac are quite labile exchanging for deuterium at alkaline pH's. Exchange of H for D occurs without Δ to Λ conversion for the <u>trans</u>-N-C$_2$ isomers. The Δ -<u>trans</u>-N-C$_2$ and Λ -<u>cis</u>-N-C$_2$ isomers convert to the Λ <u>trans</u>-N-C$_2$ isomer on treating aqueous solutions with activated carbon.

NOTES ON NORTH DAKOTA <u>ASTRAGALUS</u> SPECIES DISTRIBUTION. <u>N. K. Zaczkowski</u>, Dept. of Biology, St. John's University, St. Joseph, Minn.

Among the forbs of North Dakota the genus <u>Astragalus</u> has the largest number of species. Based on voucher specimens deposited in the NDSU herbarium, the number is 21. By comparison, 8 species grow in Minnesota and 43 in Montana. Seven species are common with state-wide distribution, <u>A. agrestis</u>, <u>A. canadensis</u>, <u>A. crassicarpus</u>, <u>A. flexuosus</u>, <u>A. missouriensis</u>, <u>A. striatus</u> and <u>A. tenellus</u>. All extend their ranges east of the Red River. <u>A. bisulcatus</u>, <u>A. gilviflorus</u>, <u>A. racemosus</u> and <u>A. lotiflorus</u> have wide distribution but are absent from the Red River valley. <u>A. pectinatus</u> is found rather uniformly distributed in the western half of the state. All other species have a much restricted distribution, growing only in specialized habitats of southwestern North Dakota.

SELECTIVE MEDIA FOR CULTURING SUCCINATE-DEGRADING RUMEN
BACTERIA. S. R. Zelie and P. P. Williams. Dept. of Bacteriol.,
NDSU and USDA, ARS, Metabolism and Radiation Research Labora-
tory, Fargo, N. D.   58102
     Selective media with and without succinate and vitamin K_1
were formulated. Growth response in these media was observed
spectrophotometrically with Bacteroides melaninogenicus (1),
Butyrivibrio fibrisolvens (2), Veillonella gazogenes (3), and
Vibrio succinogenes (4). The presence of succinate (25-2500
$\mu g/ml$ of medium) stimulated growth of organisms 3 and 4.
Succinate decarboxylation was observed when organisms 1, 3, and
4 were incubated anaerobically in a buffered mineral solution
at 37 C (5% CO_2-95% N_2, E_o^1, pH 7.4, >-30 mv) containing
succinic acid-1-4-^{14}C (20.4 mCi/mmol). No decarboxylation was
observed with 2. Addition of vitamin K_1 to the buffered
mineral solution suppressed succinate decarboxylation with cells
1, 3, 4, and with a mixed-rumen bacterial suspension (5).
Organism 3, when compared with 5, showed similar decarboxyl-
ation rates of 45.2 and 45.1%, respectively, without vitamin K_1.
By use of selective media, rumen bacteria of several genera can
be cultured without rumen fluid and can be shown to decarboxyl-
ate succinate anaerobically.

THE EFFECTS OF HE-O_2 ENVIRONMENTS AT 21 ATMOSPHERES AB-
SOLUTE UPON THE GROWTH OF GUINEA PIGS. C. A. Zogg. Dept.
of Physiology and Pharmacology, Sch. of Med., Univ. No. Dak.,
Grand Forks, No. Dak.
     The effect of He-O_2 at ambient and 21 atmospheres absolute
(ATA) upon the growth of guinea pigs was studied. Three groups of
male guinea pigs (295-395 grams each) were fed Ried-Briggs diet
ad libitum and subjected to environments of air and He-O_2 at am-
bient pressure and He-O_2 at 21 ATA. Substituting helium for at-
mospheric nitrogen at ambient pressure did not affect growth, feed
consumption or digestibility. When subjected to He-O_2 at 21 ATA,
the guinea pigs grew 137% slower than at ambient pressure condi-
tions. This was not a reflection of proportionately poorer feed
consumption nor digestibility. The growth of two normal intesti-
nal inhabiting organisms Escherichia coli and Proteus vulgaris
was unaffected by similar environmental conditions, as reported
by Waller and Zogg (Aerospace Med. Assoc. Preprint: 71-72, 1971).
Heat loss and other non-defined stresses associated with pressure
or He-O_2 at elevated pressure were responsible for halting growth
and depleting body tissues.
Supported by ONR Contract No. N00014-68-A-0499.

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Volume 27, Part II   October, 1975

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

OFFICERS AND OTHER MEMBERS OF
EXECUTIVE COMMITTEE 1972-73

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INSTRUCTIONS TO AUTHORS
FOR THE NORTH DAKOTA
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DEADLINES

Abstracts.—Both student competition and professional paper abstracts are due by the date specified by the Secretary of the Academy in the annual call for papers. Abstracts must be submitted on the prescribed form (available from the Secretary's Office) so that they can be published in time for the annual meeting of the Academy during the last week in April.

Papers.—Complete papers for student competition are due at the Office of the Secretary one month prior to the annual meeting, so that time is available for judging. Complete professional papers are due at the time of oral presentation during the last week in April.

PRESENTATION OF MANUSCRIPT

General—The general style for papers of the Proceedings will be that of the *Council of Biology Editors Committee on Form and Style* (CBE Style Manual Third Edition, 1972). Available from: American Institute of Biological Sciences, 3900 Wisconsin Avenue NW, Washington, D.C. 20016. Manuscripts that do not conform to the *Style Manual* or to the specific instructions given below will be returned to the authors for correction before consideration.

Authors are to write with clarity and conciseness so that the result is professional and consistent in style. The manuscript should be in completed, final form when submitted; changes after the galley proof is set can be made only with the approval of the Editor, and costs for these changes will be assessed to the author.

All parts of the manuscript must be typed double spaced with wide margins on 8½ inch x 11 inch white paper. Each original manuscript must be accompanied by two copies (Xerox or similar copy), including illustrations.

A separate title page, numbered one, should include the authors names, their affiliation and complete addresses, including zip codes. Subsequent pages should be number consecutively and the principal author's name should precede each page number.

A carefully organized paper should consist of the following parts introduced by major headings: ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENTS, and LITERATURE CITED; RESULTS and DISCUSSION may be treated together.

Headings.—Major headings are centered and capitalized. Subheadings are indented, underlined for italics, and followed by a period and dash (two hyphens on the typewriter) as used in these instructions.

Figures.—Maps, drawings, graphs, structural formulas and complex tables cannot be set in type and must be drafted and reproduced as line cuts. These illustrations must be drafted in India ink so they reproduce well, and should be submitted on separate sheets ordinarily not exceeding the size of the manuscript page. Therefore, larger drawings should be reduced photographically; if so, lines, lettering and symbols must be bold enough to stand the appropriate reduction.

Photographs must be unblurred and clearly show what is intended.

Each figure (drawing or photograph) must be proportioned to fit precisely on the printed page of the *Proceedings.* A full page figure should be 4⅛ x 6¾ inches to allow *adequate space* for a caption at the base of a full page figure. To reduce publishing costs, consider carefully if a full page figure is necessary, or whether a carefully cropped photograph or smaller line cut would convey the visual impression as well.

Each figure must be identified on the back with the figure number, author's name, and with the phrase ''Top of figure'' at the top of the page.

Figure captions are to be typed on a separate page and included with the manuscript. An example of a figure caption is as follows:

Figure 1.   Frequency occurrence of vegetation for each sampling station.

Tables.—Complex tables (those with vertical lines, characters on fraction of successive lines or unusually extensive characters or words) should be drafted as mentioned under Figures. Tables are to be double spaced on separate sheets, numbered (Arabic numbers) consecutively and given a short title. An example of a table caption is as follows:

Table 1.   Effect of pH on reactivity of chymotropsin.

The same material should not be repeated in tables and figures.

References.—References are to be listed at the end of the paper alphabetically and in the format of the *Style Manual.* Abbreviations of journals are in accordance with recommendations of Subcommittee Z39.5 of the American National Standards Institute. Examples of abstracting periodicals following these recommendations are *Bibliography and Index of Geology, Biological Abstracts,* and *Chemical Abstracts.* Examples of listing a book and journal are as follows:

Council of Biology Editors, Committee on Form and Style. 1972. CBE style manual. Third Edition. Amer. Inst. Biol. Sci., Washington, D.C. 297 p.

Groenewold, G. H., and F. R. Karner. 1970. Preliminary classification of concretions and nodules in the Cretaceous Hell Creek Formation, North Dakota. Proc. N.Dak. Acad. Sci. (II): 64-73.

Citations.—Citation of references in the text is by the name and year system. It may appear as Smith (1970:21) or (Smith, 1970:21). Figures

and tables are also to be cited in the text. For example: In the second and later years females grew faster than males (Table 1, Figures 2-4).

Footnotes.—Footnotes are costly and are to be avoided. Footnote material can usually be incorporated in the text or included under the major heading Acknowledgments.

Acknowledgments.—Grants and other aid are to be acknowledged under the major heading Acknowledgments.

Full papers.—Manuscripts of full papers consist of the following parts arranged in the indicated order (each page, beginning with the title page, is to be given a consecutive page number):

1. Title page (separate sheet)
2. Manuscript text
3. Tables (separate sheets)
4. Figures captions (separate sheet)
5. Figures

Other.—Words underlined in the text are placed in italics when set in type. Authors are to use the metric system for all measurements; equivalent values of the English system may be placed in parentheses.

CHARGES, GALLEY PROOFS, AND REPRINTS

For papers in excess of five printed pages, authors will be charged $10.00 per page for each page in excess of five. Exceptions may be granted in unusual cases. Authors are encouraged to include page charges in grant or other budget requests.

Galley proofs are to be corrected and returned, within three days, to the Editor. Reprints are to be ordered (at prices shown on the order form) at the time the galley proof is returned.

IN MEMORIAM

GEORGE ALONZO ABBOTT
1874-1973
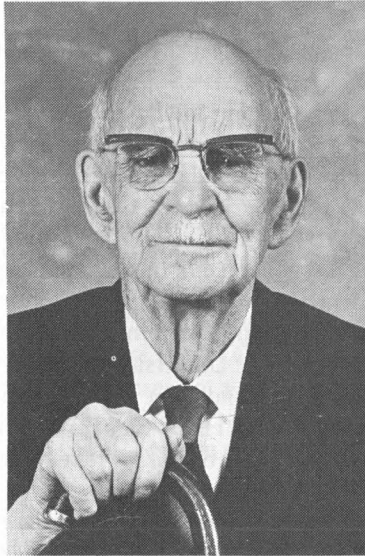
To no one does the North Dakota Academy of Science owe more than to George Alonzo Abbott. The day following the organizational meeting of the Academy, George Abbott arrived in Fargo to become a member of the chemistry faculty of the North Dakota Agricultural College, and immediately was named a member of the Academy's executive committee. From that time to the meeting of 1969, 60 years later, he did not miss a single annual meeting. For 40 of those years he was its Secretary-Treasurer.

The Academy is grateful for these 62 years of interest and service which this scholar and teacher has given to our organization. We are grateful for the many tasks well done over the years for the benefit of the society. At the 50th annual meeting of 1958 he presented to the Academy a comprehensive historical account of its founding andd record to that time which he called "The First Fifty Years." In this he suggested that the Academy warranted recognition and legal status as the "Official North Dakota Academy of Science." This recognition was granted at the State's next legislative session.

The Academy recognizes his many contributions to the field of education in which he has touched the lives of thousands of college students in the fields of science, and not only those in the classroom, but of many others. Many of us will remember his weekly radio talks over a period of 14 years which he called ''Science from the Side-Lines.'' Through his inspiration many of his students, both graduate and undergraduate, have gone on to positions of national and international recognition and leadership.

George Alonzo Abbott was born July 7th, 1874 at Alma, Illinois. He received both his Bachelor's and Master's degrees from DePauw University in 1895 and 1896. After teaching High School chemistry in Indiana and Minnesota, he returned to earn his Ph.D. degree at the Massachusetts Institute of Technology in 1908. On January 1, 1909 he became Assistant Professor of Chemistry at the Agricultural College in Fargo, North Dakota.

The following year, 1910, he came to the University of North Dakota as Professor of Chemistry and Head of the department, which positions he held until his retirement. He was awarded the honorary Doctor of Laws degree by the University of North Dakota in 1951 and the Doctor of Science degree by the North Dakota State University in 1962.

In 1962 a new chemistry building at the University of North Dakota was dedicated and named Abbott Hall. After his retirement he retained an office and private laboratory in this building where he continued his work as State Toxicologist.

In recent years his walks to the campus were less frequent. His steps were becoming slow and uncertain until in 1970 he gave up his laboratory and toxicology work. The weight of years became too heavy and on April 16th at the age of 98, this great and good man passed away.

Some excerpts from a poem Dr. Abbott wrote not long ago follows. It gives us an insight into a part of his philosophy. He called it ''On Love and Life.''

Love brought me here.
I did not ask for Life.
Love nurtured me,
A helpless creature small,
A man to be
As I grew strong and tall.

And day by day
To guide through toil and strife,
Love showed the way.
The Way; The Truth; The Life.

Is Death the end
Of all this loving care?
No! Such a trend
Assures more Life somewhere.

Somewhere above,
When Death ends this earthly strife,
I'll trust this Love
To give Eternal Life.

—E. D. Coon

PRELIMINARY STUDY OF THE CARBONATES OF THE CHERTY MEMBERS OF THE BIWABIK FORMATION, MINNESOTA

Les. R. Honeyman and Frank R. Karner

Department of Geology
University of North Dakota
Grand Forks, North Dakota 58202

ABSTRACT

Siderite (FeCO3) and ankerite (ferroan [CaMg $(CO_3)_2$]) of primary, diagenetic and metamorphic origin are the principal carbonates in the magnetite-rich cherty members (taconite) of the Biwabik Formation. Carbonate distribution that affects taconite pellet quality was studied in drill-core and pit samples from the Erie Mining Company property north of Hoyt Lakes, Minnesota. Carbonates typically occur in irregular layers and lenses in a sequence of alternating cherty and magnetitic sedimentary beds. Siderite typically occurs in three forms: microgranular, granular, and crystalline aggregates. Ankerite occurs with siderite as rhomb-shaped crystal up to several millimeters in length and as alteration rims on siderite granules. Calcite occurs only as a secondary mineral in fractures and fault zones. Siderite appears to have formed as a primary mineral during sediment deposition and diagenesis, whereas ankerite formed during epigenetic and metamorphic recrystallization.

INTRODUCTION

This preliminary study of carbonate minerals in the Precambrian Biwabik Formation utilizes samples from the Erie Mining Company property north of Hoyt Lakes, Minnesota (Figure 1). Begun after Erie had experienced periodic problems with the quality of taconite pellets apparently traceable to their carbonate content, this study summarizes part of a Master's thesis by the senior author (Honeyman, 1973). Rock and mineral descriptions and identifications were done using standard methods. Mineral abundances were determined by x-ray diffraction methods described by Karner (1968).

RESULTS

Stratigraphy and composition of carbonate-rich layers.—The Biwabik Formation is underlain by the Pokegama Quartzite and is overlain by the Virginia Slate (Figure 2). The younger Duluth Gabbro has intruded the Biwabik For-
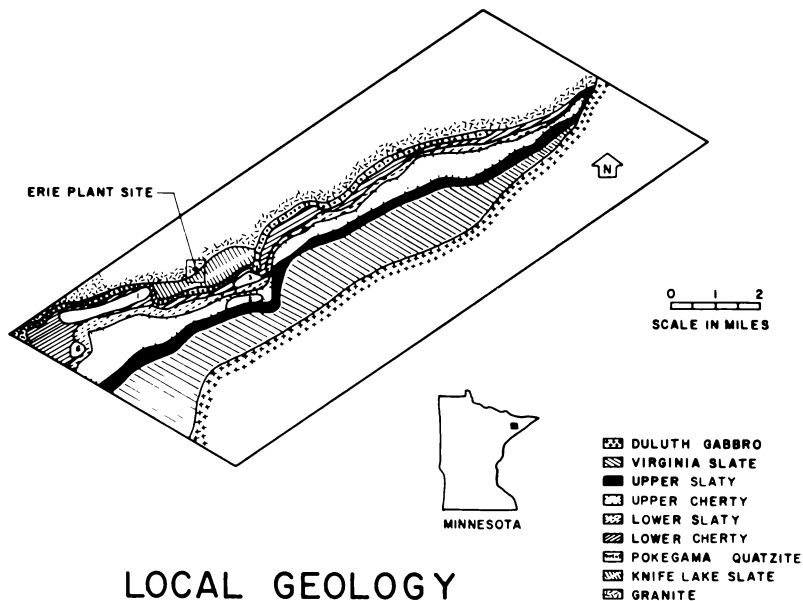
Figure 1. Location and geologic map of the study area (Honeyman, 1973). Numbers refer to pit areas of the Erie Mining Company.

mation in the eastern part of the study area (Figure 1). The Biwabik is considered to be late Precambrian and has been dated from 1.7 to 2.0 x 10^9 years before present (Goldich et al., 1961). The Biwabik is divided into four members, in ascending order: the Lower Cherty, Lower Slaty, Upper Cherty, and Upper Slaty Members (Figure 2). Each of the members is divided into several layers (Gunderson and Schwartz, 1962; Honeyman, 1973). The Upper and Lower Cherty Members are the primary ore zones and were the members of interest in this study of the carbonates. Important characteristics of these layers are summarized in Figure 2, Carbonates in layers U, T, S, and R in the Lower Cherty and L, K′, K and J in the Upper Cherty were studied in detail. Bulk mineralogical compositions of these layers are given in Table 1.

Occurrence of siderite.—Siderite is the most abundant carbonate mineral and occurs in three forms: microgranular (micrite-like), granular (oölitic), and crystalline aggregates (idioblastic or granoblastic, recrystallized) (Dimroth and Chauvel, 1973).

Microgranular siderite is less than 0.005 mm in particle size and is unbedded. It makes up the entire rock in some areas and cements siderie granules in others (Figure 3).

GEOLOGIC COLUMN

ERA	FORMATION	MEMBER	LAYER		DESCRIPTION
					GLACIAL TILL
	DULUTH GABBRO				MEDIUM TO COARSE GRAINED GABBRO COMPOSED OF FERROMAGNESIAN MINERALS AND PLAGIOCLASE FELDSPAR
	VIRGINIA SLATE				NON-MAGNETIC, GREY TO BLACK SLATE
		UPPER SLATY	A		GREY-WHITE, COARSE-GRAINED LIMESTONE
			B		NON-MAGNETIC, CHERT AND IRON-SILICATES
			C		SLIGHTLY-MAGNETIC, SLATY AND CHERTY BEDS
			D		THIN, CLOSELY SPACED MAGNETIC BEDS
			E		HIGH PHOSPHORUS LAYER
			F		SLIGHTLY-MAGNETIC, INTERBEDDED SLATY AND CHERT BEDS
PRECAMBRIAN	BIWABIK FORMATION	UPPER CHERTY	G		HIGHLY MAGNETIC. MAGNETITE OCCURS AS GRANULAR CONCENTRATIONS AND BEDS
			H		HIGHLY MAGNETIC, WAVY BEDS OF MAGNETITE
			I		ALGAL STRUCTURE
			J		MAGNETITE IN CHERT AND IRON SILICATE MATRIX
			K		THIN, WIDELY SPACED BEDS OF MAGNETITE
			DIABASE SILL		COMPOSITION SIMILAR TO GABBRO
			K'		AME AS "K" LAYER
			L		HIGHLY MAGNETIC SHARPLY DEFINED BEDS OF MAGNETITE CHERT AND IRON SILICATES MAKE UP MATRIX
			M		HIGHLY TO SLIGHTLY MAGNETIC. MIXED WAVY AND STRAIGHT BEDS OF MAGNETITE
			O		MODERATELY MAGNETIC. THICK LAYERS OF CHERT SEPARATING MAGNETIC LAYERS
		LOWER SLATY	P		NON-MAGNETIC, THIN BEDDED, DARK-GREY TO BLACK SLATE ARGILLITES
			Q		SIMILIAR TO "P" LAYER
		LOWER CHERTY	R		SLIGHTLY TO HIGHLY MAGNETIC, WIDELY SPACED MAGNETITE BEDS IN CHERT AND IRON SILICATE MATRIX
			S		HIGHLY MAGNETIC, MAGNETITE IS GRANULAR
			T		WAVY MAGNETITE BEDS, INDISTINCT BOUNDARIES
			U		HIGHLY MAGNETIC, STRAIGHT BEDS OF MAGNETITE IN AN IRON SILICATE MATRIX
			V		MODERATELY MAGNETIC CONGLOMERATIC ZONE
			W		ALGAL STRUCTURE
	POKEGAMA QUARTZITE				COARSE GRAINED QUARTZ AND QUARTZ PEBBLE CONGLOMERATE
	ALGOMAN GRANITE				INTRUSIVE GRANITE
	KNIFE LAKE SLATE				METASEDIMENTARY ROCKS
	LAURENTIAN GRANITE				INTRUSIVE GRANITE
	ELY GREENSTONE				VOLCANIC ROCKS AND BASIC INTRUSIVES

Figure 2.   Geologic column for the eastern Mesabi Range (Honeyman, 1973).

Granular siderite occurs as rounded grains 0.005 - 1 mm in diameter and is found in zones made up of both siderite and chert granules cemented by either microgranular siderite (Figure 3) or fine-grained chert.

Table 1. Bulk mineralogical composition[a] of the Upper and Lower Cherty members (modified from Honeyman, 1973).

Lower Cherty					
Layer	Siderite	Ankerite	Calcite	Quartz	Magnetite
R	6	4	1	59	29
S	5	4	0	54	31
T	4	4	0	50	35
U	8	4	1	52	33

Upper Cherty					
Layer	Siderite	Ankerite	Calcite	Quartz	Magnetite
J	5	6	1	50	29
K	5	4	1	52	31
K'	9	4	0	49	36
L	7	2	1	55	25

[a]Expressed as weight percent
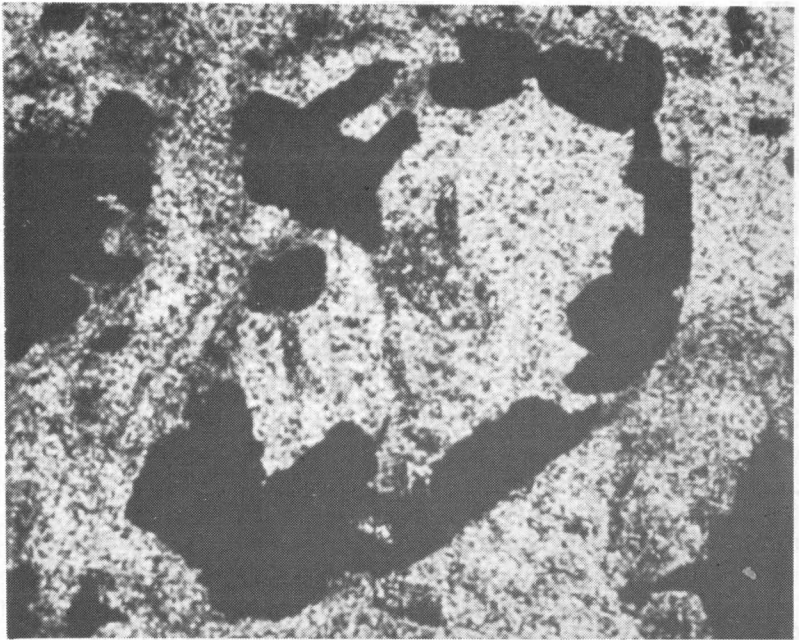
Figure 3. Granular siderite with microgranular siderite cement. Magnification 50X; plane polarized light.
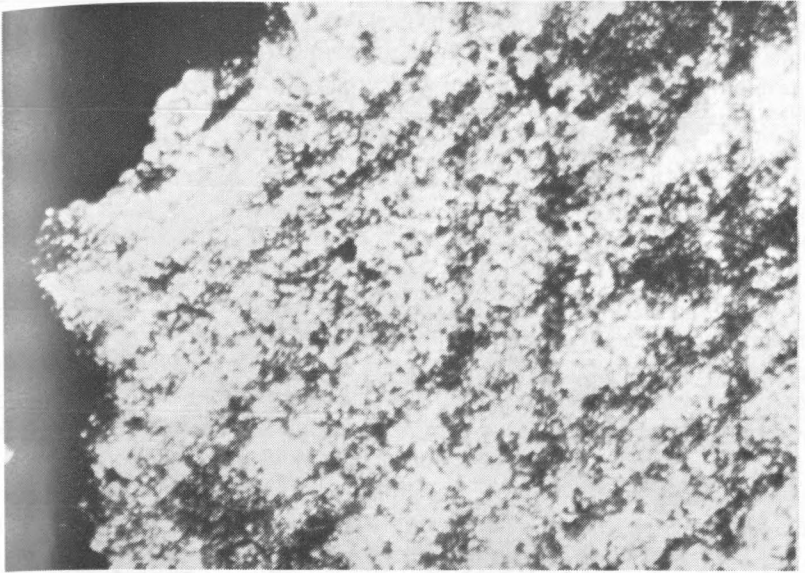
Figure 4. Crystalline aggregate form of siderite. Magnification 50X; Crossed polars.
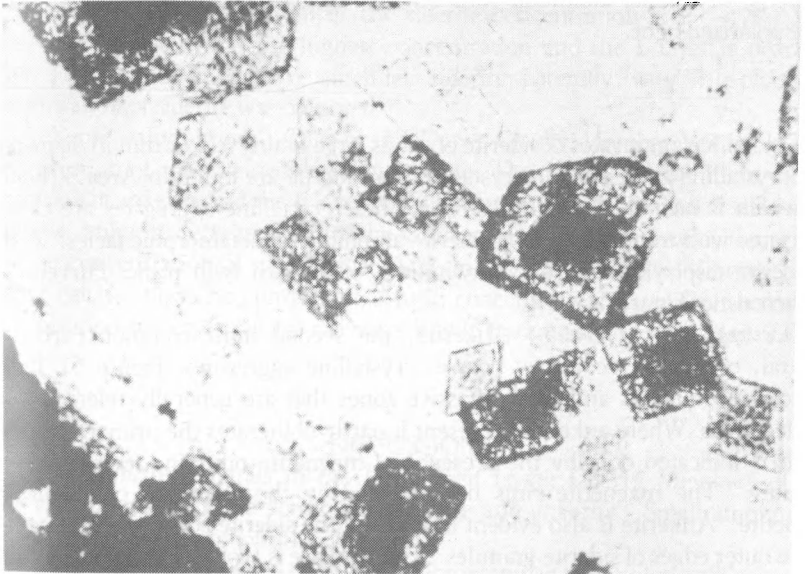
Figure 5. Coarse crystalline rhombs of ankerite. Magnification 50X; plane polarized light.
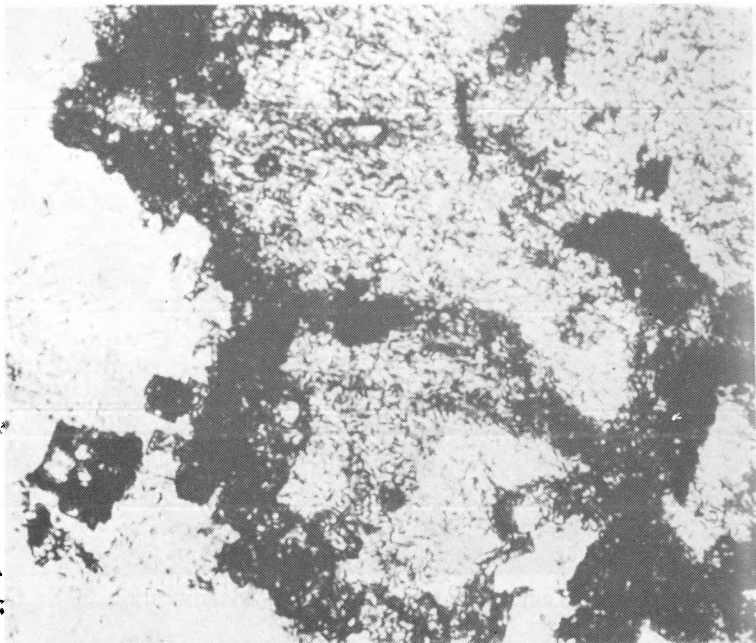
Figure 6.   Ankerite alteration rims on siderite granules. Magnification 50X; plane polarized light.

Crystalline aggregates of siderite occur as large grains 0.5 - 2 mm in diameter. The crystalline aggregates (recrystallized) of siderite are found in Area 3 (Figure 1), which is near the Duluth Gabbro.   These crystalline aggregates are closely associated with minerals of the grunerite-amphibolite metamorphic facies. Zones of siderite displaying continuous twinning, often with twin planes curved, are characteristic (Figure 4).

Occurrence of ankerite.—Ankerite, the second most common carbonate mineral, occurs as secondary, coarse, crystalline aggregates (Figure 5). It has replaced all minerals and forms massive zones that are generally referred to as mottled areas. Where ankerite is present it partly obliterates the primary texture, which is indicated only by the presence of magnetite outlining former siderite granules.   The magnetite rims have formed by the alteration of siderite to magnetite. Ankerite is also evident in many of the siderite zones where it occurs on the outer edges of siderite granules. The ankerite is typically euhedral. Where ankerite has formed alteration rims on siderite granules, it tends to be less massive and does not obliterate the texture so completely (Figure 6).

From x-ray or thin-section data ankerite could not be differentiated from dolomite. However, the results of an electron microprobe examination showed that no dolomite is present, but large amounts of ankerite occur in the iron formation on the Erie property (W.H. Tuttle, 1972, Erie mining Company, Hoyt Lakes, Minnesota).

Occurrence of calcite.—Minor amounts of calcite are formed in most pit areas of the Erie property. The calcite occurs in large fractures and fault zones and as distinct grains in small fractures. Calcite veins cut across grains of all other minerals indicating a relatively late origin.

Variation in carbonate distribution.—In the Lower Cherty Member, siderite concentration is 0 - 15%. Vertically, the U layer has the highest concentration of siderite and the S layer is the next highest (Figure 7). The lateral differences in siderite concentration are minor with one exception: the samples from Area 3 have much higher siderite:ankerite ratios with a decrease in the total amount of siderite and a parallel increase in total magnetite.

Ankerite values are 1 - 25% in the Lower Cherty Member. Vertically the ankerite concentration remains constant. Laterally, the ankerite content differs slightly in Areas 1 and 6, but is markedly lower in Area 3.

In the Lower Cherty Member the calcite concentration remains nearly constant at about 1%. The concentration of calcite is constant vertically and increases laterally where large fracture and fault zones occur, particularly in Areas 3 and 6.

In the Upper Cherty Member the siderite concentration is 2 - 49%. Vertically, the K' layer has the highest concentration and the L layer is nearly as high. The J and K layers have much less siderite. Laterally, very little change in concentration of siderite was observed.

Ankerite values are 0 - 38% in the Upper Cherty Member. Vertically, the concentration of ankerite decreases with depth. The J layer has the highest concentration of ankerite and the L layer has the least, although local reversals occur. Laterally, ankerite decreases toward the southern edge of Area 2-west (Figure 7).

The concentration of calcite is 0 - 10% in the Upper Cherty Member. Vertically, calcite shows no constant change in concentration. Laterally, the calcite concentration increases toward the more highly fractured, southern edge of Area 2 west.

DISCUSSION

The carbonate minerals in the Upper and Lower Cherty Members of the Biwabik Formation are predominantly siderite and ankerite. Small amounts of calcite also occur throughout the property.

Siderite occurs in three forms: microgranular, granular (oölitic), and crystalline aggregates (recrystallized). The microgranular and granular siderite formed with the original precipitate (a series of hydrous iron oxides or iron-silica
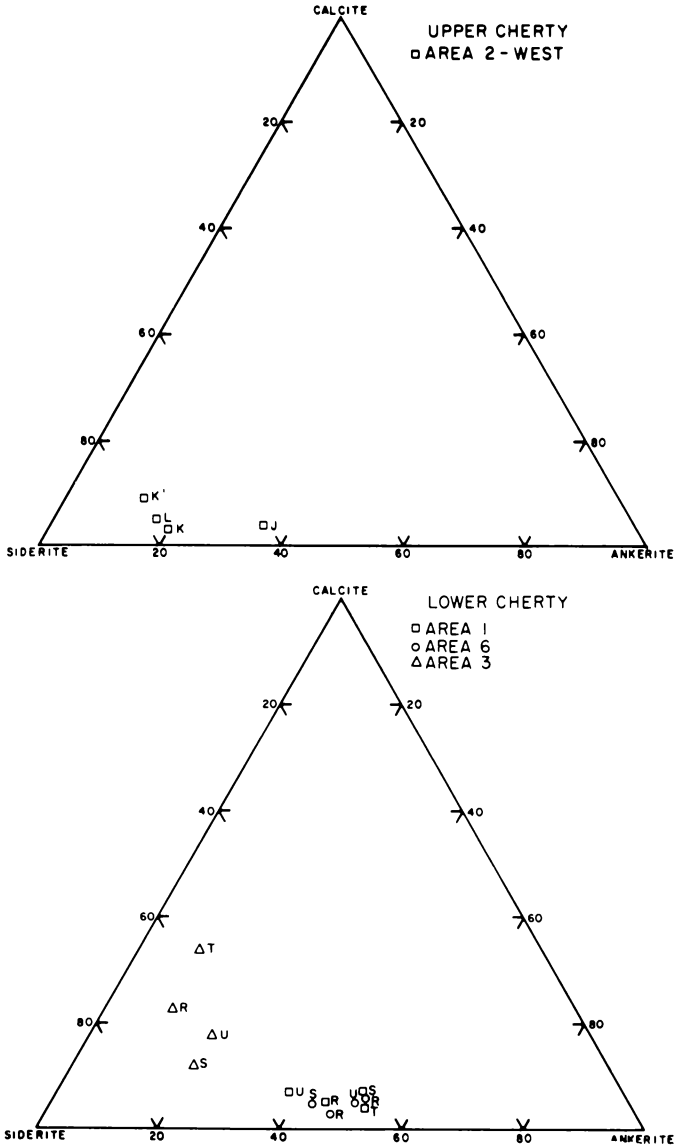
Figure 7. Triangular diagrams with relative abundance of siderite, ankerite and calcite in the Upper and Lower Cherty Members of the Biwabik Formation in the Erie Mining Company pit areas. Letters refer to layers summarized in Figure 2.

gels) and during diagenesis of the original precipitate. The crystalline aggregates of siderite formed as a result of intrusion of the Duluth Gabbro. The siderite was altered or replaced by several minerals, but the greatest amount was replaced by magnetite.

Ankerite is a secondary mineral formed by the alteration or replacement of magnetite, siderite, minnesotaite, and quartz. Ankerite occurs as alteration rims on siderite granules and as large rhombic crystals. The alteration rims are believed to be related to a period of ankeritization early in the epigenetic history of the rock. The rhombic, crystalline form of ankertie is related to a period of ankeritization much later in the epigenetic history; this crystalline form of ankerite tends to obliterate the primary texture of the rock. The only replacement of ankerite is by magnetite.

Calcite is present throughout the area with the highest concentrations where large fracture and fault systems cross the formation. Calcite appears to have been introduced into the rock much later than either siderite or ankecite.

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PRELIMINARY DESCRIPTION OF PRECAMBRIAN IRON FORMATION, PEMBINA COUNTY, NORTH DAKOTA

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ABSTRACT

Precambrian iron formation has been obtained by drilling of the 7500-gamma magnetic anomaly centered one mile (1.6 km) east of Akra in Pembina County, northeastern North Dakota. Preliminary investigation of a 348-foot (106-m) interval of NX diamond-drill core shows that the rock consists predominantly of quartz-magnetite and quartz-silicate iron formation in an inclined sequence interbedded with quartz-biotite schist. Compositional layering and schistosity are parallel and inclined at angles of 20° or less to the core axis indicating dips of 80°-90°. Schist layers are typically 0.05-2 m thick and consist of fine-grained quartz with scattered biotite, epidote and minor magnetite and chlorite. The iron formation typically consists of alternating quartz-rich and magnetite- or silicate-rich microlayers 1-5 mm thick. These rocks are mineralogically similar to those of the oxide and silicate facies of Precambrian iron formation.

INTRODUCTION

Interest in magnetic anomalies in northeastern North Dakota began in 1964 when the Topographic Branch of the United States Geological Survey reported difficulties in using magnetic compasses while mapping near Akra and Hensel, North Dakota. Members of the Department of Geology faculty at the University of North Dakota investigated these areas in the all of 1964 and located two sites of unusually intense magnetic field; in the summer of 1966, these anomalies were outlined in detail (Moore and Karner, 1969 and Figure 1). The Akra anomaly was found to be 7,500 gammas and the Hensel anomaly, 11,500 gammas in excess of the normal magnetic field (Moore and Karner, 1969).

In July of 1969, prompted by the possibility of discovering economic ore deposits, the rock bodies producing the magnetic anomalies were cored by the Amerada Hess Petroleum Corporation. This study summarizes the preliminary results of hand-specimen and thin-section examination of the core recovered from the Akra drilling site in SE¼ sec. 11, T. 161 N., R. 55W., about one mile (1.6 km) east of Akra, Pembina County, North Dakota.
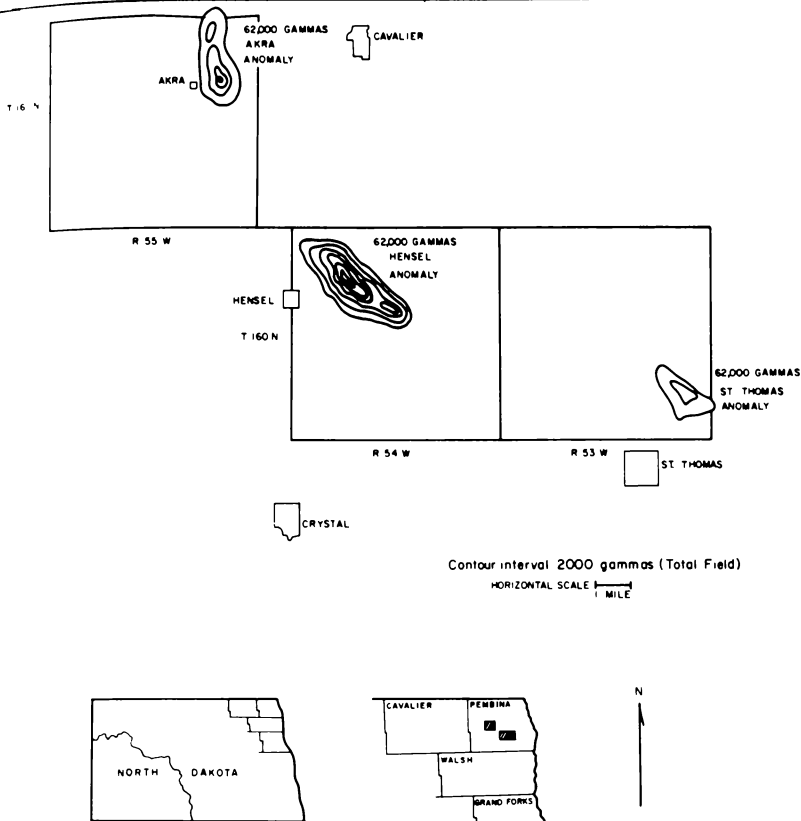
Figure 1.   Location map of magnetic anomalies (Moore and Karner, 1969).

RESULTS

General.—There are five major rock types in the Precambrian part of the Akra core: quartz-magnetite iron formation, quartz-silicate iron formation, quartz-magnetite-silicate iron formation, quartz-biotite schist, and quartz-hematite-chlorite-iron formation. Their distribution is summarized in Figure 2 and the rock types are described below. Compositional layering and schistosity are parallel and inclined at angles of 20° or less to the core axis indicating dips of 80°-90°.

Quartz-magnetite iron formation.—This type consists of well-defined, alternating, granoblastic quartz-rich and magnetite-rich layers (Figure 3). The magnetite-rich layers are 75-90 volume % magnetite with minor quartz, biotite, epidote, and stilpnomelane and are typically 1-5 mm thick. Layers that appear
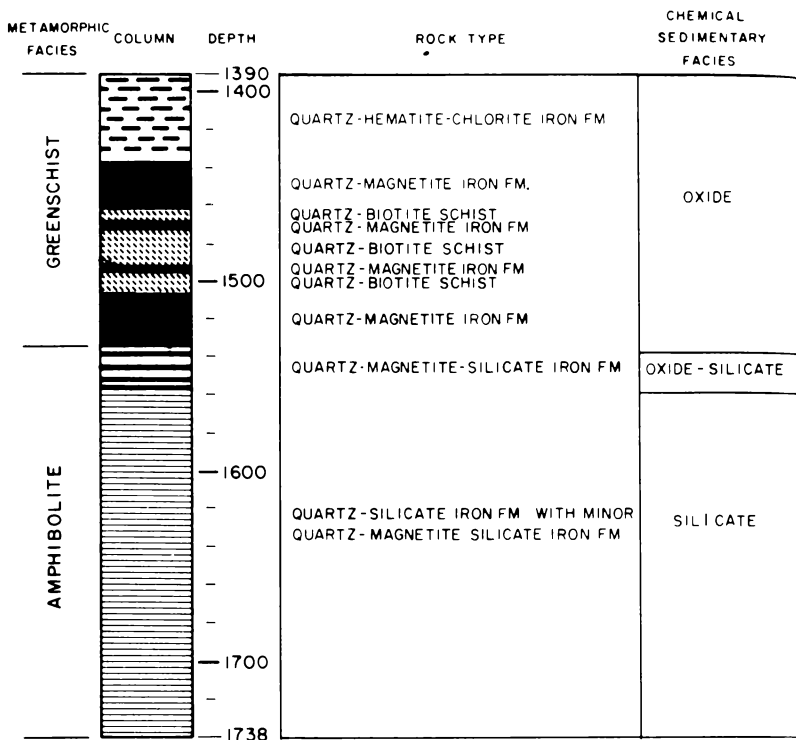
Figure 2.   Stratigraphic column, rock types, chemical sedimentary facies and metamorphic facies of Akra core. Depth given in feet below surface at drilling site.

homogeneous in the hand specimens are microscopically interbedded with quartz-rich layers. The quartz-rich layers are 1-10 mm thick and contain up to 15% magnetite and minor biotite and epidote. The quartz-rich and magnetite-rich layers are present in approximately equal amounts in the quartz-magnetite iron formation.

Quartz-silicate iron formation.—This type consists of interbedded quartz-rich and grunerite-rich layers 1-10 mm thick (Figure 4) that contain minor biotite and epidote. Usually less than 5% magnetite is present. Grunerite is an iron-rich amphibole typical of highly metamorphosed iron formation. The quartz-silicate iron formation has a predominantly schistose fabric but exhibits some rosette structures or contains radiating aggregates of prismatic crystals thought to indicate growth after removal of stress (Heinrich, 1956).

Quartz-magnetite-silicate iron formation.—This rock type is characterized by
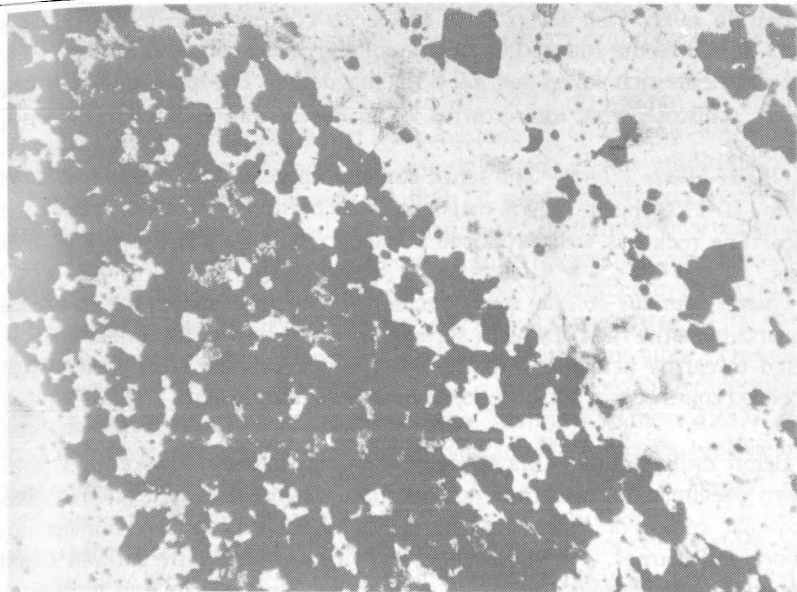
Figure 3. Photomicrograph of quartz-magnetite layers of oxide facies. Depth 1,452 feet. Magnification 50X. Plane polarized light.
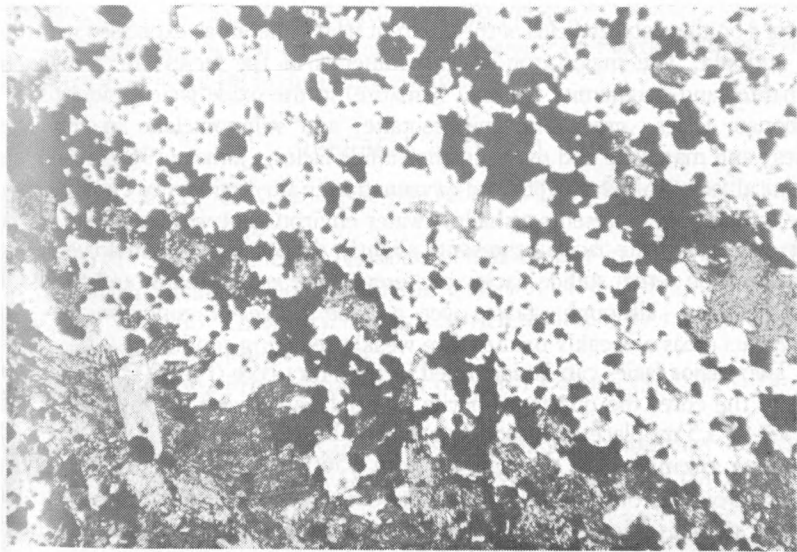
Figure 4. Photomicrograph of quartz-grunerite layers of silicate facies. Depth 1,536 feet. Magnification 50X. Plane polarized light.

alternating quartz-rich layers with grunerite concentrated in the magnetitic layers. Minor biotite and epidote are present and calcite occurs as fracture fullings. The magnetite-rich layers are ,60-85% magnetite. The predominant fabric is weakly schistose with intergrowths of grunerite and biotite-forming rosette structures.

Quartz-biotite schist.—The schist contains approximately 55% quartz, 25% biotite, 10-15% epidote, 3-5% magnetite, and minor iron silicates, sulfides, and albite. The rock has well developed schistosity and scattered quartz porphyroblasts.

Quartz-hematite-chlorite iron formation.—The upper part of the core consists of quartz-hematite rock in which, apparently, original specular hematite has been altered to earthy hematite with the fabric of the original rock preserved. Interbedded zones of quartz-biotite schist have been chloritized.

DISCUSSION

Before drilling of the Akra anomaly, it was attributed to one of three possible origins: sedimentary hematite iron formation, magnetite disseminated in gabbros or similar rocks, or magnetite-rich bodies of igneous or replacement origin (Moore and Karner, 1969). Recovery of diamond-drill core showed that the magnetic anomaly results from the presence of Precambrian iron formation, a siliceous iron-bearing metasedimentary rock.

James (1966) divided the depositional environments of Precambrian iron formation into four facies: oxide, silicate, carbonate, and sulfide. This classification is based on the stability fields of the different iron minerals as expressed in terms of Eh and pH. The major iron-bearing minerals of the facies are: magnetite, hematite, and maghemite (gamma hematite) in the oxide facies; siderite in the carbonate facies; greenalite, minnesotaite, and stilpnomelane in the silicate facies; and marcasite and pyrite in the sulfide facies. James (1966) related each chemical facies to water depth and oxygenation in the sedimentary environment. The oxide facies represents a shallow water environment with high oxygen content; the carbonate facies represents slightly deeper water with lower oxygen concentration; the sulfide facies represents deeper stagnant areas, and the silicate facies, although stable over a wide range of conditions, typically represents areas of weakly oxidizing to weakly reducing conditions.

Two major facies can be identified in the Akra core (Figure 2). In the lower part of the core, the quartz-grunerite assemblage between 1738 and 1560 feet (529.7-475.5 m) places this sequence in the silicate facies. The quartz-magnetite-grunerite assemblage in the interval from 1560 to 1533 feet (475.5-467.3 m) shows an overlap of the silicate facies and oxide facies where both iron oxide and iron silcate phases were stable in a mildly oxidizing environment. In the interval from 1533 to 1446 feet (467.3-440.8 m), the quartz-magnetite assemblage indicates the oxide facies and a shift toward more oxidizing or shallower conditions. Thin-section study of the quartz-hematite-chlorite iron formation in the upper

portion of the core, from 1446 to 1390 feet (440.8-423.7 m), indicates it to have formed from quartz specular hematite iron formation, probably as a result of weathering.

The interval from 1390 to 1385 feet (423.7-442.2 m) was not cored, but interpretation of the electric log is that it is sandstone (C. G. Carlson, 1973, North Dakota Geological Survey, personal communication). If this sandstone is near shore or even continental (alluvial) in origin, it would signify emergence of the area in later Precambrian or early Cambrian time. The upper quartz-hematite-chlorite assemblage could then be attributed to subaerial weathering processes.

Two facies of regional metamorphism have been identified in the Akra core. The unweathered interval above 1533 feet (467.3m) is characterized by the mineral assemblage quartz-magnetite-biotite-stilpnomelane with some albite. This assemblage is indicative of the greenschist facies and is analogous to the biotite subfacies of the Huronian iron formation of northern Michigan (James, 1955). Rocks below 1533 feet (467.3 m) are assigned to the amphibolite facies because of the presence of grunerite (James, 1955).

Meager data on the Precambrian basement in northeastern North Dakota makes a structural interpretation difficult. However, because of the steep dips of the rock layers it seems possible that the iron formation may have been preserved through infolding or infaulting into the surrounding and possibly underlying Ramsey gneiss terrain (Lidiak, in press).

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SHORELINE EROSION ON
LAKE ASTABULA, NORTH DAKOTA

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INTRODUCTION

Shoreline erosion on Lake Ashtabula, a 26-mile long lake in southeastern North Dakota, was studied from 1969 to 1971 to determine what processes and conditioning factors were most important on that lake. Shoreline bank erosion is one of the main determinants of the useful life of this man-made reservoir. The shoreline erosion rate also determines if vegitation can establish a protective covering along the shoreline and thus increase the aesthetic value of the lake.

Setting.—Lake Ashtabula, a U.S. Army Corps of Engineers reservoir, was formed when Baldhill Dam blocked the flow of the Sheyenne River in 1950. The depth of the lake is 30-35 feet on the inundated river floodplain near the dam and 3-6 feet on the inundated floodplain near the Griggs-Barnes County border at the upper reaches of the lake. Pool level is held at 1266.0 feet above sea level except for a 3.5-foot drawdown in the early spring to provide floodwater storage for spring-melt runoff (Baldhill Dam operating records). Lake Ashtabula has an ice cover for 5-6 months of the year. A conspicuous band of submergent vegetation (*Potamogeton* spp.) growing in water depths of 2-6 feet has developed along the entire shoreline of the lake.

The Pierre Shale of Upper Cretaceous age crops out at numberous places along the shoreline of Lake Ashtabula. Glacial till and stream deposits, mantling the Pierre Shale, also occur along the shoreline of the lake. Selective hillslope erosion of the till mantle has developed a boulder lag that has prevented shoreline erosion in places.

MATERIALS AND METHODS

Shoreline measurements.—Stations were established at 100-foot intervals around the shoreline of Lake Ashtabula. Shroeline bank sediment and shoreline use was noted at each station. Shoreline bank height and the slope of the valley wall immediately above the shoreline bank was measured at each station. Groun-

dwater regime, as evidenced by "spongy" beaches, springs, and wetted areas was also noted for each station. All information was numerically coded and evaluated using specially written computer programs (Pederson, 1971) to find the means of erosion to date for the categories listed in Table 1.

Table 1.   Analysis of bank erosion for Lake Ashtabula.

Condition	No. of stations in sample	Mean-ft^3/foot of shoreline
Shoreline use		
Cattle access	1062	40
No cattle access	492	28
Groundwater regime		
Groundwater evidence	222	54
No groundwater evidence	1332	33
Shale banks		
Groundwater evidence	100	80
No groundwater evidence	257	65
Till banks		
Groundwater evidence	100	36
No groundwater evidence	875	27
Alluvium banks		
Groundwater evidence	22	19
No groundwater evidence	200	20

Erosion to date.—Bank erosion at each station was calculated using the equation

$$Area = \frac{H^2 \cdot CotA}{2}$$

where H is bank height and A is the valley-wall slope angle. Multiplying the area by one gives the amount of erosion per foot of shoreline. This volume represents a minimum because subaqueous erosion was not considered.

Ice Push.—The effects of ice push was measured by precisely locating the positions of submerged and nearly submerged boulders in several test areas located around the shoreline of the lake in the fall and then relocating them in the spring after the ice had melted. No measurable movement was detected.

Stable-shelf profile.—Kondratfev (1966) reported the equation

$$X = ay^2 + \frac{1}{m_N}Y$$

Where

$$a = \frac{m_N - m_o}{20\,m_N m_o}$$

approximates the form of a stable-shelf profile. M_N and m_o are empirical parameters. A shelf with this profile will asue the energy of incoming waves to be expended in internal turbulence. A modification of the concept (Pederson, 1971) yields the linear equation $Y_w = -X_w \cdot \tan W$ where W is the wave-response slope (Figure 1). Wave response slope used was empirical and was measured for diffrent bank materials along the shores of Lake Ashtabula. Three other linear equations were generated to describe the repose face of the wave built terrace ($Y_r = X_r \cdot \tan R + Br$; R = repose slope), the stable-bank slope behind the strandline ($Y_s \cdot \tan S$; S = bank slope), and the slope of the valley wall ($Y_v = X_v \cdot \tan V + Bv$; V = valley-wall slope).
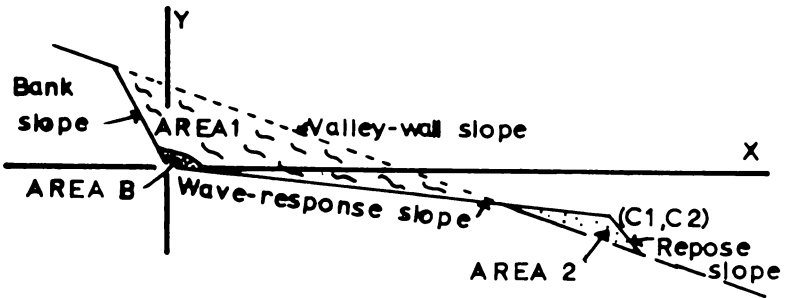
Figure 1. Diagram of stable-shelf concept.

At full development of the stable-shelf profile the edge of the wave-built terrace ((C1,C2) Figure 1) is at the depth of effective wave action (approximately 1/2 wave-length). Particles eroded from AREA 1 (Figure 1) that are too large (sand-sized and larger particles) to be carried in suspension are transported as bedload and deposited in AREA 2 or along the beach berm. AREA 2 + AREA B (Figure 1) is fractionally to AREA 1 as sand-sized and larger particles are to the total particle distribution in bank material. A computer program (Pederson, 1971:104) was used to calculate the areas and values of intercepts when stable-shelf conditions are attained for bank materials of varying particle size distribution and the possible range of valley-wall slope angles. The appropriate values were then compared to actual bank erosion at each station for the calculation of percentage-of-development to date.

RESULTS AND DISCUSSIONS

Erosion processes.—The major shoreline erosion processes observed were slumping, block separation, frost weathering, and collapse by undercutting. Most slumping is developed in the Pierre Shale and is commonly associated with groundwater activity. Fractures in the Pierre Shale provide excellent conduits by which surface runoff can enter the ground. This water, when trapped by the closing of fractures at depth, can build up large hydrostatic pressures, reducing the shearing resistance between blocks. Once movement of the slump block occurs, additional fractures develop and the process is enhanced. Swelling, followed by the contraction action of montmorillonite clays in shoreline bank material, helps to develop the fractures that lead to slumping. This swelling action also leads to the development of tabular blocks 2 - 5 inches thick along vertical shoreline banks. Wetting of the shoreline banks to a depth of a few inches by wave action and rainfall causes the montmorillonite clays to expand, lifting the face of the bank upwards and outwards. A small fracture parallel to the bank face and 2 - 5 inches from the face results from the stress with deeper unexposed bank material. Contraction of the montmorillionite clays with drying on the surface of the block curls the block much like a bimetallic strip and further opens up the fracture. The opening fracture provides a catchment for rainwater runoff and hydrostatic pressures may develop causing additional horizontal movements. The block eventually falls into the lake and the next block starts to form.

Frost weathering as described by myself (1971:19) and Harrison (1970:3407-3409) is active during the freeze-thaw cycles of early spring. Angular granules popped loose from fine-grained bank material form, in a few weeks, talus piles that nearly bury the shoreline bank face. Wave action on the thawing of the ice cover rapidly removes these talus piles.

Undercutting of shoreline banks by wave action and spring sapping with subsequent collapse of overhangs is common. The collapsed overhangs are rapidly broken up by wave action.

The fine-grained sediment from shoreline erosion is transported by turbidwater currents into the deep areas of the lake. The coarse-grained sediment is transported by longshore currents to the bay areas of the lake.

Conditioning factors.—Conditioning factor categories (Table 1) identified as playing a determinant role in shoreline erosion were shoreline use, shoreline bank material, and groundwater regime.

Cattle trample vegetation, stir up shoreline sediment, and break down bank faces. The result is an increased rate of erosion where cattle have access to the shoreline of the lake (Table 1). Limiting their access to depositional areas such as at beaches would greatly reduce their effect on erosion rates.

Protective boulder lags (beach armament) and wave-built terraces develop only where there is a supply of sand-sized and larger material in shoreline banks.

The till and alluvium banks with coarse-grained particles have developed these features. The shorelines where the Pierre Shale crops out have not developed these features. As would be expected, the total erosion of shale banks is much greater as compared to other types of bank material (Table 1).

Groundwater discharge causes increased erosion of both till and shale banks (Table 1). Weight added to the total mass, spring sapping, and expansion of montmorillonite clays are the main ways by which the groundwater affects the erosion rates on shale and till banks. Because of greater porosity, groundwater discharge has no effect on alluvium banks.

Organic activity other than cattle play an important role in shoreline bank erosion. The burrowing and hunting activities of animals living along the shores must have a significant effect because of their large numbers. The role of aquatic plants should not be overlooked. The role of the submergent vegetation-band mentioned earlier is especially important. The heights of numerous incoming waves were measured along a dock that spanned the submergent vegetation-band. Wave forms were reduced from one-fourth to one-half their original height in passing through the submergent vegetation-band. Since the energy of a wave form is directly proportional to the square of the wave height the energy loss in passing through the submergent vegetation-band is at least 50%.

Percentage of shoreline development.—Percentage of development was based on the comparison of actual erosion to the projected erosion when a stable-shelf profile is attained. The average shoreline erosion to date is 6% of the projected erosion. Headlands, as would be expected, had a greater percentage of development than bay areas. As already indicated, cattle access and ground water discharge areas had the greatest erosion and therefore the greatest percentage of development.

CONCLUSIONS

Shoreline erosion on Lake Ashtabula is greatest where cattle have access to the shoreline, where bank material is silt-sized or smaller, and where groundwater discharge is evident except in the case of alluvial bank material. The submergent vegetation-band is extremely effective in reducing wave energy. Recognition of the importance of these factors will hopefully suggest ways to reduce bank erosion and extend the useful life of the reservoir.

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GLACIAL STRATIGRAPHY OF NORTHEASTERN NORTH DAKOTA

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ABSTRACT

The glacial drift of northeastern North Dakota consists of nine formations that are differentiated by outcrop characteristics, grain size, and the coarse-sand lithology. The formations are described in ascending stratigraphic order. Unit D consists of clayey, silty till that contains many fragments of igneous and carbonate rock. Unit C consists of clayey till that contains many fragments of shale and carbonate rock. Unit B consists of silty, sandy till that contains many fragments of shale and igneous rock. Unit A consists of silty, sandy till that contains many shale fragments. Unit 1 consists of silty till that contains many fragments of carbonate rock. The lower part of the Red Lake Falls Formation consists of silty, sandy till that contains many fragments of igneous and carbonate rock. The Gardar Formation consists of silty till that contains many shale fragments. The lower part of the Red Lake Falls Formation and the Gardar Formation were deposited by an Early Wisconsinan (?) glacier that advanced from the north. The Dahlen Formation consists of silty till that contains many fragments of shale and igneous rock. It was deposited by a Late Wisconsinan glacier that advanced from the northwest. The Falconer Formation consists of silty till that contains many fragments of carbonate and igneous rock. It was deposited by a minor readvance of the latest Wisconsinan glacier.

INTRODUCTION

At least nine formations that contain glacial sediment occur in northeastern North Dakota. These formations are differentiated on the basis of outcrop charac-

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teristics, grain size, and coarse-sand lithology, particularly the shale content. In this report, each formation is described and correlated; an interpretation of the glacial history is discussed.
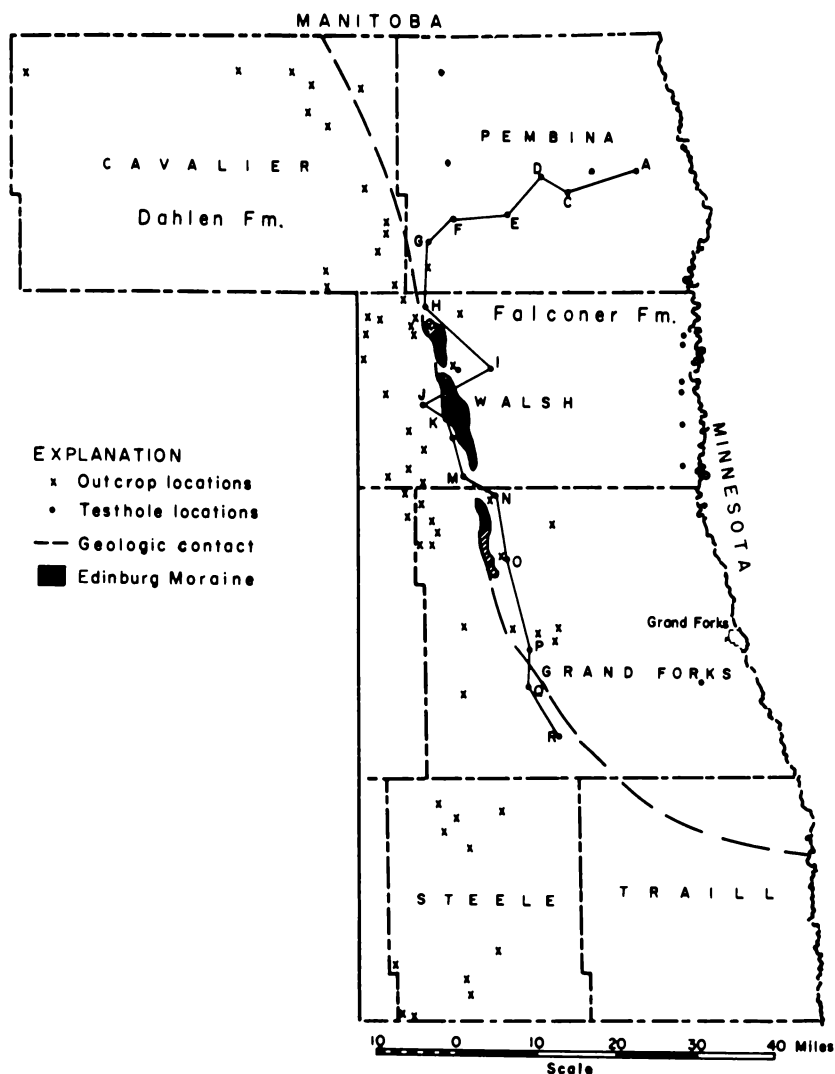
Figure 1. Map showing sample locations in northeastern North Dakota.

MATERIALS AND METHODS

About 80 outcrops in an area of 2,000 square miles were studied during the summer of 1972 (Figure 1). At each outcrop, samples were collected in a vertical sequence at intervals of three feet. Samples, collected by the North Dakota State Water Commission, were described from 28 testholes during the winter of 1972. In each testhole, the interval of sampling was five feet. Color of the samples was determined using the Munsell Soil Color Chart. The amount of sand, silt, and clay was determined for each sample using the standard sieve-pipette analysis procedures employed by the North Dakota Geological Survey. The coarse-sand fraction, herein defined as 1-2 mm, was divided into four lithologic groups: crystalline, which contained igneous and metamorphic rock types: carbonate, which contained limestone and dolostone; shale; and others, which contained a minor amount of miscellaneous rock types. About 350 grains were counted in each sample. Pebble lithology was approximated from outcrops and testhole samples.

RESULTS

Description.—Each formation is described in Table 1. The subsurface extent of these formations is shown in Figure 2.

Correlation.—Correlation of units D, C, B, and 1 with formations outside the area studied has not been attempted.

Unit A is correlated with the St. Hilaire Formation of northwestern Minnesota (Harris et al., 1974). The till in both formations contains shale pebbles and minor amounts of lignite. The shale content of the coarse sand is about 60% along the shale escarpment in northeastern North Dakota and decreases to about 30% in northwestern Minnesota.

The Gardar Formation (Salomon, in press) is correlated with the Floral Formation of Saskatchewan (Christiansen, 1968), the Minnedosa Formation of southwestern Manitoba (Klassen, 1969), the lower part of the Red Lake Falls Formation of northwestern Minnesota (Harris et al., 1974), and the Granite Falls Formation of southwestern Minnesota and northeastern South Dakota (Matsch, 1971). The till in these formations is olive gray and blocky. A boulder pavement is commonly present at the upper contact.

The Dahlen Formation (Salomon, in press) is correlated with the Battleford Formation of Saskatchewan (Christiansen, 1968), the Lennard Formation of southwestern Manitoba (Klassen, 1969), the upper part of the Red Lake Falls Formation of northwestern Minnesota (Harris et al., 1974), and the New Ulm Formation of southwestern Minnesota and northeastern South Dakota (Matsch, 1971). The till in these formations is olive gray and friable. A boulder pavement is commonly present at the lower contact.

History.—At least eight glacial advances occurred in northeastern North Dakota and northwestern Minnesota during the Pleistocene Epoch. Periods of

Table 1. Descriptions of Pleistocene formations in northeastern North Dakota.

Formation	Color	Structure	Pebble[B] lithology	Number of samples	Mean[C] grain size (%) (Sd-St-Cl)	Mean[B] coarse-sand lithology (%) (Cy-Cb-Sh)	Average thickness (feet)	Extent
Falconer[A]	Olive gray	Unbedded, friable	Cb>Cy>Sh	73	31-49-20	40-36-24	20-40	N.E. N.Dak.
Wylie[A]	Olive gray	laminated	No pebbles	0	Silty clay		5-10	N.E. N. Dak. N.W. Minn.
Dahlen[A]	Olive gray	Unbedded, friable	Sh>Cy>Cb	111	35-45-20	31-21-48	10-30	E.N. Dak.
Gardar[A]	Olive gray	Unbedded, blocky	Sh≥Cy≅Cb	98	35-43-22	13 -9-78	40-70	E. N. Dak.
Lower Red Lake Falls	Olive gray	Unbedded, blocky	Cb>Cy≫Sh	15	42-39-19	43-54- 3	30-60	N.E. N.Dak. N.W. and W. central Minn.
Unit 1	Olive gray	Unbedded, hard	Cb≫Cy>Sh	6	32-48-20	32-67- 1	10-30	N.E. N. Dak.
Unit A[A]	Olive gray	Unbedded, blocky	Sh>Cy≅Cb	39	34-37-29	21-17-62	40-80	N.E. N. Dak.
Unit B	Olive gray	Unbedded, friable	Cb>Sh>Cy	24	35-37-28	25-21-54	30-60	N.E. N. Dak.
Unit C	Olive gray	unbedded, hard	Cb>Sh>Cy	11	30-32-38	29-30-41	30-40	N.E. N.Dak.
Unit D	Olive gray	Unbedded, hard	Cb>Cy>Sh	7	28-34-38	35-44-21	10-40	N.E. N. Dak.

[A]Formation cropping out in northeastern North Dakota.
[B]Cy=Crystalline, Cb=Carbonate, Sh=Shale.
[C]Sd=Sand, St=Silt, Cl=Clay.
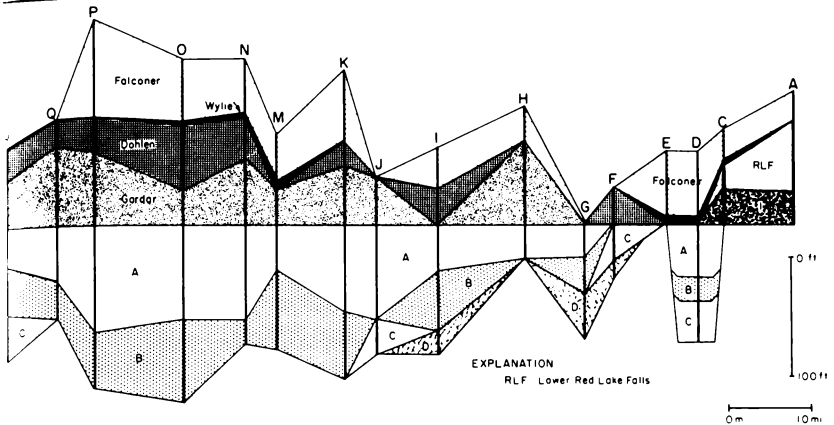
FIGURE 2. Stratigraphic cross section of Pleistocene formations in north-eastern North Dakota. Datum is the top of unit A-base of the Gardar Formation. The location of this cross section is shown in Figure 1.

erosion, varying in duration and degree, followed each glacial advance. Lake Climax formed in the Red River Valley between the last two glacial advances, and Lake Agassiz was formed after the last glacial advance (Moran and Clayton, 1972).

The history of units D, C, and B is not known because of the sparse occurrence of these formations. The till of each formation was deposited by a glacier that probably advanced from the north during pre-Wisconsinan time.

The till of unit A and of the St. Hilaire Formation was deposited by a glacier that advanced from the northwest, probably during pre-Wisconsinan or Early Wisconsinan time. Shale and lignite fragments within the till of the St. Hilaire Formation suggest a northwest source area because shale and lignite occur only to the west and northwest of northwestern Minnesota.

The history of unit 1 is not known because of the sparse occurrence of this formation. The till of unit 1 was deposited by a glacier that probably advanced from the north during pre-Wisconsinan or Early Wisconsinan time.

Extensive erosion followed the deposition of the till of unit 1. This erosion produced a boulder accumulation that was later reworked to form the boulder pavement that locally occurs at the base of the Gardar Formation, and commonly occurs at the base of the Red Lake Falls Formation. Abundant weathered bedrock, mostly shale, was exposed to the glacier that advanced following this period of erosion. As a result, the till of the Gardar Formation contains very large amounts of shale. In Cavalier, western Walsh, and western Grand Forks Counties, where the base of the Gardar Formation is exposed, the till overlies

shale, with or without thin sand and gravel at the contact. At a few sites the upper surface of the till underlying the Gardar Formation has a weathered zone characterized by iron and manganese staining. This weathered zone is thicker and more intense than the Holocene weathering zone at these sites.

The till of the Gardar Formation and the lower part of the Red Lake Falls Formation was deposited by a glacier that advanced from the north probably during Early Wisconsinan time. Radiocarbon dates from sediment of the Floral and Granite Falls Formations indicate this advance occurred before 40,000 years B. P.

An advance from the north explains the difference in composition between the till of the Gardar Formation and the till of the lower part of the Red Lake Falls Formation. The till of the Gardar Formation contains 70%-80% shale because much Cretaceous shale is present in eastern North Dakota. The till of the lower part of the Red Lake Falls Formation contains 0%-5% shale because no Cretaceous shale is present in northwestern Minnesota.

Erosion, following this glacial advance, produced a boulder accumulation that was later reworked to form the extensive boulder pavement that occurs at the top of the Floral, Minnedosa, Gardar, and Granite Falls Formatons. Bedrock was not extensively exposed during this period of erosion. In northeastern North Dakota, the Gardar Formation is commonly present beneath the Dahlen Formation. The restricted exposure of bedrock is also reflected in the till of the Dahlen Formation, the shale content of which is relatively lower than the shale content in the till of the Gardar Formation.

The till of the Dahlen Formation and of the upper part of the Red Lake Falls Formation was deposited by a glacier that advanced from the northwest during Late Wisconsinan time. Washboard moraines, flutings, and drumlins composed of sediment of the Dahlen Formation occur throughout northeastern North Dakota, and indicate that the glacier advanced from the northwest. Radiocarbon dates in Iowa (Ruhe, 1969:201,212), for materials under till that has been correlated with the Dahlen Formation (Matsch, 1972; Moran and Clayton, personal communication), indicate that this glacier advanced into northeastern North Dakota and northwestern Minnesota before 20,000 years B.P.

As the glacier stagnated, lakes formed between large areas of stagnating ice and bedrock highs. The Wylie Formation (Harris et al., 1974) was deposited in Lake Climax, an ice-walled lake in the Red River Valley.

The till of the Falconer Formation (Harris et al., 1974) was deposited during latest Wisconsinan time by a readvance of the glacier that had deposited the Dahlen Formation. The western extent of this readvance is marked by the Edinburg Moraine (Figure 1). Radiocarbon dates in North Dakota indicate that the readvance occurred before 13,500 years B.P. (Harris et al., 1972).

Following the readvance, the ice stagnated. Lakes existing between stagnating ice coalesced to form Lake Agassiz, which eventually drained to the north when the last ice dam melted.

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BLACK MAGNETIC SPHERULES FROM FLETCHER'S ICE ISLAND (T-3, ARCTIC OCEAN) AND IMPLICATIONS OF SPHERULE STUDIES

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ABSTRACT

Black magnetic spherules are present in the glacial and sea ice of Fletcher's Ice Island (T-3) in the Arctic Ocean. These opaque, spherical, magnetically susceptible, highly reflective particles were measured and counted to determine their size distribution, sedimentation rate and average diameter. The diameters of the spherules from the T-3 glacial ice are 5 - 160 μ and average 21 μ. The diameters of spherules from the sea ice are 5 - 145 μ and average 23 μ.

These spherules can be derived by ablationary processes from meteoritic and perhaps cometary sources. A meteoritic origin has been generally accepted but it cannot account for the total number of spherules present in most collections. The hypothesis that some black magnetic spherules are cometary in origin may have important implications for the explanation of geomagnetic field reversals

If cometary bodies have caused geomagnetic field reversals, cometary debris (including black magnetic spherules) should be more abundant in those deep-sea sediments deposited when polarity reversals occur. To test this hypothesis, it is proposed that a study be made of black magnetic spherule abundance in deep-sea sediments for which paleomagnetic information is already known. Such in formation may provide additional independent support for the Heezen Glass hypothesis that comet-earth interactions producing tektites have caused some reversals of the earth's magnetic field.

INTRODUCTION

Fletcher's Ice Island (Figure 1) is a 30-km² block of glacial icezt ihmvvxos with the sea ice in the Beaufort gyre of the Canada Basin in the Arctic Ocean. In the summers of 1968 and 1970, ice cores were collected to determine if spherules were present in sea ice and if the two types of ice, having different modes of formation, are significantly different in the content of black magnetic spherules.

Black magnetic spherules are opaque spherical grains, some of which are unquestionably of extraterrestrial origin (Schmidt and Keil, 1966, Finkelman,

1970). Interest in these particles has been due mainly to this accepted origin and because no sediment has been found devoid of such spherules (Crozier, 1960).

The sizes of spherules vary widely; the range is normally 5-250 μ although spherules as large as 650 μ have been reported.

The hypotheses for the extraterrestrial source of these spherules have been numerous. Some of the more important sources may be meteoroid ablation products (Carr, 1970), interplanetary dust (Cosby and Lyle, 1965), or cometary body ablation (Wright and Hodge, 1968; Schmidt, 1965).

MATERIALS AND METHODS

From selected sites on Fletcher's Ice Island (Figure 1), 28 ice cores were placed in containers and allowed to melt. After a minimum of 48 h settling time,
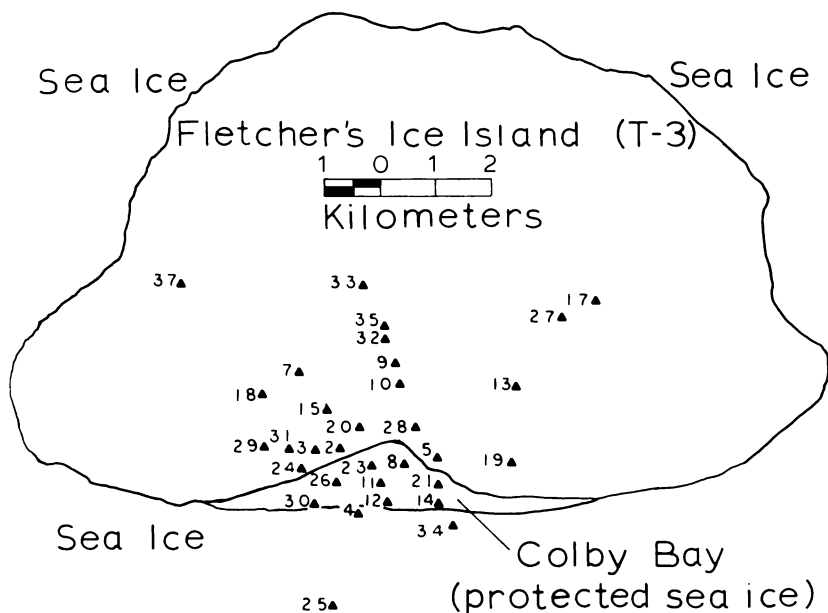
Figure 1. Sketch map of Fletcher's Ice Island (T-3), made in 1965, showing location of core samples listed in Table 1.

water above 10 cm was siphoned off and discarded. The remaining water and particulate matter was returned to the laboratory.

Black magnetic spherules were concentrated using a Frantz isodynamic separator and a wet separation method; such spherules have a high magnetic susceptibility. The magnetic portion was filtered within a clean box to prevent sample contamination; the filtered sample was mounted on a microscope slide and scanned systematically. Using standard techniques the spherules were measured; the resulting data were analyzed statistically.

Studies of spherules require microscopic methods and careful examination, especially of samples that frequently have large amounts of terrestrial material with high magnetic susceptibility.

The elemental composition of the spherules was determined by using the electron microprobe. Since no standards were used, the microprobe data presented in this paper is qualitative. Trace element data were not included unless peak values, minus the background values, were greater than three times the square root of the background value. Background counts were made at 0.5 degrees from the strongest lines.

RESULTS AND DISCUSSION

Table 1 shows the size distribution data for the glacial and sea ice of Fletcher's Ice Island (T-3). The average diameter is approximately 20 μ and the total mass of such material is generally on the order of micrograms.

Elemental analysis of the surfaces of six magnetic spherules from glacial ice sample 15 (Table 1) is shown in Figure 2. Clearly, the most important element is iron. All others are present as trace elements. Ironically, these elemental trace quantities are essential in interpreting an origin for such particles. The primary chemical criterion that has been used for confirming the cosmic origin of such spherules as these is the presence of nickel (Schmidt and Keil, 1966), although the absence of nickel does not preclude an extraterrestrial origin (Finkelman, 1972). One spherule (6 in Figure 2) contains titanium, an element considered to be indicative of a terrestrial (volcanic) origin (Wright and others, 1966). Volcanic spherules, besides containing titanium, are usually reddish when viewed through a microscope.

How, then, does one explain those spherules that have no identifiable nickel or cobalt? Unless these particles are from stony meteorites (Finkelman, 1970), there is the necessity to search for some other source, perhaps a cometary source.

Size measurements of these particles offer information that may be overlooked. For example, the sedimentation rates for spherules from the T-3 glacial ice, as calculated from size measurements, are 1.1×10^4 - 1.1×10^5 metric tons per year (Scattolini, 1973), extrapolated for the entire earth's surface as is the standard procedure. Similarly calculated sedimentation rates for the sea ice near T-3 are 5.0

Table 1. Statistical parameters for spherules from T-3 glacial and sea ice cores.

Sample number	Length of core (cm)	Total mass (g/m of core ×10⁻⁶)	Average diameter (μ)
		T-3 glacial ice	
2	1.0	4.5	16.2
3	1.0	5.9	20.7
5	3.0	4.3	17.6
7	3.0	1.9	22.3
9	3.0	3.1	22.6
15	3.0	1.8	24.7
17	3.0	.8	19.1
18	3.0	2.1	21.4
19	3.0	3.3	23.3
20	3.0	1.1	20.8
24	2.0	7.8	30.9
27	3.0	.8	22.8
29	1.0	1.3	16.4
32	6.0	2.9	19.8
33	1.0	2.2	20.6
35	8.0	.8	16.8
37	8.0	2.0	23.8
Average		2.3	20.6
		T-3 sea ice	
4	3.0	.4	24.4
8	3.0	1.9	19.8
1	3.0	.2	21.4
12	3.0	1.2	26.4
14	3.0	.1	18.2
21	3.0	1.0	24.4
23	3.0	1.4	30.9
25	3.0ᴬ	1.9	25.9
26	2.0	2.0	23.3
30	3.0	4.5	22.6
34	3.0	1.8	21.6
Average		1.5	22.6

ᴬPack ice sample. Other sea ice cores are from Colby Bay (Figure 1).
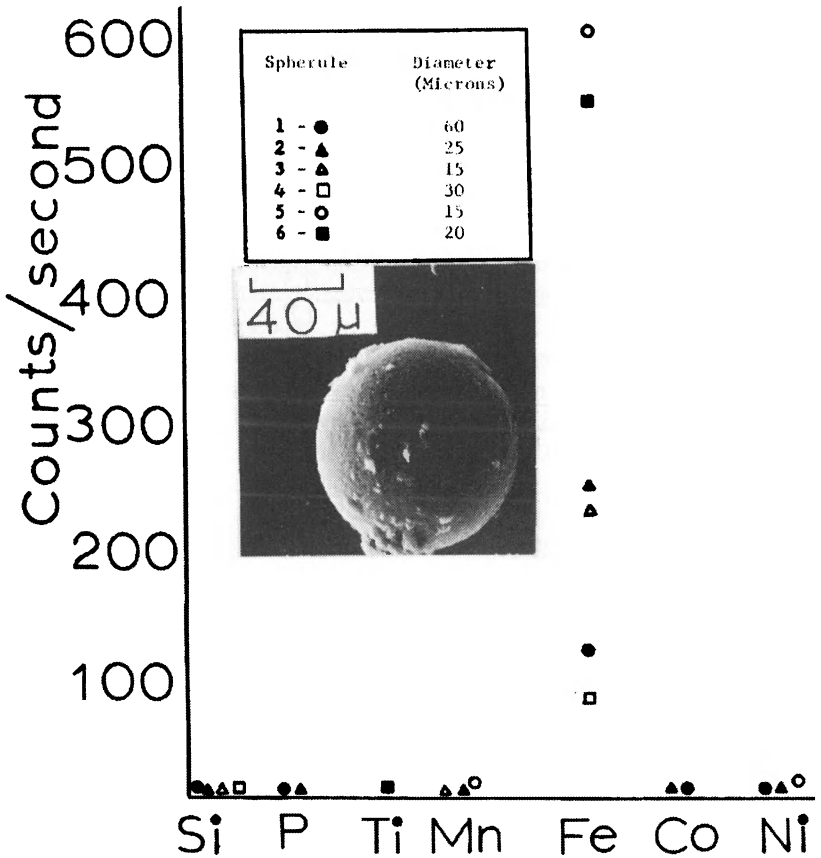
Figure 2. Preliminary elemental x-ray microanalysis of the surfaces of 5 black magnetic spherules and one (number 6) reddish spherule. Spherules analyzed are all from sample 15. A typical black magnetic spherule (scanning electron photomicrograph) is shown in the inset.

x 10^3 - 1.6 x 10^5 metric tons per year (Scattolini, 1973). A comparison of the sedimentation rates of both types of ice shows good agreement (Figure 3). This range is similar to what other investigators have observed (Table 2).

Recently, it has been suggested that such values are too high. Space craft data indicate that fewer micrometeorites actually reach the earth's surface than the sediments indicate (Finkelman, 1972; Parkin and Tilles, 1968). This controversy is far from settled and is perplexing. Numerical data from the sediments yield surprisingly consistent results which are frequently within an order of
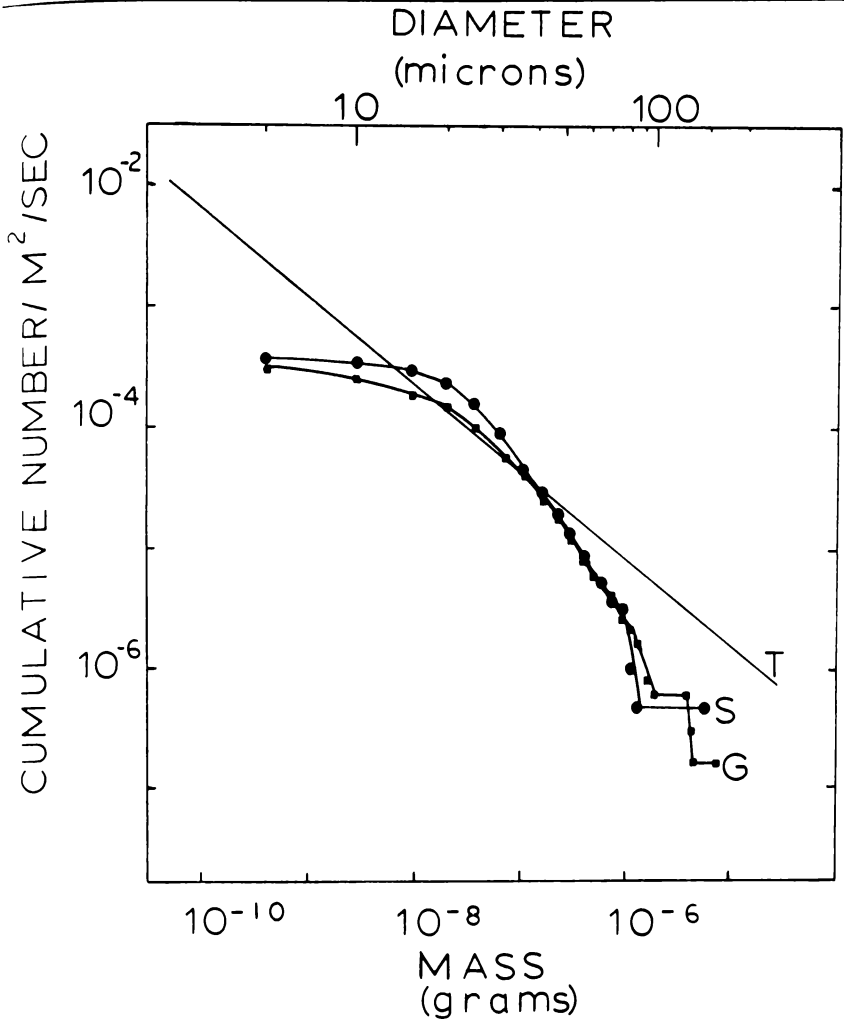
Figure 3. Sedimentation rates and mass values for the average glacial(G) and sea (S) ice samples from T-3 compared with the theoretical curve (T) as proposed by Laevastu and Mellis (1961).

magnitude (Table 2), yet electron microprobe and x-ray diffraction data suggest that relatively few spherules can be from iron and stony meteorites. It may, however, be possible to resolve this dilemma; the solution may lie in the

suggestions by Wright and Hodge (1968) that some of these particles are from cometary bodies.

The hypothesis of Wright and Hodge (1968) proposes that black magnetic spherules that cannot be shown clearly to be meteoritic or volcanic are cometary in origin. These spherules may be produced from nearly complete ablation of a large body in the atmosphere. Certainly, there is some evidence for this. Carr (1970) has shown that meteor events can be quite different; the Revelstoke fall in 1965, a carbonaceous chondrite (Folinsbee and others, 1967), produced large numbers of spherules, yet the Allende fall in 1969 produced very few spherules. If cometary bodies are involved, perhaps complete ablation is not unreasonable.

Table 2. Sedimentation rates of black magnetic spherucles as calculated by various workers.

Type of spherule collection	Sedimentation rate (metric tons/year)	Source of data
Atmosphere	8 to 1.29×10^5	Buddhue, 1950
Oceanic sediments	1.25	Laevastu and Mellis, 1955
Oceanic sediments	$(2.4 \text{ to } 5.0) \times 10^3$	Pettersson and Fredriksson, 1958
Atmosphere	9×10^4	Crozier, 1961
Antarctic ice	1.2×10^5	Schmidt, 1963
Atmosphere	1.6×10^5	Crozier, 1966
Antarctic ice	1.8×10^5	Theil and Schmidt, 1961
Upper atmosphere	2×10^5	Wright and Hodge, 1962
Greenland ice	2×10^5	Wright, Hodge and Langway, 1963
Arctic atmosphere	5×10^5	Hodge and Wildt, 1958
Greenland ice	$(2.1 \text{ to } 6.6) \times 10^5$	Langway, 1967
Atmosphere	Approximately 2×10^3	Vittori, 1970
Atmosphere and Paleozoic salts	$(1 \text{ to } 2) \times 10^5$	Ivanov and Florenskiy, 1970
Atmosphere	$(2 \text{ to } 6) \times 10^6$	Baranov and Vilenski, 1968
T-3 Glacial ice	$(.1 \text{ to } 1.1) \times 10^5$	Scattolini, 1973
T-3 Sea ice	$(.05 \text{ to } 1.6) \times 10^5$	Scattolini, 1973

Black magnetic spherules (along with glassy spherules) were discovered in the soil at the site of the Tunguska event in 1908 (Krinov, 1966). Black magnetic spherules have also been found in association with tektites and microtektites (Glass and Heezen, 1967). Tektites have been found associated with two

paleomagnetic reversal boundaries, the Brunhes-Matuyama (0.7 m.y.) and the Jarmillo magnetic event (0.9 m.y.) (Glass, 1972; Glass, 1967).

Over the last decade, it has become well accepted that reversals of the earth's main dipole field have occurred in the geologic past. Even though the earth's core is believed to produce the main dipole field and polarity reversals are suspected to be governed by dynamo oscillations, there is no general theory (Rititake, 1966). An encounter of a cosmic body with the earth could have mechanical or electro-magnetic consequences on the magneto-hydrodynamic motions of the earth's core (Glass and Heezen, 1967). Herein lies the hint of a possible test for the Wright-Hodge hypothesis. If cometary explosions have been responsible for magnetic field reversals, large numbers of cometary particles must have been introduced onto the earth. This suggests that the cometary (glassy) black magnetic spherule sedimentation rate should undergo signficant variations at or near paleomagnetc field reversal boundaies, especially in deep-sea sediments. If comets are an important source of black magnetic spherules, then the investigation and analysis of samples across paleomagnetic boundaries may show evidence of this relationship. Not only would positive results for this test lend credence to the cometary origin for some black magnetic spherules, but it may also lend support to the Heezen-Glass hypothesis that cometary explosions were involved in past reversals of the earth's magnetic field.

Among the many problems that would have to be considered in a study undertaken to determine if this relationship holds, are the effects of bottom currents and burrowing benthic fauna that may rework the sediments (Glass and Heezen, 1967), and the mechanical effects associated with manganese nodule formation (Finkelman, 1972).

SUMMARY

Black magnetic spherules are present in the glacial and sea ice of Fletcher's Ice Island (T-3). Very preliminary analysis indicates that it is difficult to explain all spherules by a meteoritic origin. If cometary bodies produce black magnetic spherules and if paleomagnetic field reversals were caused by comet-earth interactions, significant changes in the sedimentation rates of such spherules should be present across paleomagnetic boundaries. It is proposed that such a study be undertaken on deep-sea sediments.

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PROGRESS REPORT ON BIRD LOSSES AT THE OMEGA TOWER, SOUTHEASTERN NORTH DAKOTA

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INTRODUCTION

Bird migration and losses at the U.S. Coast Guard's Omega Navigation Station near LaMoure, North Dakota, have been studied since the completion of the 336-m transmitting tower in the fall of 1971. This paper is a progress report summarizing the findings through the fall of 1972. The study is being sponsored by the Northern Prairie Wildlife Research Center in cooperation with the Department of Zoology at North Dakota State University.

The Omega Navigation Station is about 1.6 km west of the James River and 3.2 km west of LaMoure. The tower is in an area that is mostly marsh but includes some grassy upland. A gravel area 46 m in radius surrounds the base of the tower. Three sets of five guy wires spaced 120°.° apart support the tower. The guy wires are anchored along three service roads which radiate northeast, south, and west from the gravel area 312 m into the marsh. In each set of five guy wires, two are anchored 122 m from the base of the tower, two others at 213 m, and the last one is anchored 297 m away. These are attached to the tower at heights of 53, 109, 167, 228, and 293 m. In addition, 16 evenly spaced transmitting cables extend from the top of the tower to the circular perimeter road 732 m away.

The tower is equipped with five, red, non-flashing obstruction lights and four, red, flashing beacons. A service building adjacent to the tower is equipped with four white lights that illuminate the area around the base of the tower.

METHODS

Searches for dead and injured birds were made several times a week during the fall of 1971. Daily searches at dawn were conducted during the spring and fall of 1972. Since the marshy habitat made it impossible to search the entire area (168 ha) under the guy wires, a sampling plan was devised. The plan was based in part upon findings of previous tower mortality studies that have shown that most dead birds are found within 62 m of the central structure. The central gravel area within 46 m of the tower was examined completely for birds. The three service roads were also included in this stratum since it was felt that these roads, lying under the three sets of supporting guy wires, might receive a disproportionately greater number of dead birds than areas between the sets of guy wires.

Other strata were formed by concentric circles with radii of 91.5 m, 183 m and 732 m. Two compass lines, one north-south and the other east-west, divided each of these strata into four sub-strata. This division resulted in 12 substrata beyond the central area. Two square sample plots, 12.4 m on a side, were located randomly in each substratum. These plots were large enough to be likely to contain birds if a large kill occurred, yet sufficiently small to enable quick inspection. The 19 sample plots in moist areas consisted of nylon netting suspended by a steel frame. The remaining five sample plots consisted of smooth, gravel surfaces on upland sites.

Nighttime observations of migratory birds were made during the spring and fall of 1972 by means of a portable ceilometer technique described by Gauthreaux (1969). Most observations were made with a 20x60 spotting scope but were supplemented at times with 7x35 binoculars to follow the movements of individual birds over a greater area. Although some of the spring watches took place at the tower, most were made 305 m away near the end of the northeast service road. During the watches in the fall, each hour was divided into three 20-minute periods. Observations were made at the tower during one period and away from it during another period; the third period was used for rest and changing locations. Weather conditions were noted during all observation periods.

Analysis of variance was used on the ceilometer data from the fall of 1972, the 5% level of probability being accepted as significant. No statistical treatment was made on the spring data since the number of watches made at the tower were too few.

Removal of tower-killed birds by predators and scavengers was assessed by placing dead birds collected from other sources on the inner gravel area, the roads and some of the sampling plots. These birds were tagged, and those that were not taken overnight by predators and scavengers were picked up the next morning.

Tests of a commercial sound device, an Av-Alarm unit model TAV-60 (Av-Alarm Corp., Mountain View, Cal.), as a deterrent to migrants were conducted during the spring and fall of 1972. Daytime tests consisted of determining the

responses of birds that were perched, feeding, or in flight. The only nighttime trial was conducted during the night of 4-5 October when many birds were fluttering around the lights of ths tower and the building adjacent to it. On this occasion, the sound unit was located near the base of the tower.

RESULTS AND DISCUSSION

Size and composition of losses.—During the fall of 1971 and the spring and fall of 1972, 633 birds and 5 red bats (*Lasiurus borealis*) were found dead or injured at the tower site. On the basis of the 409 birds found on sampling areas, the total estimated kill for this period was 3062. Table 1 shows that the size of the kill remained fairly constant throughout the three migration seasons. This is somewhat surprising since during the fall, many more migrants are present, and usually most of them are immature. Previous investigators have reported greater losses at towers in the fall than in the spring (e.g. Caldwell and Cuthbert, 1963; Stoddard and Norris, 1967). Also, spectacular kills, sometimes involving thousands of birds, have been reported during September and October (e.g. Kemper, 1964; Laskey, 1969; Bagg, 1971), but not during spring months.

Table 1. Bird losses at the Omega tower during three migration seasons.

	Fall 1971	Spring 1972	Fall 1972	Total
Total birds found	152	255	226	633
Number found on sampling areas	113	141	155	409
Total estimated loss	941	1084	1037	3062

Table 2. Percent composition by family of the bird losses at the Omega tower for three migration seasons.

Family	Fall 1971	Spring 1972	Fall 1972
Anatidae	2	6	3
Rallidae	3	11	12
Troglodytidae	3	7	1
Turdidae	0	4	5
Vireonidae	15	1	3
Parulidae	40	11	40
Icteridae	3	7	3
Fringillidae	27	49	26
Others	7	4	7

In contrast to the size of the kill, the composition of the kill exhibited marked seasonal variation (Table 2). Close agreement exists between the fall figures in percentages of parulids and fringillids, but sharp dissimilarities exist between them and the spring figures for these families.

A look at the 10 most frequently killed species (Table 3) illustrates the variation at the species level. Only the Sora, American Coot, and Tree Sparrow did not show considerable seasonal variation. Of the remaining seven species, the Yellow Warbler and Mourning Warbler had greater fall totals, whereas the others exhibited the opposite trend. A complete list of species killed through the fall of 1972 is given in Table 4.

During their study, Stoddard and Norris (1967) found that unlike most species, some birds suffered greater losses in the spring than in the fall. They noted (p. 71) that 'a relatively large spring kill seems more likely to pertain to species that breed abundantly with us...than to ones that travel farther north to their breeding ground.' Our findings to date appear to confirm this (Table 3).

Table 3. Seasonal totals of the 10 most frequently killed species at the Omega Tower.

Species	Fall 1971	Spring 1972	Fall 1972	Total
Savannah Sparrow	8	22	10	40
Yellow Warbler	15	1	19	35
Sora	3	14	12	29
Clay-colored Sparrow	2	20	7	29
Common Yellowthroat	3	16	8	27
Grasshopper Sparrow	0	21	3	24
American Coot	1	8	13	22
Mourning Warbler	15	0	6	21
LeConte's Sparrow	2	16	3	21
Tree Sparrow	5	8	7	20

Four of the five species having spring totals at least twice as great as their fall totals (Savannah Sparrow, Grasshopper Sparrow, Clay-colored Sparrow and Common Yellowthroat) are common breeding birds in this state (Stewart and Kantrud, 1972). The fifth species, LeConte's Sparrow, is considered a locally common breeder (R.E. Stewart, personal communication). In fact, 61% of the birds found dead or injured at the tower site during the spring of 1972 were of species regularly observed inhabiting the marsh and grassy uplands around the tower (Table 4). These percentages for the falls of 1971 and 1972 were 18% and 33%. This may indicate that in the spring, migrants seek their characteristic feeding and resting habitat more so than in the fall.

Other factors that may influence the size and composition of tower kills include seasonal routes of migration, weather conditions during the peak periods of movements, relative abundance of species migrating through an area, altitudes at which various species migrate, whether migration is nocturnal or diurnal, and how various species are influenced by tall, lighted structures. Interestingly, ducks

Table 4.   Complete species list of the bird loss at the Omega tower through the fall of 1972.

Species	Fall 1971	Spring 1972	Fall 1972	Total
Eared Grebe (*Podiceps nigricollis*)			1	1
Western Grebe (*Aechmophorus occidentalis*)		2		2
Pied-billed Grebe (*Podilymbus podiceps*)ᴬ		1		
American Bittern (*Botaurus lentiginosus*)ᴬ			2	2
Mallard (*Anas platyrhynchos*)ᴬ	1		1	2
Pintail (*Anas acuta*)ᴬ		1	1	2
Blue-winged Teal (*Anas discors*)ᴬ	1		1	2
Northern Shoveler (*Anas clypeata*)			1	1
Lesser Scaup (*Aythya affinis*)		5	2	7
Ruddy Duck (*Oxyura jamaicensis*)			1	1
Marsh Hawk (*Circus syaneus*)ᴬ			1	1
Virginia Rail (*Rallus limicola*)ᴬ		7	2	9
Sora (*Porzana carolina*)ᴬ	3	14	12	29
Yellow Rail (*Coturniceps noveboracensis*)			1	1
American Coot (*Fulica americana*)ᴬ	1	8	13	22
Common Snipe (*Capella gallinago*)ᴬ			1	1
Mourning Dove (*Zenaida macroura*)			1	1
Yellow-billed Cuckoo (*Coccyzus americanus*)	1	1		1
Black-billed Cuckoo (*Coccyzus erythropthalmus*)		1		1
Yellow-bellied Flycatcher (*Empidonax flaviventris*)			1	1
Traill's Flycatcher (*Empidonax traillii-alnorum* complex)	1		1	2
Least Flycatcher (*Empidonax minimus*)		1		1
Tree Swallow (*Iridoprocne bicolor*)		1		1
Barn Swallow (*Hirundo rustica*)	1			1
Brown Creeper (*Certhia familiaris*)		1		1
House Wren (*Troglodytes aedon*)	2	6	2	10
Long-billed Marsh Wren (*Telmatodytes palustris*)ᴬ	2	12		13
Short-billed Marsh Wren (*Cistothorus platensis*)ᴬ	1	1		2
Gray Catbird (*Dumetella carolinensis*)		1		1
Brown Thrasher (*Toxostoma rufum*)		2		2
Hermit Thrush (*Catharus guttatus*)			1	1
Swainson's Thrush (*Catharus ustulatus*)		4	4	8
Gray-cheeked Thrush (*Catharus minimus*)		3	5	8
Veery (*Catharus fuscescens*)		1	1	2
Golden-crowned Kinglet (*Regulus satrapa*)	1		3	4

Species				
Ruby-crowned Kinglet (*Regulus calendula*)		2	3	5
Bell's Vireo (*Vireo bellii*)			1	1
Solitary Vireo (*Vireo solitarius*)	2			2
Red-eyed Vireo (*Vireo olivaceus*)	14	1	4	19
Warbling Vireo (*Vireo gilvus*)	6	1	2	9
Black-and-white Warbler (*Miniotilta varia*)	1	1	3	5
Golden-winged Warbler (*Vermivora chrysoptera*)			1	1
Tennessee Warbler (*Vermivora peregrina*)	**2**	2	1	4
Orange-crowned Warbler (*Vermivora celata*)	7		9	16
Yellow Warbler (*Dendroica petechia*)	15	1	19	3
Magnolia Warbler (*Dendroica magnolia*)			1	1
Myrtle Warbler (*Dendroica c. coronata*)	2		13	15
Black-throated Green Warbler (*Dendroica virens*)			1	1
Blackburnian Warbler (*Dendroica fusca*)				1
Bay-breasted Warbler (*Dendroica castanea*)			2	2
Blackpoll Warbler (*Dendroica striata*)		2	2	4
Palm Warbler (*Dendroica palmarum*)		3	9	12
Ovenbird (*Seiurus aurocapillus*)	5	1	6	12
Northern Waterthrush (*Seiurus noveboracensis*)	1	1	2	4
Mourning Warbler (*Oporornis philadelphia*)	15		6	21
Common Yellowthroat (*Geothlypis trichas*)[a]	3	16	8	27
Wilson's Warbler (*Wilsonia pusilla*)	10		4	14
Canada Warbler (*Wilsonia canadensis*)	3		1	4
American Redstart (*Setophaga ruticilla*)		2	2	4
Bobolink (*Dolichonyx oryzivorus*)[a]		3		3
Western Meadowlark (*Sturnella neglecta*)[a]		2		2
Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)[a]		7	1	8
Red-winged Blackbird (*Agelaius phoeniceus*)[a]	1	1	1	3
Orchard Oriole (*Icterus spurius*)		1	1	2
Baltimore Oriole (*Icterus g. galbula*)	2		1	3
Brown-headed Cowbird (*Molothrus ater*)[a]	1	4	2	7
Common Redpoll (*Acanthis flammea*)	1	2		3
Rufous-sided Towhee (*Pipilo erythropthalmus*)		1		1
Lark Bunting (*Calamospiza melanocorys*)		2		2
Savannah Sparrow (*Passerculus sandwichensis*)[a]	8	22	10	40
Grasshopper Sparrow (*Ammodramus savannarum*)		21	3	24
Baird's Sparrow (*Ammodramus bairdii*)		2		2
LeConte's Sparrow (*Ammospiza leconteii*)[a]	2	16	3	21
Sharp-tailed Sparrow (*Ammospiza caudacuta*)[a]		1	1	2
Vesper Sparrow (*Pooecetes gramineus*)	1	2	2	6

Slate-colored Junco (*Junco h. hyemalis*)		4	4	8
Tree Sparrow (*Spizella arborea*)	5	8	7	20
Chipping Sparrow (*Spizella passerina*)		4	1	5
Clay-colored Sparrow (*Spizella pallida*)[A]	2	20	7	29
Harris' Sparrow (*Zonotrichia querula*)	4	2	3	9
White-crowned Sparrow (*Zonotrichia leucophrys*)		1		1
White-throated Sparrow (*Zonotrichia albicollis*)	1	3	3	7
Fox Sparrow (*Passerella iliaca*)			1	
Lincoln's Sparrow (*Melospiza lincolnii*)	6	4	4	14
Swamp Sparrow (*Melospiza geogiana*)[A]	1	1	3	5
Song Sparrow (*Melospiza melodia*)[A]	1	4	2	7
Lapland Longspur (*Calcarius lapponicus*)	7	5	5	17
Smith's Longspur (*Calcarius pictus*)	1		1	2
Unidentified	8	6	2	16
Total (87 species)	152	255	226	633

[A]Inhabitant of marsh or grassy uplands surrounding the tower.

comprised only 2-6% of the total kill (Table 2) despite their abundance. Perhaps they are influenced less by the tower than are other nocturnal migrants.

Ceilometer observations.—The spring ceilometer data show that more migrants were seen at the tower than at 305 m to the northeast under all types of cloud cover (Table 5). The difference was greatest on overcast nights and least on clear nights. Observations in the fall of 1972 were more extensive and revealed that on overcast nights throughout the season, significantly more birds per hour were seen at the tower than 305 m northeast of it (Table 6). Conversely, on clear nights, significantly more birds per hour were observed away from the tower than at it. The greater number of birds per hour away from the tower on partly cloudy nights was not significant.

Most of the birds observed at the tower on overcast nights milled about in a seemingly disoriented manner. The largest kills generally occurred during such nights. An exception to this was the night of 14-15 May 1972 when the largest loss of the spring took place under a clear sky. Although many birds were seen at the tower that night (49 birds per hour average), they did not seem disoriented. Favorable winds (southerly in the spring and northerly in the fall) were also characteristic of nights during which sizable kills occurred.

Scavenger and predator activity.—Removal by scavengers and predators of dead birds placed on sampling areas resulted in only 2 of 82 birds (2.5%) and 4 of 82 birds (4.9%) taken during the first night in the spring and fall of 1972. Thus, it was believed that daily searches for tower-killed birds kept losses to scavengers and predators at an acceptably low level, and no correction was applied to the estimated kill totals.

Table 5. Ceilometer observations at the Omega tower and 305 m to the northeast during spring 1972.

Sky condition	Hours observed		Birds seen		Birds/ hour	
	Tower	305m	Tower	305m	Tower	305m
Overcast	2.1	12.2	69	72	32.2	5.9
Partly cloudy	0.3	7.5	4	37	12.6	5.0
Clear	2.4	15.1	29	148	12.0	9.8
All nights	4.8	34.8	102	257	21.0	7.4

Table 6. Ceilometer observations at the Omega tower and 305 m to the northeast during fall 1972.

Sky condition	Hours observed		Birds seen		Birds/ hour	
	Tower	305m	Tower	305m	Tower	305m
Overcast	9.2	6.9	602	13	65.4	1.9
Partly cloudy	15.5	14.8	17	25	1.1	1.7
Clear	24.6	24.6	31	68	1.3	2.8
All nights	49.3	46.3	650	106	13.2	2.3

Daytime observations.—Throughout the study, observations showed that diurnal migrants were able to avoid colliding with the tower and guy wires. Large flocks of blackbirds passed through the area without difficulty as did groups of sparrows, longspurs, shorebirds and ducks. Some flocks of geese passed directly over the tower, but most detoured around it, staying approximately 400-600 m away. These birds were flying at altitudes below 366 m and saw the tower as an obstruction, whereas those which passed over it probably were flying above 366 m and thus did not have to change direction or altitude to avoid the tower.

Av-Alarm trials.—Tests of the Av-Alarm unit during daylight hours gave mixed results on birds that were on the ground or perched on trees or on utility wires. When the sound was turned on, individuals within 60 m of the speaker generally were startled and flew away. Birds, mainly blackbirds and ducks, more than 60 m away either continued feeding or became alert and moved slowly away but usually did not fly away. Birds in local or migrational flight generally exhibited little response to the sound. Usually individuals heading toward the sound would continue their flight over the speaker, at times passing as close as 15 m.

During the night of 4-5 October 1972, when hundreds of birds were milling

around the tower, the unit was turned on for short periods of time, and the responses of the birds were observed. Although no quantitative data were obtained, the general impression was that birds within a radius of about 30 m above and to the sides of the speaker responded by leaving the area of intense sound. Effects on birds at greater distances were not as conclusive. Each time the unit was turned on, the birds that were fluttering around the bright white lights of the service building immediately left the lighted area. When the sound unit was turned off, the birds began almost at once to return to the area. After a few minutes, as many birds were back in the lighted area as there had been before the sound was applied.

General.—The attraction of night migrants to the red lights of tall towers and the resulting mass mortality have been reported since the late 1950s (Cochran and Graber, 1958; Kemper, 1958), and the ceilometer results reported in this paper confirm these earlier observations. However, no solution to the problem has yet been forthcoming. Bright white strobe lights are currently being used on some towers as aircraft warning lights instead of the conventional red ones. Their effects, if any, on birds are unknown.

As more and more tall towers are erected, ths hazards posed to nocturnal migrants increases. Research into the effects on birds of various wave-lengths in the visible spectrum, intensities and flash rates of lights is presently lacking. It is hoped that through the continuation of the study at the Omega tower and through new studies elsewhere, the adverse effects of tall, lighted structures on nocturnal migrants can be largely eliminated.

SUMMARY

Investigations through the fall of 1972 revealed that:

1. The total estimated kill for the three migration seasons from fall 1971 through fall 1972 remained fairly constant at about 1000 birds per season.

2. The species composition of the kills showed sharp seasonal variations; parulids dominated the fall kills and fringillids the spring kill. The species most commonly involved in the mortality were the Savannah Sparrow, Yellow Warbler, Sora, Clay-colored Sparrow, and Common Yellowthroat.

3. Largest losses generally occurred on overcast nights with winds favorable for migration. However, the largest loss during the spring of 1972 took place on a clear night.

4. Ceilometer observations during the fall of 1972 showed that birds were influenced by the tower on overcast nights, resulting in birds-per-hour counts averaging more than 30 times greater at the tower than 305 m to the northeast. Most birds seen at the tower during these nights fluttered in a seemingly disoriented manner.

5. On clear and partly cloudy nights, the attractive effect of the tower was less. On clear nights during the fall of 1972, significantly more birds were seen

305 m northeast of the tower than at it. This suggests that migrants avoided the tower under those conditions.

6. Daily pick-up of tower-killed birds kept removal by predators and scavengers to a level that did not appear to greatly affect the estimate of total birds killed.

7. Observations of diurnal migrants indicated that they were able to avoid colliding with the tower and guy wires.

8. Tests of the Av-Alarm sound unit were successful in dispersing low-level birds that were milling around the white lights on a building adjacent to the tower. Further tests are needed before the effectiveness of the unit as a deterrent to nocturnal migrants can be fully determined.

ACKNOWLEDGMENTS

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GROWTH, PRODUCTION AND BROWSE UTILIZATION CHARACTERISTICS OF SERVICEBERRY (*AMELANCHIER ALNIFOLIA* NUTT.) IN THE BADLANDS OF SOUTHWESTERN NORTH DAKOTA

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ABSTRACT

Serviceberry (*Amelanchier alnifolia* Nutt.) was studied in the Little Missouri River Badlands of southwestern North Dakota from 1971 to the present. Data for annual twig growth, annual production (leaves and twigs), and browse utilization are from the 1971 growing season. Average annual twig growth was 2.84 cm. Average annual growth was greatest in seedlings (6.60 cm) and least in young shrubs (2.38 cm). Total annual yield was 93.84 kg/ha (172.31 lbs/acre) for all sites, age and form classes combined. Leaves constituted 82 % of the total annual production. Mature shrubs contributed 78 % of the total production. Shrubs in form classes 4 (mostly available, little or no hedging) and 7 (mostly unavailable) contributed 74% of the total production. Less than 20% of the total production was available as deer browse. Average utilization for all sites was 11 %. Clones of this species appear to be deteriorating due to environmental influences such as snow breakage, rust fungus, and damage from domestic livestock and small game.

INTRODUCTION

The big game range of western North Dakota has been said to be one of the most thoroughly studied ranges in the western United States (McKenzie and Samuelson, 1967:21). This may be true in terms of animal census and evaluation of browse condition and trend transects, but far more information concerning the growth, productivity, and utilization characteristics of the important browse species is essential for proper game management. Surveys of the primary mule deer range (Badlands Management Unit) by the State Game and Fish Department showed that the condition of the browse is trending downwards and the desirable browse species are being replaced by less desirable species (McKenzie, 1966:22). If such a downward trend exists, its continuance will mean a lowered carrying capacity for the badlands mule deer ranges (McKenzie and Samuelson; 1967:21). As serviceberry is recognized as a highly desirable browse species, this study was instituted to examine the growth, productivity, and utilization characteristics so proper management may occur. From the analysis of data for the 1971 growing season, it is anticipated that analytical procedures will make possible the develop-

ment of predictive statements on the growth and forage production of serviceberry by age and form class, and by site.

Physiographically, the North Dakota Badlands are a part of the Missouri Plateau Section of the Northern Great Plains Province. Fenneman (1931) described the area as interstream uplands imperfectly peneplaned in a series of partial cycles and locally dissected into badlands. Relief is most rugged in the northern extent and nearest to the Little Missouri River; away from the river or south the relief becomes less (Edwards and Ableiter, 1934:4).

Considerable variation is a striking feature of the badlands vegetation associations. The variations in topography and exposure result in an equally varied number of microclimatic situations, which are reflected in the development of numerous major soil and site characteristics. Shrub and tree communities occur mostly on slope habitats where more favorable moisture and temperature conditions exist, usually at the lower parts of slopes or on north-facing slopes.

Mixed grass prairie usually borders these woody communities on the uplands and on the edge of the bottomlands. The major woody community types in western North Dakota were categorized by Nelson (1961) as: green ash, juniper slope, cottonwood bottomland, sagebrush flat, and brushy coulee. His more restricted types were: ponderosa pine, big sagebrush, aspen, and oak. Although these woody types occupy a small percentage of the total vegetation, they play a vastly important role as a source of food and cover for big and small game as well as non-game animals. Deer are perhaps the dominant user of these woody vegetation types; trees and shrubs provide excellent cover and a main winter food source. Serviceberry is interspersed throughout these types in varying amounts depending upon the desirability of the site for the establishment and growth of this species.

The serviceberry geus (*Amelanchier*) is almost world-wide in distribution but probably best represented in the western United States where perhaps 24 species occur (Dayton, 1931, p. 60). *Amelanchier* is a member of the rose family, which contains many important browse species. Serviceberry or Juneberry is a slender shrub, 0.9-3.0 m (3-10 feet) high. Serviceberry or juneberry ranges from western Ontario and Michigan to the Yukon and British Columbia and south to California, New Mexico, the Dakotas, and Minnesota (Dayton, 1931, p. 60). It is a common shrub in woods, coulees, and on hillsides.

MATERIALS AND METHODS

All sites for this study are in the Little Missouri River Badlands in Billings County, North Dakota. Sites were on slopes, the areas that receive primary utilization by mule deer. Seven sites were selected on the basis of the abundance of serviceberry and the probability that mule deer would use an area. Most of the slopes selected for study were of a northerly exposure and of a moderate steepness to provide a good heterogeneous shrub stand. At each site, study plots were laid

out for individual growth (measurement of total growth), yield (production of leaves and twigs), and browse utilization studies. Study plots were 3.04 m (10 feet) wide to 22.81 m (75 feet).

All shrubs tagged for the individual studies were assigned Dasmann (1951) age and form classes. The age classes used were: (1) seedling, (2) young shrub, (3) mature shrub and (4) decadent shrub. The form classes used were: (1) all available, little or no hedging; (2) all available, moderately hedged; (3) all available, heavily hedged; (4) mostly available, little or no hedging; (5) mostly available, moderately hedged; (6) mostly available, heavily hedged and (7) mostly unavailable. These form classes represent a composite rating of degree of hedging and availability. In shrubs that are not browsed or browsed very lightly a characteristic rounded crown develops. When a shrub is browsed, the apical buds are nipped off; this stimulates the lateral buds and a hedged or 'flat top' appearance eventually develops. Heavy hedging indicates poor browse condition. Availability as browse is a result of height, location and/or density of plants (Dasmann, 1951).

Two parameters were used to establish a growth index: annual growth and annual yield. A total of 240 shrubs were tagged for growth measurements, and 240 shrubs were tagged for yield clippings. Ten shrubs were selected at each site from each of the following size classes: (1) 0-.30 m (0-1 foot); (2) .31-.60 m (1-2 feet); (3) .61-1.52 m (2-5 feet); and (4) more than 1.52 m (5 feet). Three of the sites studied had shrubs in all four size classes. The remaining four sites lacked shrubs in the fourth size class.

To determine the total growth, the current season's twig growth was measured at the end of August. On shrubs in size classes one and two, all current growth was measured and recorded; on shrubs in size classes 3 and 4, the current growth on two marked branches was measured. The tagged shrubs in the yield plots were clipped in late August near the end of the growing season. All of the current growth was clipped from shrubs in size classes 1 and 2, and approximately one-third of the current growth was clipped from shrubs in size classes 3 and 4. Clippings for each shrub were oven dried, separated into twigs and leaves, and weighed and recorded. Additional data taken on shrubs in growth and yield plots are as follows: stem diameter, crown dimensions (used to calculate relative yield), crown density, leaf count, degree of rust infection, and degree of dead material in the crown.

The twig measurement method described by Aldous (1945) was used to determine the current utilization. This method is most useful on shrubs whose seasonal growth tends to be linear and easily descernible. Winter utilization of serviceberry was measured for the winter of 1971-72. Two measurements of current growth were taken, the first in late August to determine amount of current growth available as deer browse, and the second in mid-April to determine how much of the current growth was utilized. A total of 120 shrubs were tagged for

the study; 5 shrubs were selected from each of the size classes present at each site. All the current growth was measured on shrubs in size classes 1 and 2, and on shrubs in size classes 3 and 4 the current growth was measured on three tagged branches. The formula used to determine the percent of current growth that was utilized as browse is as follows: percentage utilization equals the total fall twig length, minus the total spring twig length, divided by the total fall twig length, times one hundred (Hladeck, 1971:38).

RESULTS AND DISCUSSION

All shrubs were classified into the Dasmann (1951) age and form classes (Table 1). The majority of serviceberry shrubs were in the young and mature age classes (88.5 %), and in form classes 1 (all available, little or no hedging) and 4 (mostly available, little or no hedging, 65.2%). A small percentage of shrubs were in the seedling age class (9.0%), which is an indication of the shrub's ability to reproduce either vegetatively or by seeds. Very few of the shrubs studied were in the decadent age class (2.5 %). If no seedlings were present and the majority of the shrubs were in the mature or decadent age class, a downward trend would be indicated. A light to moderate degree of utilization can stimulate growth to a certain degree. However, heavy utilization physiologically weakens a shrub, decreases its growth, and ultimately causes a decline in vigor to a poor condition. Factors other than utilization may influence a plant's form. Some of these may be snow damage, livestock damage, and site factors. As in the case of skunk bush, the hedged appearance was due mainly to a site factor adaptation mechanism instead of browse utilization (Sanford, 1970:125).

Table 1. Number of individual plants of serviceberry (*Amelanchier alnifolia*) in each age[A] and form[B] class studied in Badlands of southwestern North Dakota during 1971 season.

Age	Number in class	%	Form	Number in class	%
S	54	9.0	1	262	43.6
Y	340	56.7	2	93	15.5
M	191	31.8	3	39	6.5
D	15	2.5	4	130	21.6
			5	10	1.6
			6	1	0.1
			7	65	11.1

[A]S=seedling, Y=young, M=mature, D=decadent.
[B]Dasmann (1951) form classes are explained under Materials and Methods.

Table 2. Growth and yield (dry wt.) from different age[A] classes of serviceberry (*Amelanchier alnifolia*) in Badlands of southwestern North Dakota during 1971 season.[B]

Age	% in class	Mean av. ann. growth (cm)	Mean av. total growth (cm)	Mean total weight twigs (gm)	Mean total weight leaves (gm)	Mean total yield weight (gm)
S	9.1	6.60a	10.33a	0.23a	0.52a	0.75a
Y	60.0	2.38b	34.06b	1.64b	6.03b	7.67b
M	28.8	2.60c	98.06c	8.57c	43.04c	51.61c
D	2.1	2.52d	74.27d	7.30d	21.26d	28.56d

[A] S = seedling, Y = young, M = mature, D = decadent.
[B] Values for age classes, followed by the same letter, are not significantly different at the 0.10 level.

Table 3. Growth and yield (dry wt.) from different form[A] classes of serviceberry (*Amelanchier alnifolia*) i Badlands of southwestern North Dakota during 1971 season.[B]

Form	% in class	Mean av. ann. growth (cm)	Mean av. total growth (cm)	Mean total weight twigs (gm)	Mean total weight leaves (gm)	Mean total yield weight (gm)
a	43.4	3.23a	39.46a	2.43a	8.68a	11.11a
2	27.9	2.36a	53.28b	3.20b	10.91b	14.11b
3	7.9•	2.45a	61.22c	3.88c	8.70c	12.58c
4	12.5	3.16a	70.70d	5.67d	27.44d	33.11d
5	4.1	1.24a	37.25e	0.00	0.00	0.00
6	0.4	2.27a	107.10f	0.00	0.00	0.00
7	3.8	3.47a	92.46g	5.78e	33.84e	39.62e

[A] Dasmann (1951) form classes are explained under Materials and Methods.
[B] Values for form classes, followed by the same letter, are not significantly different at the 0.10 level.

Data presented for growth and yield studies is for the 1971 growing season. The results for growth and yield were broken down by age and form classes (Table 2 and 3). Values for growth and yield parameters (Table 2) were significantly different (P$\geq$0.10) for the age classes. In the form class breakdown of growth (Table 3), only the values for mean average total growth were significantly different P= 0.10). Values for the yield parameters were significantly different (P$\geq$0.10) for the form classes (Table 3). Mean average annual growth, and mean average total growth seem to be characteristic of the different age classes (Tables 2 and 3). Average twig growth is greatest in seedlings (6.60 cm) mainly because growth is primarily on one twig. As the shrub ages, more twigs are produced, and the mean average annual growth drops in the young age class (2.38 cm). At maturity the average annual growth was observed to be 2.60 cm. As the shrub eventually becomes decadent, the normal growth is impaired due to environmental or physiological stresses and the average annual growth decreases (2.52 cm). Since values for mean average annual growth for the form classes are not significantly different, no correlations between form and average twig growth can be made. Mature shrubs had the largest mean average total growth (98.06 cm) and seedlings the least (10.33 cm). This is because mature shrubs had many more twigs than did the seedlings. Young shrubs were intermediate in total growth between seedlings and mature shrubs. In decadent shrubs the mean total growth began to decline, due to various stresses. Form classes 1 and 4 (lightly hedged categories), had smaller mean total growth than form classes 3 and 6 (heavily hedged categories, 110.16 cm versus 168.32 cm. This can be explained as being due to browsing that stimulated the growth of lateral buds, hence more total growth in the heavier hedged categories.

The total annual yield was 93.84 kg/ha (172.31 lbs/acre) for all sites. Mean total yield for respective age classes corresponds to the total growth for the age classes (Tables 2 and 3). In mature shrubs the mean total growth was greatest and likewise the mean total yield (51.61 g); in seedlings, the mean total growth was least as was the mean total yield (0.75 g). Mean total yield was also greater in form class 3 (heavily hedged) than in form class 1 (lightly hedged), 12.58 g versus 11.11 g. This again can be explained on the basis of more total growth in the heavily hedged classes. Mature and young shrubs contributed 94% of the total annual production (78% and 16% respectively). Seedlings constituted a minimal percent (0.32 %) and decadent shrubs accounted for a little more than 5 % of total yield. Shrubs in form classes 1 (all available, little or no hedging) and 4 (mostly available, little or no hedging) contributed to 61 % of the total yield. Form classes 2 (all available, moderately hedged) and 3 (all available, heavily hedged) accounted for a little over 8 % of total yield. Due to unavailability (form class 7), 29 % of total production could be considered as not providing deer browse. Leaves constituted approximately 80 % of the total annual production, leaving less than

20 % of total production available as deer browse, after the leaf fall. During the summer of 1971, *Gymnosporangium* rust infections were noted on serviceberry shrubs. This rust infection causes early defoliation, shoot dieback, and malformations of the fruits, decreasing both quantity and quality of the browse (Furniss and Krebill, 1971, p. 219).

Table 4. Utilization of serviceberry (*Amelanchier alnifolia*) by age[A] and form[B] classes in Badlands of southwestern North Dakota during winter of 1971-72.[C]

Age	% in class	Mean % util.	Form	% in class	Mean % util.
S	6.7	2.96a	1	6.1	11.05a
Y	63.2	13.10a	2	4.2	17.05a
M	30.1	8.69a	3	5.0	24.70a
D	0.0	0.00	4	18.4	10.26a
			7	11.4	4.56a

[A]S=seedling, Y=young, M=mature, D=decadent.
[B]Dasmann (1951) form classes are explained under Materials and Methods.
[C]Values for age and form classes, followed by the same letter, are not significantly different at the 0.10 level.

Average utilization of serviceberry for all sites was 11 %. Values ranged from no use to 28 % utilization. Utilization was also broken down by age and form classes and statistically analyzed (Table 4). Values for mean percentage utilization were not significantly different (at high levels) for various age and form classes; however, they do show a definite trend. Shrubs in the young age class received the highest utilization (13 %) and seedlings the lowest utilization (2.9 %) mainly because snow would cover most seedlings. Utilization of mature shrubs was intermediate (8.6 %) between seedlings and young shrubs. The difference in utilization percentages of various age shrubs could be explained on the basis of the numbers of each age shrub available to deer. Shrubs in form class 7 (mostly unavailable) had the lowest utilization (4.5 %) due to the height of the shrubs. Shrubs in which available browse was more than 1.52 m (5 feet) in height are classed as mostly unavailable. Shrubs in form classes 2 (all available, moderately hedged) and 3 (all available, heavily hedged) had the highest utilization (17 % and 24 %), whereas shrubs in form classes 1 (all available, little or no hedging) and 4 (mostly available, little or no hedging) had lesser degrees of use (11 % and 10 %). Shrubs in moderate to severely hedged form classes indicate moderate to heavy past utilization and this degree of use should continue, giving a high present

utilization value. Shrubs in a form class in which hedging is very little, or not at all, indicate little past browse utilization, so current utilization values could be expected to be lower.

SUMMARY

Results presented in this paper are from the basic analysis of data for the 1971 growing season. The majority of the shrubs tagged for growth, yield, and utilization studies are in young and mature age classes, and in lightly hedged classes. This, combined with the fact that there are some seedlings, would indicate that serviceberry is in good condition. In both growth and yield data, shrubs in the heavier hedged classes had more total growth and so more yield. Most of total production (80 %) was unavailable to deer as winter browse, as this portion was the leaves. This left less than 19 kg/ha (34 lbs/acre) dry weight for deer in the form of twigs after the leaf fall. Utilization ranged from no use to 28 % utilization, the average of all sites being 11 %. Clones of this species appear to be deteriorating due to environmental influences, such as snow breakage, rust fungus, and damage from domestic livestock and small game.

ACKNOWLEDGMENTS

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THE STATISTICAL ESTIMATION AND COMPARISON OF GROWTH RATES USING STRAINS OF THE MALLARD *ANAS PLATYRHYNCHOS*

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ABSTRACT

Many biological investigations require the comparison of growth rates of organisms. Biological growth curves are typically sigmoid in shape, a fact which renders calculation of rate difficult because the first derivative, a standard measure of rate, varies with time. However, if the data are adequately described by one of the usual growth curves, such as the logistic, the von Bertalanffy or the Gompertz, then a unique parameter defining growth rate can be identified. The logistic curve was shown to provide the most satisfactory fit to body weights of two mallard (*Anas platyrhynchos*) strains. Parameters were estimated by both nonlinear least squares, a technique with some optimal statistical properties but requiring fairly large amounts of computer time, and linear least squares applied to transformed variables, a less exact but faster technique. There was no significant difference in the average growth rates of the two mallard strains.

INTRODUCTION

This investigation was prompted by an earlier study (Greenwood, 1973) that compared morphological and physiological characteristics of wild and game-farm strains of the mallard. Although this previous study had established that game-farm mallards were heavier than wild birds, both at hatching and at maturity, it was not known if growth from time of hatching to maturity proceeded at the same rate in both strains of birds. Accordingly, an analysis was undertaken to statistically estimate and compare growth rates of the two strains.

The data available included weights recorded on the day of hatch and at nine

weekly intervals thereafter, for a total of 10 measurements, and a "mature" weight taken at the 20th week. Greenwood (1973) noted that weight growth after the seventh measurement became somewhat erratic because the rapid maturation of flight feathers then diverted energy from overall body growth. Because of this perturbation, only the first seven measurements for each bird were used in most analyses.

Greenwood (1973) investigated four groups of birds: wild, game-farm, hybrid and backcross. For the purposes of this report, however, I chose nine males from each of the first two groups for the sample. Curves indicating average weights for each group throughout the period are displayed in Figure 1.
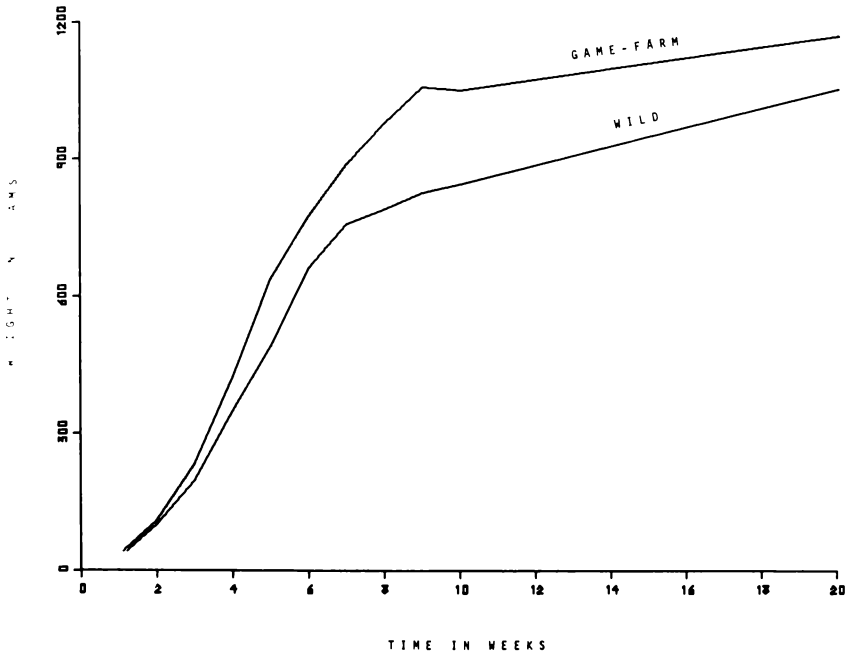
Figure 1. Average weights recorded at weekly intervals for nine game-farm and nine wild mallards.

DESCRIPTION OF GROWTH CURVES

Weights recorded regularly throughout the growth period of an organism can, as any set of points, be fitted to some arbitrary mathematical function, such as a polynomial. Ideally, however, the data should fit some curve that is based on biological considerations and from which useful inferences can be drawn. Three families of curves have been regularly used to fit early-phase sigmoid growth data:

the logistic, the von Bertalanffy and the Gompertz. Growth often continues at a linear rate beyond this early sigmoid phase (Laird et al., 1965).

The following notation will be adopted in the description of each curve:

$W(t)$ =weight at time t,
A =asymptote, the weight at which leveling off occurs,
b =a parameter related to initial weight,
r =growth rate.

According to Andrewartha and Birch (1954:347), the first use of the logistic curve was to describe the growth in numbers of human populations, which were observed to grow explosively at early stages and at a lesser rate as the population became large. It was later applied to populations of other organisms, and eventually to growth in weight of individual animals. The function describing the curve can be derived by assuming that at time t the rate of growth is proportional to the size already attained, W, and also to the proportion of growth yet to be achieved, $(A-W)/A$. Thus,

$$\frac{dW}{dt} = rW(A-W)/A \ ,$$

which leads to a function (Equation 1) featuring exponential growth early and a gradual leveling off as the asymptote is approached (Figure 2). The curve is symmetric about $\frac{1}{2}A$.

$$W(t) = A/[1 + b \times exp(-rt)] \ , \tag{1}$$

where $exp(x)$ denotes the exponential function, e^x.

The von Bertalanffy function (Fabens, 1965) is derived from the assumption that the rate of growth is equal to the difference between an anabolic rate and a catabolic rate. It is further assumed that catabolism varies directly as the total weight of the organism, whereas anabolism occurs at a rate proportional to the surface area of the organism, approximately the two-thirds power of weight. Thus,

$$\frac{dW}{dt} = constant \ W^{2/3} - constant \ W \ ,$$

resulting in

$$W(t) = A[1 - b \times exp(-rt)]^3 \ . \tag{2}$$

Although it is now recognized that the underlying assumptions are somewhat unrealistic (e.g., Hubbell, 1971:277), the resulting function nevertheless provides a close approximation to certain growth curves occurring in nature. As can be seen from Figure 2, this curve is distinguished from the logistic by more
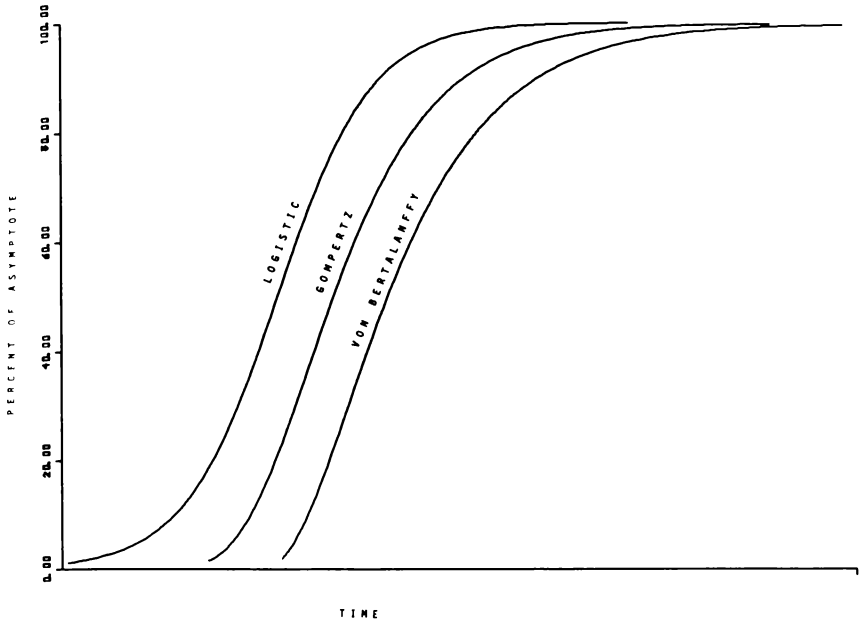
Figure 2. Graphical representation of logistic, Gompertz, and von Bertalanffy growth curves (after Ricklefs, 1967).

rapid growth early in the period and a more gradual approach to the asymptote.

Laird et al. (1965) described the Gompertz function. The empirical observation leading to it was that the specific growth rates, $\dfrac{1}{W}\dfrac{dW}{dt}$,

declined linearly when plotted against time on semilog graph paper. This suggests a curve with exponential growth that is damped decreasingly at a rate which is itself exponential. That is,

$$\frac{dW}{dt} = vW \quad,$$

where v is function of t such that

$$\frac{dv}{dt} = -rv \quad,$$

resulting in

$$W(t) = A \times \exp[-b \times \exp(-rt)] \quad.$$

This curve is intermediate between the logistic and the von Bertalanffy (Figure 2).

The usage of consistent notation for parameters should not suggest that values derived from different curves are comparable. The meaning of the asymptote A is the same regardless of the function used, but this property is not shared by the other parameters. The parameter r measures the rate at which the asymptote is approached, but as Ricklefs (1967:981) pointed out, growth rates "are comparable only among species whose growth curves are fit by the same equation." The parameter b is in each case a function of $y(0) = W(0)/A$, the proportion of weight achieved by time $t = 0$. In the logistic equation,

$$b = [1 - y(0)]/y(0), \tag{4}$$

whereas in the Gompertz equation,

$$b = -\log[y(0)] \tag{5}$$
$$= 1 - y(0), \text{ approximately,}$$

and in the von Bertalanffy equation,

$$b = 1 - [y(0)]^{1/3} \tag{6}$$

Natural logarithms are used throughout this report. The parameter b is often a "nuisance parameter" because it varies with the time origin used, which may be arbitrary.

FITTING DATA TO A GROWTH CURVE

For the mallard data, it was first necessary to determine which of the three growth curves was most appropriate. This was done by fitting growth data for three individual birds from each group to the logistic, Gompertz, and von Bertalanffy curves by nonlinear least squares (e.g., Draper and Smith, 1966; Conway et al., 1970). The entire sample was not analyzed in this manner because of computer time requirements. The average residual sum of squares (RSS) for the Gompertz curve was somewhat smaller than for the logistic and the von Bertalanffy (Table 1), but only the logistic curve yielded reasonable estimates of the asymptotes (Table 2). Because growth continued at a linear rate beyond the initial period of sigmoid growth, a realistic asymptote for the sigmoid phase should lie somewhere between the 10th weight (which is still in the sigmoid phase) and the weight at maturity (after the linear phase). But, as Table 2 demonstrates, estimates of the asymptote derived under the Gompertz and von Bertalanffy models regularly exceeded the weight at maturity. Accurate estimation of the asymptote is critical even though interest is in the growth rate, because the two parameters are highly correlated. The nonlinear least squares computer program employed in this study (GAUS, developed at the University of Wisconsin) allowed only one constraint on each parameter, so the asymptote could be constrained to exceed the 10th weight, but not also to be less than the weight at maturity. Because estimates under the Gompertz and von Bertalanffy models were not realistic when fitting seven weights, some fits using all 10 weekly weights were made, despite the irregularities occurring in the latter weeks. The

Table 1.   Average residual sum of squares after fitting three individuals from each strain of the mallard to various models.

Strain	Logistic	Gompertz	von Bertalanffy
Wild	2,325	1,747	2,288
Game-farm	5,010	3,394	4,876

Table 2.   Average estimated asymptotes for various models and actual weights at 10 weeks and at maturity for each strain of the mallard.

	Estimated asymptotes			Actual weight	
Strain[A]	Logistic	Gompertz	von Bertalanffy	10th week	maturity
Wild	1,050[B]	1,375	1,875	1,003	1,253
Game-farm	1,262	1,473	1,820	1,262	1,380

[A]Three individuals of each strain were used.
[B]Weights in grams.

weights during these latter weeks began to stabilize, indicating the approximate level of the asymptote. By using 10 weights the asymptotes were more realistically estimated and, of the three curves, the logistic provided a superior fit.

For the above reasons the logistic model was chosen for future analysis of these data. It should be recognized, however, that another model may prove more appropriate in similar studies.

Nonlinear least squares is an acceptable procedure if the number of observations is small, but because the technique has extremely large computer memory requirements and is relatively expensive in terms of computer time, short-cut procedures are needed for large samples. Also, nonlinear least squares programs have not been universally implemented, so an approximation using readily available techniques is desirable.

If the asymptote is known, it is possible for each of the growth equations considered to transform the data so that the parameters can be estimated by linear least squares. Let $y(t) = W(t)/A$ be the proportion of the asymptotic weight achieved by time t. Then the following transformations linearize the model:

$$\log (1/y(t) - 1) = \log b - rt \text{ for the logistic,}$$
$$\log (-\log y(t)) = \log b - rt \text{ for the Gompertz,}$$
$$\log (1 - [y(t)]^{1/3}) = \log b - rt \text{ for the von Bertalanffy.}$$

Notice that the right-hand side of each equation is identical; only the form of the transformation changes depending upon which growth function is employed. Estimates of $\underline{r}$ and log $\underline{b}$ are thus obtained by a linear regression on time of the appropriate transformed values.

Linearization has several drawbacks, however; the most apparent is the requirement that the asymptote be known. In some cases, obvious estimates of $\underline{A}$ are available and can be used. Otherwise various trial values can be used and one providing the best fit to the data chosen. A further restriction is that $\underline{y(t)} < 1$, or $\underline{W(t)} < \underline{A}$, for each $\underline{t}$. Thus, if the asymptote is approached closely, a small measurement error or random fluctuation could yield $\underline{W(t)} > \underline{A}$, and result in a value impossible to transform. Another deficiency of linearization is that biases may develop when nonlinear transformations are made. For example, if $u = f(\underline{x})$ is a non linear transform, then $u \neq f(\underline{\overline{x}})$ and $f^{-1}(\underline{\overline{u}})$ is not equal to x and is biased for estimating the expected value of x. Finally, the error structure implicit in the linearized model may not be appropriate. For example, if the errors in the original measure are normally distributed with a common variance, a nonlinear transformation will cause this useful property to be lost.

An alternative method was given by Stevens (1951) to directly estimate the parameters in the expression $a + dc^t$. By making the transformation $1/\underline{W(t)}$ for the logistic, log $(\underline{W(t)})$ for the Gompertz, or $(\underline{W(t)})^{1/3}$ for the von Bertalanffy, estimates of a, d, and c can be obtained directly. A further transformation of the parameters is required to convert these values into estimates of A, b and r. Stevens' procedure has been incorporated into a widely distributed computer program (BMD06R—Dixon, 1968). Ricklefs (1967) presented a graphical procedure for estimating growth rates for each of the three equations. His method requires A to be estimated independently, and the adequacy of this estimate is determined by how closely the transformed values follow a straight line.

COMPARISON OF GROWTH RATES

The theory of hypothesis testing in nonlinear situations has not been developed to any extent. Lacking exact methods, one can employ procedures that are appropriate for linear models and hope that the nonlinearity does not result in misleading conclusions.

Under the logistic model it appears reasonable to take the weight at the $\underline{t}$-th time for the $\underline{j}$-th bird in the $\underline{j}$-th group as

$$\underline{W}_{ij}(\underline{t}) = \underline{A}_{ij}/[1 + \underline{b}_{ij} \times exp(-\underline{r}_{ij}\underline{t})] + \underline{e}_{ijt} \, , \qquad (7)$$

where $\{\underline{e}_{ijt}\}$ are error terms independent and normally distributed with zero mean and variance $\hat{\delta}$. i.e.

$$\underline{e}_{ijt} \sim N(0,\sigma^2) \text{ independent.}$$

The parameters $\{\underline{A}_{ij}\}$ and $\{\underline{b}_{ij}\}$ are not of direct interest; instead, one tests certain hypotheses about the $\{\underline{r}_{ij}\}$.

It is most realistic to consider the observed $\{\underline{r}_{ij}\}$ as samples from a population with means $\{\rho_j\}$. Interest centers on the hypothesis

$$H_0: \quad \rho_1 = \rho_2 ,$$

against the alternative

$$H_1: \quad \rho_1 \neq \rho_2 .$$

Because the computer storage requirements for model fitting under H_0 were enormous, I was not able to carry out this test.

An alternative test of group differences can be constructed by estimating the individual growth rates $\{\underline{r}_{ij}\}$ and comparing the difference in the group averages to the variation within groups. This leads to the usual $\underline{t}$ test:

$$\underline{t} = (\overline{r}_1 - \overline{r}_2)[\underline{s}^2(1/\underline{n}_1 + 1/\underline{n}_2)]^{-\frac{1}{2}} ,$$

where $\underline{s}^2$ is the pooled estimate of variance within groups. The degrees of freedom for this test are $n_1 + n_2 - 2$. Because $\{\underline{A}_{ij}\}$ and $\{\underline{b}_{ij}\}$ are also estimated, the $\{\underline{r}_{ij}\}$ may be measured wih varying precision, so that the $\underline{t}$ test is only approximate.

For the mallard data, $\underline{\overline{r}}_1 = 0.7765$ (wild birds), $\underline{\overline{r}}_2 = 0.7504$ (game-farm birds), $\underline{s}^2 = 0.00445$, resulting in $\underline{t} = 0.83$, which is non-significant on 16 degrees of freedom.

DISCUSSION

The reasons for fitting growth data to a curve fall into two broad categories. The first is to gain an understanding of the biological processes that culminate in growth. The second purpose is simply to compare the parameters of growth among various groups. The direction the analysis should take depends upon the intent of the study.

Weiss and Kavanau (1957) provided an outstanding example of model-building in order to understand a phenomenon. They considered the fundamental physiological components of an organism which, together with their interrelationships, determine the growth of that organism. By examining the differential equations describing these component systems, they were able to formulate a model that was biologically realistic, but yet was amenable to analysis. Researchers should not accept the adequacy of fit to a model as conclusive evidence that the assumptions of the model are correct, however, because differing sets of assumptions often give rise to the same model.

If the purpose is to compare growth parameters, then it may not be necessary to construct a biological model describing growth. Leech and Healy (1959) fitted orthogonal polynomials to growth data for different groups and tested for differences in main effects (which relate to average weight), linear components (which relate to the growth rate), and quadratic components (which relate to the shape of the growth curve). Their method assumes no biological structure to the data, but it is not clear how comparisons at one level, for example the linear, can be made when differences in higher-degree components exist. Box (1950) took the differences between values of the growth curves at successive, equally spaced times and assumed that these were independent. He performed an analysis of variance on them to detect linear differences in growth rate, and concluded by testing the underlying assumptions. However, the examples considered by Box were primarily linear and did not exhibit the sigmoid shape that is characteristic of most biological growth. To overcome this limitation, Rao (1958) generalized Box's procedure by transforming time to a metameter so that average growth in each interval of the time metameter was constant. He then tested for differences in the linear components between groups. His method is applicable if all individuals grow according to the same pattern, regardless of the pattern. Rao also developed some likelihood ratio tests that are more efficient for large samples. Allen and Grizzle (1970) presented a multivariate approach to the comparison of growth rates under a nonlinear model that is appropriate in the latter stages of growth.

The advantage gained by fitting the data to a growth curve is the existence of a unique parameter defining rate of growth. A broader class of growth curves might be examined by employing self-modeling nonlinear regression, a promising procedure recently described by Lawton et al. (1972). The technique is distinctive in that a single set of data is used to select the optimal *form* of the model from a broad class of models as well as to estimate the parameters associated with the chosen model. It is conceivable that the method, if applied to growth data, would select the optimal model (logistic, von Bertalanffy or some intermediate type) and, at the same time, estimate the growth rate and other parameters.

ACKNOWLEDGMENTS

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AQUATIC MALACOGEOGRAPHY OF NORTH DAKOTA

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ABSTRACT

At least 44 species of aquatic mollusks inhabitat North Dakota—13 mussels (unionacean bivalves), 9 pill clams (sphaeriid bivalves) and 22 snails (4 prosobranch and 18 pulmonate gastropods). The aquatic mollusks serve to define three zoogeographic provinces proposed here: Northeastern, Intermediate, and Southwestern. The Intermediate province corresponds to the Missouri Coteau, a hilly region with nonintegrated drainage trending northwesterly through the central part of the state; tentatively, the Turtle Mountains are placed within this province. Fewest species occur in the Intermediate province and mussels are lacking. The Northeastern province is one of integrated and nonintegrated drainage and hilly, undulating, and dissected topography. Most species occur in this province; unique to the province are such mussels as *Fusconaia flava* (Rafinesque), *Quadrula quadrula* (Rafinesque), and *Proptera alata* (Say). The Southwestern province is characterized by essentially integrated drainage and dissected topography. It is intermediate in number of species; one mussel, *Proptera laevissima* (Lea), is unique to this province. Species distribution patterns in the state are of three types: cosmopolitan (generally throughout all provinces), sporadic (at widely separated localities in one or more provinces), and restricted (confined to part of a province). The present fauna probably originated within about the last 9,000 years; mussels most likely are derived from the Mississippi River system, and pill clams and snails possibly populated the state from the south and east. Primary ecological factors limiting aquatic mollusks include availability of suitable water body (especially as related to size and permanency), bottom instability, possibly high total chlorides and sulfates, fish host (mussels), and man's activities.

INTRODUCTION

Little but species lists and notes on species were published on the aquatic mollusks of North Dakota prior to the 1960s. Say (1825, p.11) noted a snail (*"Planorbis armigerus"*) for the "Red River." Owen (1852:177) noted five mussels for the Red River below the mouth of the Red Lake River. Lea (1858) noted two mussels from the Missouri River at Fort Clark. Hayden (1862:180) listed one mussel from the White Earth River, one from the Missouri at Fort Clark, two from the James River (in North Dakota?), and two snails from Fort Berthold. Forebel (1870:72) mentioned a mussel, a pill clam and four snails from small creeks and ponds in the state. Dawson (1875:350) listed seven mussels from the Red River; Call (1885) reported (*fide* Ortmann, 1919:281) a mussel

from presumably the same river at Pembina; and Grant (1885:115-119) recorded eight mussels from the Red at Wilkin County, Minnesota. Dall (1905:125-136) listed 14 mussels and two pill clams for the Red River drainage—many of the occurrences, however, were for Manitoba. Coker and Southall (1915:15) reported six mussels from the Red River at Fargo and four from the Sheyenne River at Lisbon. Ortmann (1919:17,31,203,292) noted four mussels from the Sheyenne River at Argusville. Winslow (1921) listed 49 mollusks from the Devils Lake region, Turtle Mountains, and westernmost North Dakota and incorporated notes on general molluscan habitats.

Tuthill (1962, 1963) compiled a list of the state's aquatic mollusks based largely on previous publications; in 1963, he and Laird published the first ecologic paper on aquatic mollusks in North Dakota. Since 1966 my associates and I have reported on the distribution and ecology of the aquatic mollusks, concentrating initially upon the mussels (Cvancara, 1966, 1967, 1970a, 1970b; Cvancara and Erickson, 1968; Cvancara, Erickson, and Delimata, 1972; Cvancara and Harrison, 1966; Cvancara, Heetderks, and Iljana, 1967; Cvancara, Norby, and Van Alstine, 1975; and Groenewold, 1971). Peterka (1972) discussed two genera of pill clams and four genera of snails from Lake Ashtabula, a man-made reservoir on the Sheyenne River. Clarke (1973) reported many species of mollusks from 18 localities in northeastern North Dakota.

My main purpose in this paper is to list the aquatic mollusks presently known in North Dakota and suggest that these organisms can serve to delineate three provinces in the state. I will also introduce specific patterns of distribution and suggest reasons for the general and specific distributions. Elsewhere, I intend to describe and illustrate all species, give distribution maps for each, and analyze limiting ecological factors in some detail.

MATERIALS AND METHODS

Two hundred ninety-two collecting stations (Figure 1), examined for mollusks during the summers of 1965 through 1969, form the basis for this study; fifteen additional stations for each of the Turtle (1964) and Forest (1966-70) Rivers and six semiaquatic stations for terrestrial mollusks (1969) were used to supplement data from the basic stations. More stations are on streams than on lakes or ponds because streams were emphasized in the early phases of this study.

Collecting time at a station was generally one-half to two hours. Mussels were collected primarily by handpicking; in relatively clear water, these animals were more easily located with a Turtox Fishscope, an aluminum alloy cylinder (0.61 m long by 0.15 m in diameter) fitted with a glass plate at one end. A 2.4-m crowfoot dredge (Cvancara, 1970a:62) or basket dredge was used in the deeper rivers and lakes. Mussel abundance was determined as live individuals collected per hour by hand-picking. Snails and pill clams were collected from plants and sediment with a screened food strainer in shallow water (about 1 m and less) and with an Ekman
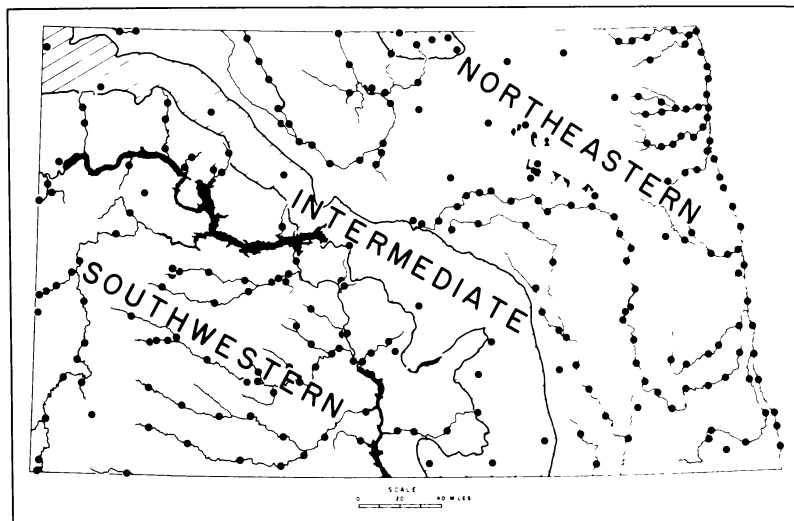
Figure 1. Aquatic molluscan provinces in North Dakota and collecting stations. Boundaries of the Intermediate province, excepting the Turtle Mountains, correspond to those of the Missouri Coteau and have been modified from Colton, Lemke and Lindvall (1963).

dredge in deep water. The relative abundance of these mollusks was estimated in the field as abundant, common, uncommon or rare. It is difficult to be quantitative about these abundance terms because of collecting these mollusks at the same time that mussels were being collected, searching for various snails on the bottom as well as on plants, and other factors. However, ''rare'' generally means observing three specimens or less, and ''abundant'' generally means observing about twenty specimens or more.

Chemical factors (such as pH, total alkalinity, and total chlorides) and turbidity were measured at many stations with a Taylor pH Slide Comparator, Model T-1, and a Hach Chemical Company protable chemical kit (Model DR-E). Specific conductance was also measured at many stations with a Beckman Solu-Bridge, Model RB3-338. Observations were also made on bottom sediment, associated organisms, and other factors.

Specimens collected are in the paleontological collection of the Department of Geology, University of North Dakota.

RESULTS

Composition of fauna. —At least 44 species of aquatic mollusks inhabit North Dakota (Table 1)—13 mussels (unionacean bivalves), 9 pill clams (sphaeriid bivalves), and 22 snails (gastropods); only four of the snails (species 23 to 26 in Table 1) are operculate, gill-breathing (prosobranch) forms, the remainder being nonoperculate, lung-breathing (pulmonate) forms. The most frequently occurring mussel, pill clam, and snail are *Anodonta grandis, Pisidium (Cyclocalyx) compressum,* and *Physa gyrina.* All of these species are substantiated by specimens I have collected; no attempt has been made to verify all the species previously reported in the state (Tuthill, 1962, 1963). Probably a few other species will be added to the list (Table 1) with further collecting; the most likely are pill clams and snails from stagnant (nonflowing) water bodies from which fewer collections were made.

Distribution of fauna. —Three malacogeographic provinces for North Dakota are proposed here: Northeastern, Intermediate, and Southwestern (Figure 1). For convenience, the boundaries of the provinces correspond to those of physiographic subdivisions. The Intermediate province corresponds to the Missouri Coteau (Clayton, 1967), a hilly region of nonintegrated drainage with numerous potholes or sloughs and lakes. The Turtle Mountains, resembling a wooded Coteau, are tentatively placed within this province; further collecting may require placement of the Turtle Mountains in a separate province or subprovince. The Northeastern province, a region of nonintegrated and integrated drainage and hilly, undulating, and dissected topography, encompasses several physiographic subdivisions (Kume and Hansen, 1965:6). It is primarily within the Hudson Bay drainage except for the area drained by the James River and its tributaries (Mississippi River drainage). The Southwestern province, a drier region dominated by dissected topography with integrated drainage and with relatively few stagnant water bodies, is entirely within the Mississippi River drainage.

The three molluscan provinces are not completely distinct, but they are distinguishable by notable differences in species number and composition. The Northeastern province has the most species (41), representing all major molluscan groups; unique species are several mussels, such as *Fusconaia flava, Quadrula quadrula,* and *Proptera alata.* The Southwestern province contains an intermediate number of species (26); it has fewer mussels, fewer pill clams, and presumably fewer snails than the Northeastern province. Unique to the Southwestern province is the mussel *Proptera laevissima.* The Intermediate province has the fewest species (23); no mussels were found in this province although Winslow (1921:15) mentioned empty shells of *Anodonta pepiniana* Lea and ?*A. Kennicotti* Lea (both=?*Anodonta grandis*) from Gravel Lake in the Turtle Mountains. This province contains about the same number of pill clams as the Northeastern province and perhaps fewer snails.

Table 1. North Dakota aquatic mollusks with occurrence by province and general habitat.

SPECIES	PROVINCE			GENERAL HABITAT (N.Dak.)^A				
	North-eastern	Inter-mediate	South-western	1	2	3	4	5
Unionacean bivalves (mussels)								
1. *Amblema plicata* (Say)	X						X	X
2. *Fusconaia flava* (Rafinesque)	X						X	X
3. *Quadrula quadrula* (Rafinesque)	X							X
4. *Anodonta grandis* Say	X		X			X	X	X
5. *Anodontoides ferussacianus* (Lea)	X		X				X	X
6. *Lasmigona complanata* (Barnes)	X		X				X	X
7. *L. compressa* (Lea)	X						X	X
8. *Strophitus undulatus* (Say)	X		X				X	X
9. *Lampsilis ovata* (Say)	X		X				X	X
10. *L. radiata* (Gmelin)	X						X	X
11. *Ligumia recta* (**Lamarck**)	X							X
12. *Proptera alata* (**Say**)	X							X
13. *P. laevissima* (**Lea**)			X				X	
Sphaeriid bivalves (pill clams)								
14. **Sphaerium (Musculium)** *lacustre* (**M**üller)	X	X		X		X	X	
15. *S.* (M.) *transversum* (Say)	X	X			X	X		
16. *S. simile* (Say)	X		X			X	X	
17. *S. striatinum* (Lamarck)	X		X			X	X	
18. *Pisidium (Cyclocalyx) casertanum* (Poli)	X	X	X	X		X?	X	
19. *P.* (C.) *compressum* Prime	X	X	X	X		X	X	X
20. *P.* (C.) *ferrugineum* Prime		X		X?				
21. *P.* (C.) *nitidum* Jenyns	X	X		X		X	X	
22. *P.* (C.) *ventricosum* Prime		X		X?			X	

Gastropods (snails)

#	Species	1	2	3	4	5
23.	Valvata tricarinata (Say)	X		X	X	X
24.	Cincinnatia					
	cincinnatiensis (Anthony)	X				X
25.	Probythinella lacustris (Baker)	X		X		X
26.	A. limosa (Say)	X	X	X	X	X
27.	Fossaria obrussa (Say)	X	X	X	X	X
28.	Stagnicola caperata (Say)	X	X	X	X	X
29.	S. elodes (Say)	X	X	X	X	X
30.	Lymnaea stagnalis (Say)	X	X	X	X	X
31.	Ferrissia rivularis (Say)	X	X	X	X	X
32.	Gyraulus parvus (Say)	X	X	X	X	X
33.	G. circumstriatus (Tryon)	X	X	X	X	X
34.	Armiger crista (Linnaeus)	X	X	X	X	
35.	Helisoma (Helisoma) anceps (Menke)	X	X	X	X	X
36.	H. (Pierosoma) trivolvis (Say)	X	X	X	X	X
37.	Promenetus exacuous (Say)	X	X	X	X	X
38.	P. umbilicatellus (Cockerell)	X		X	X	
39.	Planorbula armigera (Say)	X	X	X?	X?	
40.	P. campestris (Dawson)	X		X	X	
41.	Aplexa hypnorum (Linnaeus)	X	X	X	X	X
42.	Physa gyrina (Say)	X	X	X	X	X
43.	P. integra Haldeman	X	X	X	X	X
44.	P. jennessi Dall	X	X	X	X	X
	Total Species	41	23	26		

*1=permanent ponds and lakes; 2=intermittent ponds and lakes; 3=intermittent streams; 4=small streams; 5=large streams (permanent). Permanent water bodies are in existence 50% or more of the time and intermittent water bodies are those not in existence 50% or more of the time. Large streams are the Missouri, Red and lower Sheyenne Rivers; all other streams in the state are small. An italicized X signifies that habitat in which a species occurs in greatest numbers and so may be considered a preferred habitat. Question marks indicate an uncertain habitat or an uncertain preferred habitat.

Taking all groups together, aquatic mollusks are decidedly most similar in the Northeastern and Southwestern provinces (Table 2). By group, pill clams are most similar in these two provinces but snails may be more similar in the Intermediate and Northeastern provinces.

Apart from occurrences with province, mollusk distributions can be grouped into three types: cosmopolitan, sporadic, and restricted. Cosmopolitan species occur generally throughout all provinces, such as the snail *Physa gyrina* (Figure 2). Sporadic species occur at widely separated localities throughout the state or within a single province, such as the mussel *Strophitus undulatus* (Figure 3). Restricted species are confined to part of a province, such as the mussels *Proptera alata* and *P. laevissima* (Figure 4).

DISCUSSION
Origin of molluscan fauna

To evaluate the possible origin of aquatic mollusks in a region, recent geologic history, ease of dispersal, and possible sources of the fauna might be considered.

North Dakota was affected by several glacial ice advances during the late Pleistocene (Clayton, 1966:4-8), leaving three-fourths or more of the northeastern part of the state covered by glacial drift. The resulting hilly and undulating topography has given rise to numerous water-filled depressions, particularly in the Northeastern and Intermediate provinces. Only the southwestern one-fourth or less of the state, a region of dissected topography with few stagnant water bodies, was unglaciated. The present drainage of the state, excepting that of the unglaciated region, developed after the late Wisconsinan (Bluemle, 1972) within about the last 9,000 years when glacial Lake Agassiz drained from the upper Red River Valley (Elson, 1966, Table 6 and Fig. 6).

Table 2. Jaccard's similarity coefficients[A] comparing numbers of molluscan species by group and province.

Mollusk Group	Jaccard's Similarity Coefficient (x 100)		
	Southwestern-Intermediate	Intermediate-Northeastern	Northwestern-Southwestern
Mussels	0	0	38
Pill clams	25	44	57
Snails	65	77	73
All mollusks	44	49	60

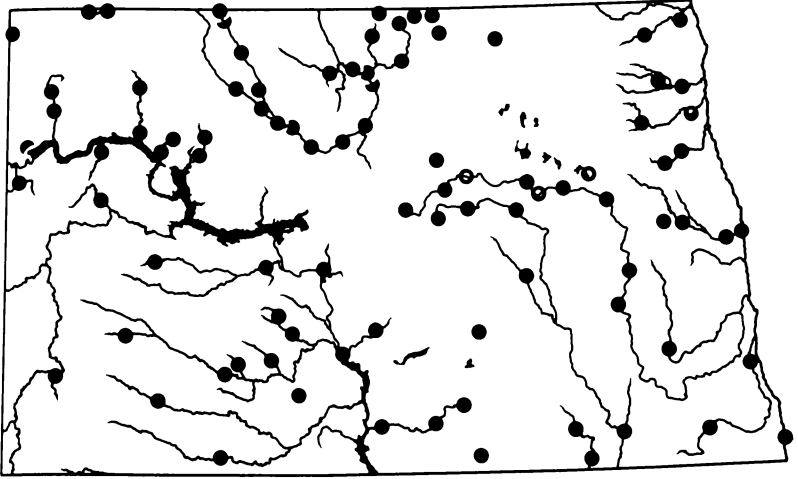[A]Cheetham and Hazel (1969).

Figure 2. Distribution of a cosmopolitan mollusk, the snail *Physa gyrina* Say, in North Dakota. Open circles indicate dead occurrences and closed circles indicate live occurrences.
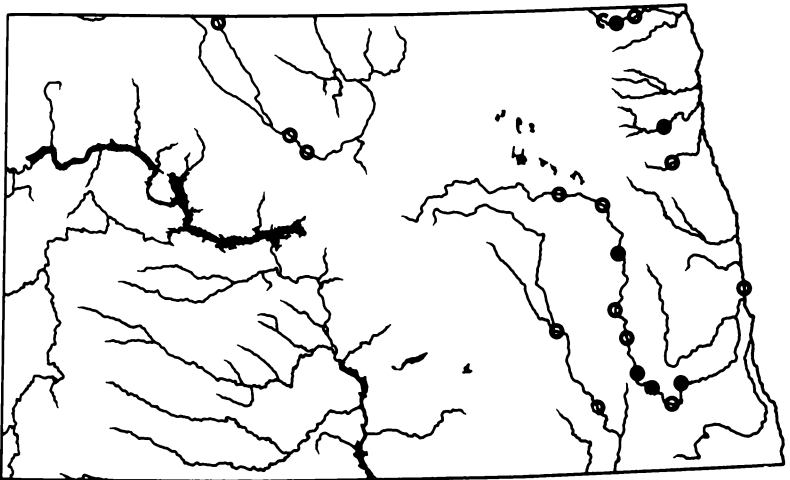
Figure 3. Distribution of a sporadic mollusk, the mussel *Strophitus undulatus* (Say), in North Dakota. Open circles indicate dead occurrences and closed circles indicate live occurrences.
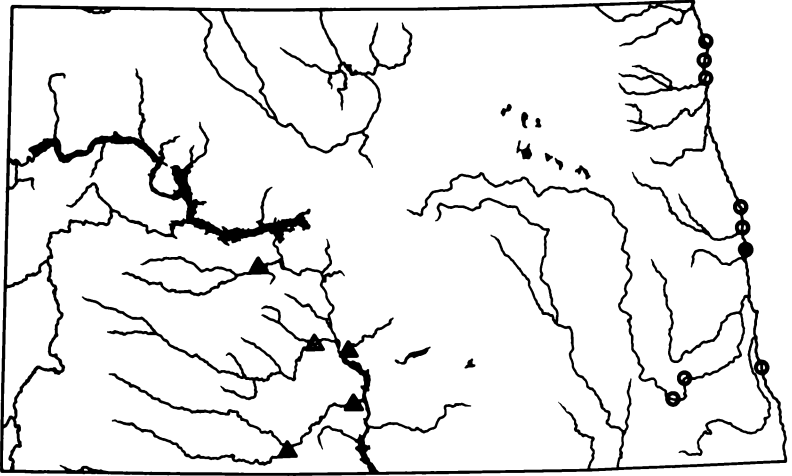
Figure 4. Distribution of two restricted mollusks, the mussels *Proptera laevissima* (Lea) (triangles) and *P. alata* (Say) (circles), in North Dakota. Open triangles and circles indicate dead occurrences and closed triangles and circles indicate live occurrences.

Mussels generally undergo a glochidial larval stage that is usually dependent upon a fish host. Dispersal occurs primarily during the larval stage and seems to require a direct stream connection for passage from one region to another (Van der Schalie, 1939), although mussels have been observed attached to birds and other animals (Kew, 1893:78-81,83). Pill clams and snails, however, do not have a host-dependent larval stage and are readily dispersed overland, perhaps chiefly by birds (Kew, 1893:46-47,51-54,81; Baker, 1945:39-40) and locally by other animals (Kew, 1893:61-77,84-89). Flooding between drainages and dispersal by man may, at times, affect the relative ease by which all groups of mollusks are moved about.

The oldest known Quaternary mollusks in the state are about 13,500 years old (Cvancara, Norby, and Van Alstine, 1975). Although aquatic mollusks existed in the state for many thousands of years, the present fauna is probably a relatively recent one: Quaternary geologic history of North Dakota includes several changes significant for molluscan occurence, including a modern drainage that bears little resemblance to earlier Quaternary patterns (Bluemle, 1972); and most mollusks can readily disperse into new areas if a means of introduction is available and ecological requirements are satisfied.

All the mussel species of North Dakota occur in the Mississippi River system to the south and east. Those of the Southwestern province were derived from that system as well as presumably those of the Northeastern province (Cvancara, 1970a:86-87).

The pill clams and snails of North Dakota generally occur throughout much of central and northern North America; they probably entered North Dakota from the east and south where more diverse assemblages of these mollusks exist today. Certain anomalous occurrences or nonoccurences, however, are known for these easily dispersed groups. For example, two snails, *Helisoma campanulatum* (Say) and *Bulimnea megasoma* (Say), inhabit western Minnesota but do not seem to occur in North Dakota. *Helisoma campanulatum*, however, lived in the state on the Missouri Coteau at least as recently as about 9,000 years ago (Tuthill, 1967:74,78).

Ecological factors affecting molluscan distribution

Because aquatic mollusks are generally readily dispersed (but mussels less so than pill clams and snails), the distribution of species tends to be limited by the degree of adaptation of individual species to various ecologic factors. Widely adaptable species tend to be cosmopolitan (Figure 2); less adaptable species tend to be sporadic (Figure 3) or restricted (Figure 4).

Physical factors.—Availability of suitable water body, especially as related to size and permanency, is presumably a primary physical factor, because certain types of water bodies are inhabited by certain species (Table 1). Mussels probably have not been found in the Intermediate province because of the lack of streams serving as avenues of dispersal. Discharge is an indicator of stream size and permanence. Small and large streams contain characteristic species, as well as species common to both; and, more species of mussels occur in the larger streams. Intermittent streams (with long periods of no discharge) tend to be inhabited by composite assemblages of flowing and stagnant-water forms; mussels, however, are lacking or usually represented by a single species, *Anodonta grandis*. Size and permanency of stagnant water bodies also relates to certain aquatic molluscan assemblages, and this relationship is suggested provincially (Figure 5). The Northeastern province has the most species because it contains the greatest variety of suitable water bodies—both flowing and stagnant.

Bottom instability may be a limiting factor in distribution; it may explain the apparent lack of mollusks in the Missouri and Little Missouri Rivers. Water quality seems satisfactory and mollusks occur in the tributaries of both rivers where the bottoms are relatively stable. Mollusks, too, occur in Lake Trenton, and oxbow lake of the Missouri, and they may inhabit certain backwater areas. Elsewhere in the state, I have seen mollusks only rarely on a shifting, unstable bottom. In the Little Missouri, erratic discharge and periodic, high turbidity may contribute to the apparent lack of mollusks. High turbidity may cause the scarcity of certain mollusks in the lower reaches of other streams, as the tributaries of the Red River (Cvancara, 1970:83), but the highly turbid Red River proper is generally rich in mussels.

Chemical factors.—High total chloride content seems to relate to fewer
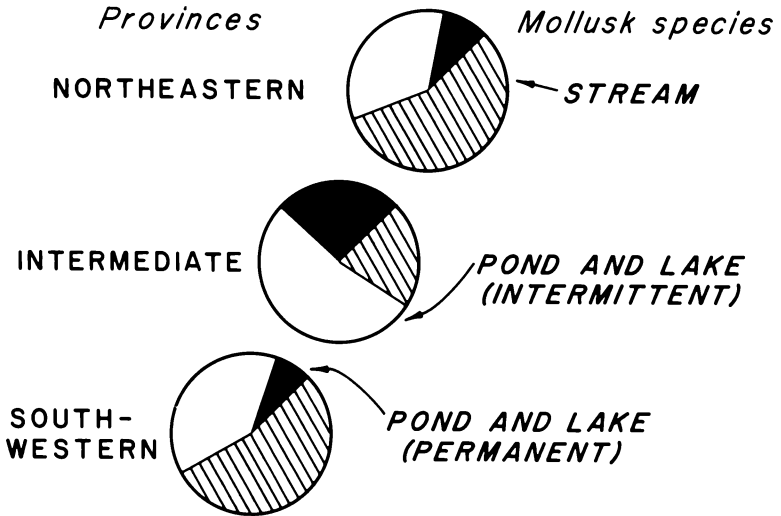
Figure 5.   Percentages of predominantly stream, intermittent pond and lake, and permanent pond and lake species of mollusks for each province in North Dakota. Italicized habitat occurrences in Table 1 are the basis for this figure.

species of mollusks. Mussels were few or absent in the lower reaches of the Turtle, Forest, and Park Rivers (Northeastern province) where the chloride content is high (Cvancara, 1970:83,87); pill clams were similarly affected. I have not collected live mussels or pill clams where the total chloride content was greater than 160 mg/l. Snails, however, are more tolerant; I have collected one snail species (*Physa integra*) where the total chloride content was nearly 2200 mg/l. Imlay (1973) believed that the potassium ion is toxic to mussels and perhaps more so than the chloride ion (p. 109). He pointed out (p. 108) that in the lower Park River, where the chloride content was high and mussels were absent, the potassium content was also high.

High total sulfate content may also relate to fewer mollusks; here, also, snails seem to be more tolerant of salts. I have not collected live mussels or pill clams where the total sulfate content was greater than 1300 mg/l (Southwestern province), but two snail species were present in water with a total sulfate content of about 3600 mg/l (Northeastern province). Waters in the state are generally highly alkaline (pH generally about 7.5-10) and the generally high total alkalinity and total hardness suggests sufficient carbonates for shell secretion.

Biological factors.—Fish host may be a significant biological factor for mussel distribution in the state. Regionally, this probably accounts for the presence of mussels in the Missouri River tributaries although these mollusks do not seem to inhabit the river itself. The restricted occurrence of *Proptera laevissima* or *Prop-*

tera alata (Figure 4) may be related to an appropriate fish host; however, more information on mussels and their hosts is needed before this can be determined. Locally, the concentration of mussels may be related more to the place where larvae leave the fish host than bottom sediment type (Cvancara, 1970a:88). Predation, parasitism, and other biological factors are presumed to be of lesser importance.

Man's activities.—Alteration or destruction of habitat, pollution, and drainage changes are among man's activities that are ecologically limiting to mollusks and affect their distribution. The building of dams (such as Baldhill on the Sheyenne River) encourages stagnant-water species at the expense of flowing-water forms; habitat may also be altered or destroyed by channel dredging or straightening (as in the Forest River), or by the draining of ponds and lakes.

Water pollution affects the occurrence of mollusks and is restrictive to mussels in parts of the Red River Valley (Cvancara, 1970a:88). It appears to be also restrictive to mussels at Minot. At the downstream edge of the city, 1.8 mussels (one species) were collected (1968) per hour. About 7 river miles upstream from the city, 144 mussels (three species) were collected (1967) per hour. Other mollusks are not always adversely affected (nine species of pill clams and snails at Minot and six species above it) and may be tolerant of or thrive on organic pollution, as the limpet *Ferrissia rivularis* (Basch, 1963:430).

Man's alteration of drainage patterns could have marked effects on the occurrence of mollusks. The irrigation Garrison Diversion Project in North Dakota will result in the mixing of the waters of the Missouri and Red River drainages. Certain mussels will presumably pass from one drainage to another on their fish hosts. Seven mussels characteristic of the Northeastern province (Table 1) might move into the Southwestern province, and one presently unique to the Southwestern province may pass into the Northeastern province—provided that certain ecological requirements are met. Will these and other possible changes in the species distributions tend to homogenize the molluscan fauna of the state?

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STUDIES ON HELMINTHS OF NORTH DAKOTA. VI. *CENTROVARIUM LOBOTES* (MAC CALLUM, 1895) IN THE FLESH OF THE COMMON SHINER MINNOW, *NOTROPIS CORNUTUS* (MITCHILL)

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ABSTRACT

In eastern North Dakota, metacercariae of the trematode *Centrovarium lobotes* often encyst in the flesh of the common shiner minnow, *Notropis cornutus* (Mitchill). Host-parasite relationships revealed cysts with an inner hyaline layer and an outer fibrous connective tissue wall. This wall was continuous with the connective tissue of the host's perimyseal septa. Relative proportions suggest that the hyaline layer develops before the outer wall. Granular degeneration of the fibrous wall was noted ofte. Incidence of infection was high (97.5%) with intensity variable (1-269 worms/infected fish). The longest and heaviest size classes of fish possessed the most worms, indicating accumulation of parasites with age. Progenesis was noted in 1.2% of the metacercariae examined.

INTRODUCTION

The trematode *Centrovarium lobotes* (Cryptogonimidae) is widely but sporadically distributed from the Midwest eastward to Quebec and New York. Hoffman (1967) listed adult flukes as occurring in the digestive tract of several genera of piscivorous fish (*Esox, Ictalurus, Anguilla, Ambloplites, Micropterus, Perca,* and *Stizostedion*). Of these, the northern pike, *E. lucius* L., smallmouth bass, *M. dolomieu* Lacépède, walleye pike, *S. vitreum* (Mitchill), and sauger, *S. canadense* (Smith), appear to be preferred hosts. The University of North Dakota Parasite Collection includes adult specimens of *C. lobotes* (738-742) taken from northern pike of the Turtle River in Grand Forks County, North Dakota.

Encysted metacercariae occur in the flesh of various small fishes, but especially in such cyprinids as *Notropis* and *Pimephales*. Locally, the common shiner minnow, *N. cornutus* (Mitchill), is often infected. The most westerly records of *C. lobotes* are from the Turtle (Hoffman, 1953,1967), Goose (Voth and Larson, 1968), and Forest Rivers (Woods, 1971) of eastern North Dakota. The snail host and cercarial stage are unknown.

This study concerns the tissue relationships between the encysted fluke and the host. Observations are also included on the incidence and intensity of infection in the common shiner minnow from the Forest River.

MATERIALS AND METHODS

Minnows were collected at Turtle River State Park near Arvilla, and from the Forest River near Inkster, North ·Dakota. Specimens were maintained until needed in aerated tanks at 10° C in a Sherer-Gillett environmental chamber.

Fish used for histological study were decapitated. Small pieces of infected muscle were preserved in AFA (alcohol-formalin-acetic acid), 10% formalin, or Bouin's fixative. Tissues were embedded in paraffin, sectioned at 10 microns, and stained with Mallory's triple connective tissue stain according to Humanson (1962).

Forty common shiner minnows were selected randomly and examined for incidence and intensity of infection. These fish were killed by severing the spinal cord immediately behind the head. Body lengths and filet weights were taken after removal of the head, tail and viscera. Subsequently, filets were carefully dissected in 0.6% saline to recover encysted flukes. These were sorted, counted and identified after vital staining in aqueous neutral red.

RESULTS AND DISCUSSION

Histological observations.—Cyst walls surrounding 10 randomly chosen metacercariae were measured and examined for histological structure. Light microscopy indicates that there are two layers: (1) an inner acellular, hyaline wall presumably secreted by the parasite and averaging 5.5 microns in thickness (range 1.8-17 microns), and (2) an external wall of host origin containing collagen fibers and fibroblasts (Figure 1). The outer layer was 3.0-35.7 microns thick, with an average of 13.4. The perimyseal connective tissue separating host muscle bundles was continuous with the outer cyst wall (Figures 2, 3). In some cyst walls the fibrous material appeared to be degenerating into a granular, amorphous layer (Figure 4). The cause of this deterioration is unknown, and apparently has not been noted for other species of encysted flukes.

There are structural similarities between the cysts of *C. lobotes* and those of several strigeoid trematodes occurring in fish. However, the literature indicates some disagreement and much variability in the timing and sequence of cyst wall formation. Hunter and Hamilton (1941) reported that the inner hyaline cyst formed 2-4 days after the metacercariae of *Uvulifer ambloplitis* (Hughes, 1927) Dubois, 1938 reached the perimyseal septa, with the collagenous outer cyst becoming apparent two weeks following exposure. For the same species held at 24° C, Hoffman and Putz (1965) reported the reverse sequence with the hyaline layer forming 9-14 days after the outer cyst. They also noted that cooler temperatures increased the time needed for cyst formation. According to Hoffman (1956), a similar sequence of development (i.e., host cyst followed by parasite cyst) occurs with *Crassiphiala bulboglossa* Van Haitsma, 1925. *Posthodiplostomum minimum centrarchi* (MacCallum, 1921; Dubois, 1936) Hoffman, 1958, and *Neogogatea kentuckiensis* (Cable, 1935) Hoffman and Dun-
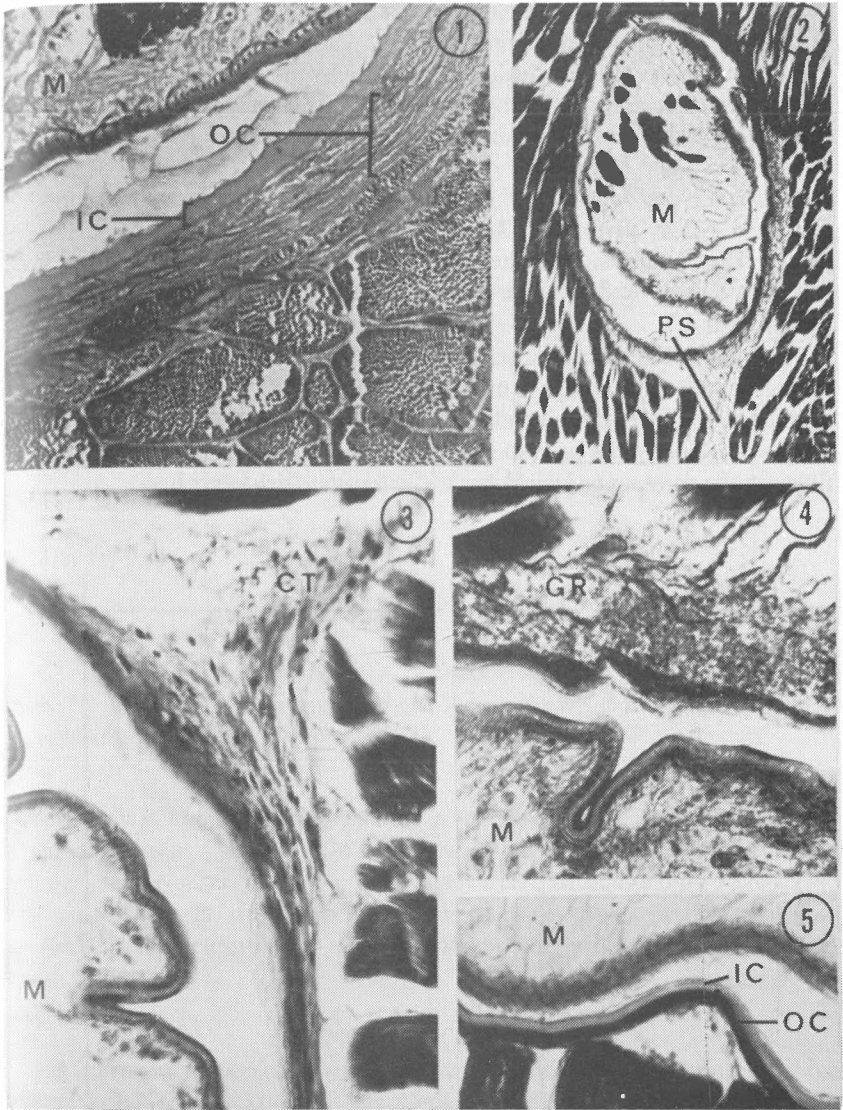
Figure 1. *Centrovarium lobotes* metacercaria (M) with inner cyst (IC) and outer cyst walls (OC). X530.   Figure 2. Metacercarial cyst (M) in association with perimyseal septum (PS). X55.   Figure 3. Continuity of host connective tissue (CT) with outer cyst wall. X465.   Figure 4. Granulation (GR) of outer cyst wall. X410.   Figure 5. Thin, early cyst walls. X625.

bar, 1963, are opposite examples wherein the parasite cyst precedes the host cyst (Hoffman, 1958; Hoffman and Dunbar, 1963). In sectioned material of *C. lobotes*, it appears as if the hyaline layer precedes the host cyst. This hypothesis is based on smaller cysts having hyaline and fibrous layers of nearly equal thickness (Figure 5), whereas larger and presumably older cysts have proportionately thicker host cysts (Figure 1).

Incidence and intensity of parasitism.—Thirty-nine of 40 common shiners from the Forest River (97.5%) possessed *C. lobotes* metacercaiae. These fish yielded 1,243 worms. The intensity of parasitism was 1-269 cysts per infected fish with a mean of 30.7. The average number of worms per gram of fish (filet weight) was 4.7.

Fish compared by size classes showed the highest intensity of parasitism in the heaviest and longest groups of minnows (Figures 6, 7). If one assumes that bigger minnows are older, these results suggest an accumulation of worms with age. Such a relationship has been shown by Chappell (1969) for metacercarial strigeoid flukes in the three-spined stickleback, *Gasterosteus aculeatus* L.
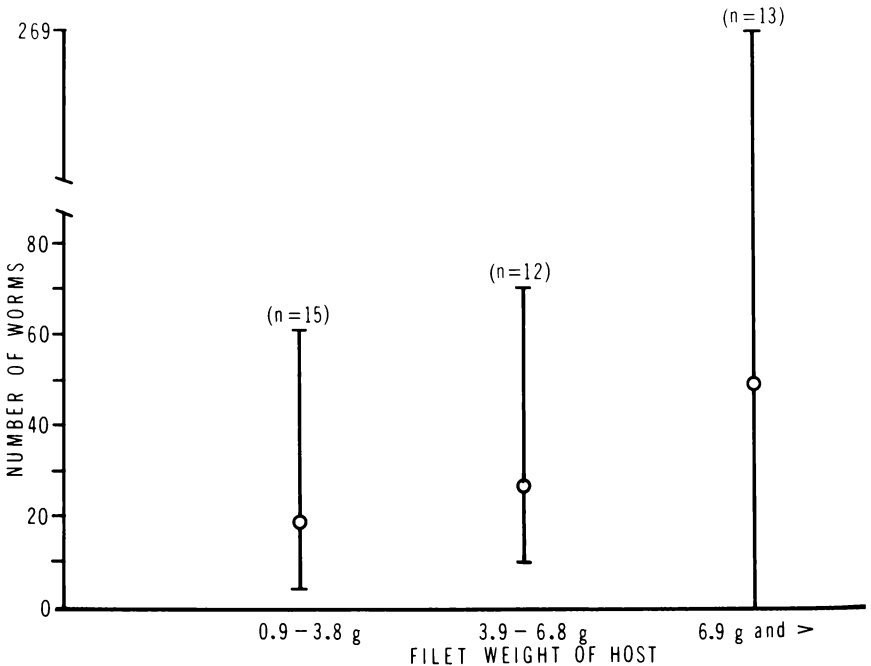
Figure 6. Range and mean number of *Centrovarium lobotes* per filet weight class of host fish.
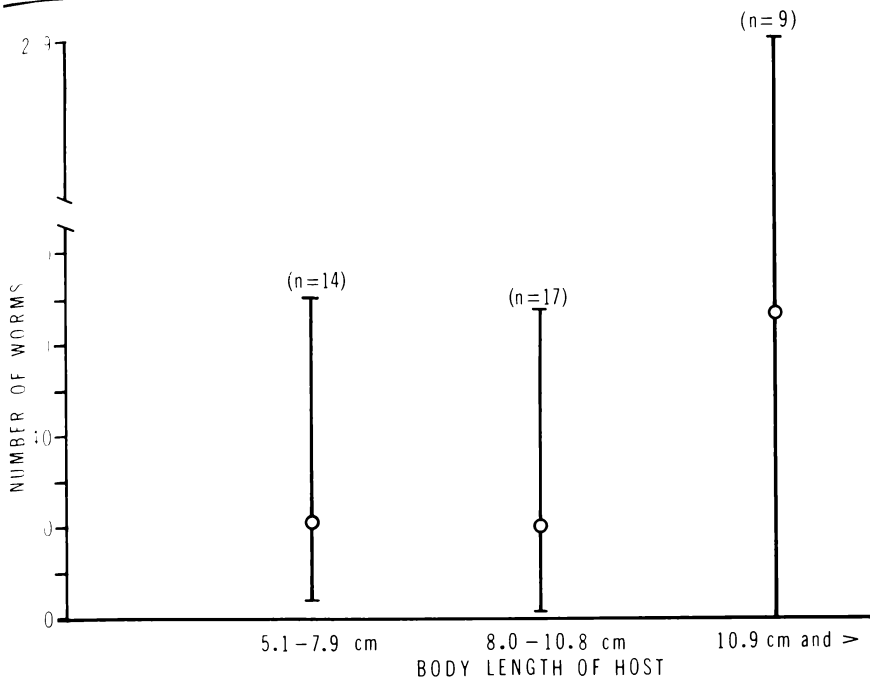
Figure 7.   Range and mean number of *Centrovarium lobotes* per body length class of host fish.

Progenesis is known to occur among some flukes'in at least 16 families of digenetic trematodes (Manter, 1967). Of the 39 infected minnows in this study, four harbored one or more progenetic metacercariae. Although only 15 of 1,243 worms possessed eggs, this precocious condition raises the interesting possibility of *C. lobotes* completing its life cycle in the absence of northern pike or other piscivorous hosts.

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SURFACE CONTOURS OF THE SUBARACHNOID SPACE IN THE DOG AS REVEALED BY SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Young dogs were perfused with buffered aldehydes at fluid pressures approaching normal physiological values. A silicone rubber compound (MICROFIL) was injected to prevent the collapse of thin-walled subarachnoid veins. Surface tissue facing on the subarachnoid space from the cerebral and cerebellar cortices, brainstem and spinal cord were prepared for scanning electron microscopy using critical point drying and gold-palladium coating.

Pia of cerebrum and cerebellum exhibit a multilayered cellular and fibrous organization. The pial surface is smooth, but connective tissue fibrils, blood vessels, macrophages, fibroblasts and free pial cells below its surface impart a wrinkled appearance. Fenestrations with associated macrophages and fibrils are observed on the pia of cerebral and cerebellar cortices. The arachnoid also has a multilayered arrangement of cells and connective tissue fibrils. However, the arachnoid is flatter, rougher and more fibrous than the pial surface. Surfaces of nerve exits, which may be double or single according to level, are very complex. Arachnoid membrane, trabeculae and fenestrations complicate observable surface contours. Numerous macrophages showing extreme pleomorphism were observed lying upon the meningeal linings. They are found on all areas lining the subarachnoid space.

INTRODUCTION

The subarachnoid space and its leptomeningeal linings have been studied extensively at both the light and transmission electron microscopic levels. Early light microscopists (Wislocki, 1932, 1938; Tilney and Riley, 1938; and others) accepted the existence of the subarachnoid space and other smaller fluid spaces, and interpreted these 'spaces as structureless voids with open communication. Later light microscopists (Patek, 1944; Woolam and Millen, 1954; Millen, 1963; Rosen et al., 1967) agreed with the existence of a subarachnoid space

freely available to flow of fluids, but objected to previous interpretations of the existence of smaller voids or spaces (reviewed by Frederickson and Low, 1969).

Transmission electron microscopic interpretation of the subarachnoid space and the cellular and extracellular elements lining this space has confirmed and refined these later light microscopic observations. The original electron microscopic description of meningeal fine structure, in which the pia mater was described as a single, thinly attenuated layer of cells, was done by Pease and Schultz (1958). Ramsey (1965) showed discontinuities in the pia mater, and Iida (1966) reported a variety of cellular and fibrous organizations of the pia of the cerebral and cerebellar cortices. Other investigators (Klika, 1966; Waggener and Beggs, 1967; Shabo and Maxwell, 1968, 1971; Anderson, 1969; Anderson and Hoyt, 1969) expanded on the description of the leptomeninges in a limited number of gross areas of the central nervous system. Morse and Low (1972) reexamined the pia mater at the fine structural level with emphasis on regional variation. They reported wide variations in cellular thickness, amount of extracellular connective tissue, and overall thickness of pia in regions of the brain and cord. Frederickson and Haller (1971) evaluated the subarachnoid space with respect to a fine structural classification of its leptomeningeal lining components. Their study resulted in an interpretation of the subarachnoid space as a modified portion of the connective tissues. This latter interpretation agreed with earlier fine structure studies (Waggener and Beggs, 1967; Frederickson and Low, 1969) in which the resemblance of leptomeningeal cells to connective tissue cells was pointed out. Prior to the conceptual interpretation of the subarachnoid space as part of the space containing the connective tissues (Low, 1964; Frederickson and Low, 1969; Haller and Low, 1971; Himango and Low, 1971; Haller et al., 1972) the subarachnoid space was generally described as a special epithelial-lined cavity similar to the coelomic cavity (Bloom and Fawcett, 1968).

With the advent of scanning electron microscopy (SEM), the next approach with respect to the investigation of the subarachnoid space is examination of the surface contours bounding this space at the scanning electron microscopic level. This study places emphasis upon subarachnoid macrophages and trabeculae of the brain and spinal cord, nerve root exits of the spinal cord and leptomeningeal cellular and extracellular elements of the pia-arachnoid of the brain.

MATERIALS AND METHODS

Seven young dogs of both sexes and varying ages (6-14 weeks) were anesthetized by either intraperitoneal or intrathoracic injection of 6.5% pentobarbital (0.5 cc-kg - 32.5 mg/kgm body weight) and perfused with buffered aldehyde solutions (Karnovsky, 1965) by techniques modified from Rosen et. al. (1965). The aldehydes were administered by inserting a large-gauge (no. 18) hypodermic needle directly into the exposed left ventricle of the heart. Initial fixation was obtained with a washout solution of a cacodylate-buffered para-

formalehyde-glutaraldehyde mixture. Procaine hydrochloride (0.1%) added to the washout solution (Forssman et al., 1967) prevented peripheral vaso-constriction. After allowing 400-500 ml of the washout solution to flow through the circulatory system, perfusion was continued with Karnovsky fixative (1500 ml). Then MICROFIL (100 ml; Canton Bio-Medical Products, Boulder, Colo.) was introduced by perfusion into the vascular system to prevent collapse of thin-walled subarachnoid veins.

The animals were decapitated, and the surface of the skull was stripped bare of soft tissue. For the opening of the cranial cavity, a dental drill was used to cut through the calvaria to expose the external surface of the dura mater. With the head immersed in fixative, the dissection was continued until the dural covering of the brain was free except on its ventral aspect. The brain was then lifted from the cranial cavity as the cranial nerves, infundibulum, and blood vessels were cut. Selected nervous tissue samples were taken from the brainstem and the cerebral and cerebellar cortices.

To obtain the spinal cord, nerve roots and nerve root exits, a laminectomy was performed along the full extent of the spinal column. A midline incision was made through the dura-arachnoid. Cutting the arachnoid trabeculae, denticulate ligaments, and nerve rootlets near their exits allowed removal of the intact spinal cord with attached nerve rootlets. Alternatively the nerve root exits were obtained by longitudinally bisecting the cord and vertebral bodies. Each half of the spinal cord was removed by cutting nerve rootlets close to the cord and severing denticulate ligaments. Nerve trunks were cut in the intervertebral foramina lateral to the dorsal root ganglia, and the remaining dura-arachnoid membranes with exiting nerve roots were pulled free. Selected samples of the spinal cord, nerve rootlets and nerve root exits were taken.

Lining tissues were prepared for SEM by post-fixing for half an hour in 2 OsO_4 buffered with 0.114 M sodium cacodylate (Karnovsky, 1965). Immersion of tissue samples in amyl acetate (15 min) was preceded by dehydration in an ascending alcohol series and followed by critical point drying in a Sorvall Critical Point Drying System (Sorvall Co., Newton, Conn.) connected to a cylinder of liquid CO_2. After drying, tissues were mounted on specimen stubs with Electrodag (an electrically conductive adhesive, Acheson Colloids Company, Port Huron, Mich.) and coated with carbon and gold palladium (Denton Vacuum DV-502 high vacuum evaporator, Denton Vacuum, Inc., Cherry Hill, N.J.). They were viewed in a Cambridge Stereoscan S4 Scanning Electron Microscope.

OBSERVATIONS

The subarachnoid space, through which cerebrospinal fluid circulates in life, is lined by leptomeningeal cells (actually connective tissue cells) and associated fibrils. SEM confirms that the connective tissue lining is more cellular than fibrous. Discontinuities exist in this leptomeningeal layer in the form of

fenestrations and gaps between leptomeningeal cells and their processes. Connective tissue fibrils appear in these and other areas of the subarachnoid space, but they are usually separated from it by an almost continuous layer of leptomeningeal cells. Macrophages appear in association with the lining cells and fibrils. The relationship of the subarachnoid space to the pia mater, arachnoid mater, and trabeculae, and the interrelationships of the meninges themselves are shown in Figure 1.
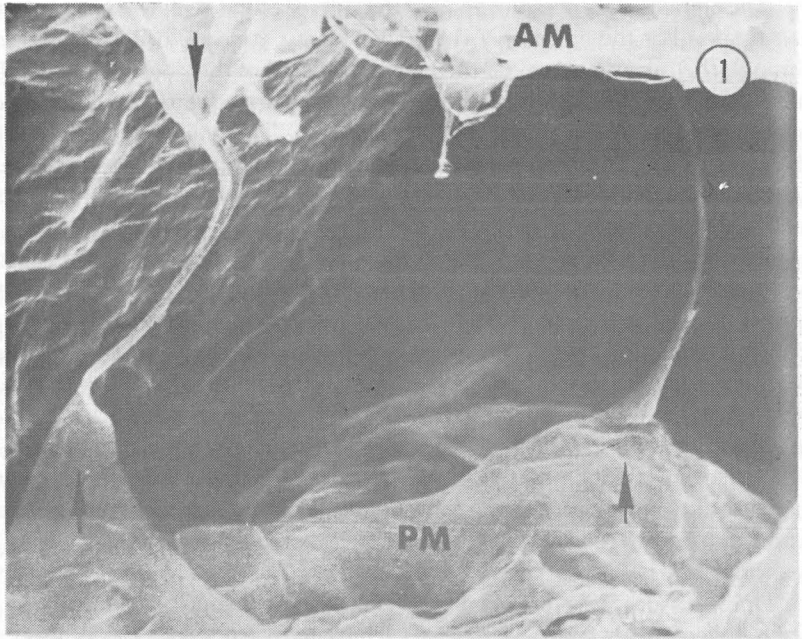
Figure 1.   Subarachnoid space. The arachnoid mater (AM) is above and the pia mater (PM) covering the brain is below. Arachnoid trabeculae traversing the subarachnoid space are formed by pial and arachnoid cell extensions (arrows) lifted away from the respective surfaces. (500X)

Pia mater.—The pia mater, forming the inner boundary of the subarachnoid space and the outer boundary of the brain and spinal cord, usually exhibits a multilayered cellular and fibrous organization (Figures 2, 4). The pial lining of the subarachnoid space, however, is predominately cellular (Figure 2). When fibrils are present, they are not always obvious. This portion of the cellular meninges is relatively smooth but fibrils, blood vessels, probable macrophages and other cellular elements below the surface give it an irregular (wrinkled) appearance or variation in surface relief. In certain areas of the pia, small fenestrations or concavities are observed in which fibrils and macrophages are
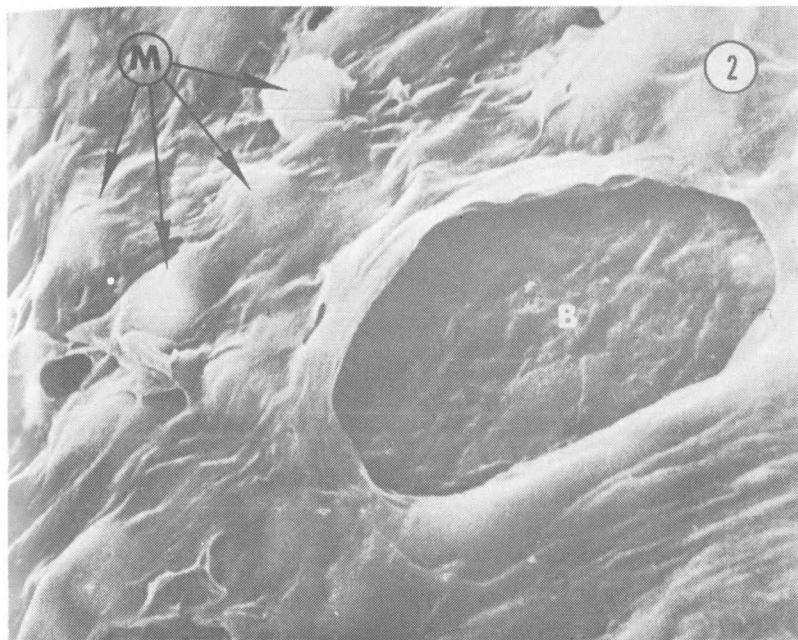
Figure 2.   Pia mater of the brain. Macrophages (M) above and below the pial surface border a fenestration that exposes the cerebellum (B). Smaller fenestrations are seen on this pial surface. (1,000X)

associated. Larger pial fenestrations often bordered by macrophages are observed in both the cerebral and cerebellar cortices (Figure 2. These fenestrations seldom have closely associated fibrils, and may offer a direct communication with the brain. In areas where the pial layer is incomplete or where fenestrations exist, macrophages, fibroblasts and free pial cells are observed among groupings of connective tissue fibrils (Figure 3). Deeper pial layers parallel the pial surface layer (subarachnoid space lining) with pial cells and pial connective tissue fibrils mingled between these layers (Figure 4).

   Arachnoid mater.—The arachnoid mater, forming the outer boundary of the subarachnoid space, has a multilayered arrangement of arachnoid cells and connective tissue fibrils. The inner layer of the arachnoid membrane lining the subarachnoid space is rougher and more fibrous (Figures 5, 6) than the opposite pial surface. Arachnoid cells and cellular processes constitute a layer with jagged cellular margins and fibrils producing an irregular, fibrous appearance (Figure 5). Adherent to or lying free upon overlapping arachnoid cells are macrophages (Figure 6). Concavities or small fenestrations similar to those on the pial surface are observed on the arachnoid surface (Figure 6). Deeper arachnoid layers appear
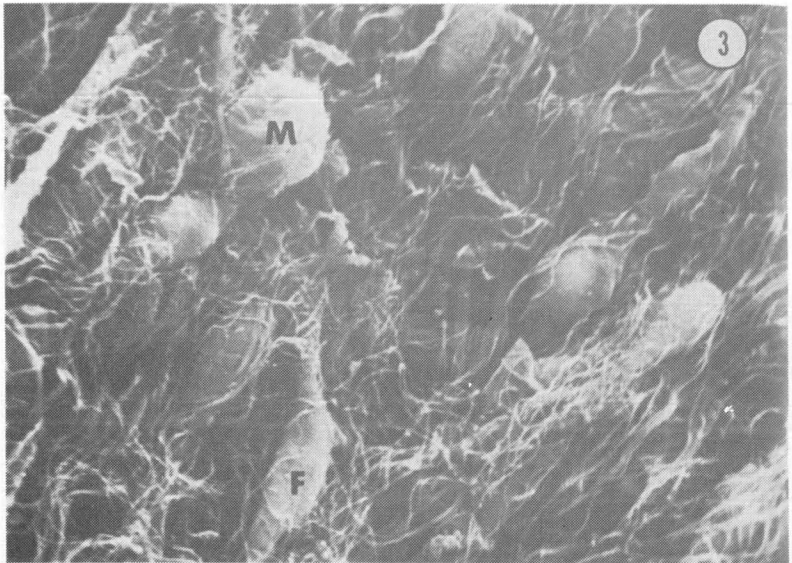
Figure 3.   Pial connective tissue elements. Macrophages (M) and fibroblasts (F) are situated among connective tissue fibrils. This arrangement was observed between cellular pial layers in the cerebral and cerebellar cortices. (1,000X)
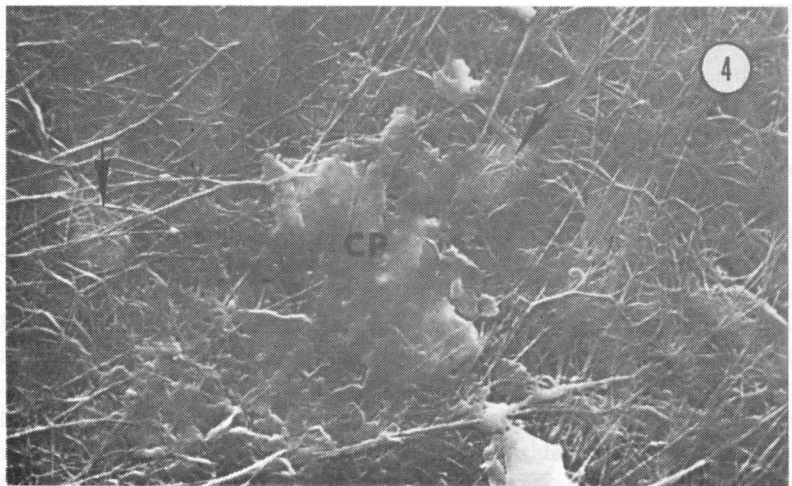
Figure 4.   Pia mater of the brain. Pial cells (CP) and pial connective tissue fibrils are mingled between layers of cellulr pia mater. The impression of individual pial cells (arrows) comprising a deeper pial layer is evident. (1,000X)
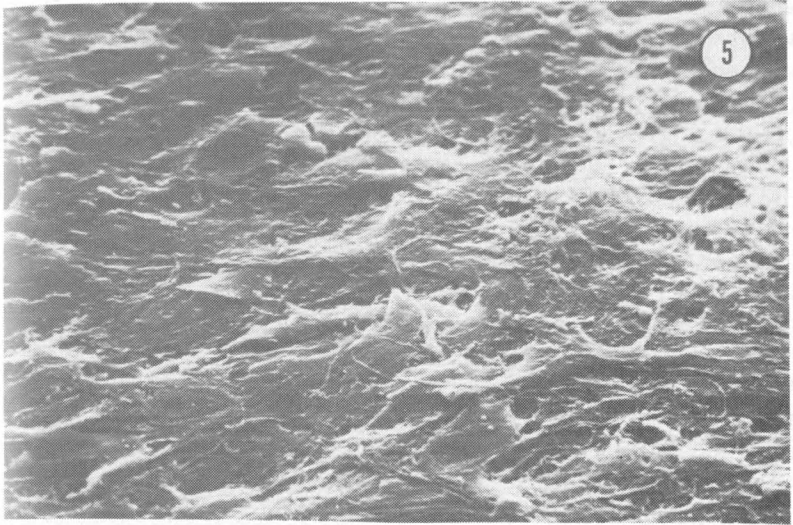
Figure 5.   Arachnoid mater. The inner layer of the arachnoid membrane, forming the outer margin of the subarachnoid space, is rough and fibrous. Arachnoid cells overlap to give a rough, scale-like appearance, and connective tissue fibrils extend onto the arachnoid surface to add to the irregularity of the surface. This area is devoid of trabeculae, but in adjacent regions, cellular processes and fibrils contribute to and are continuous with trabeculae and pia. (1,000X)
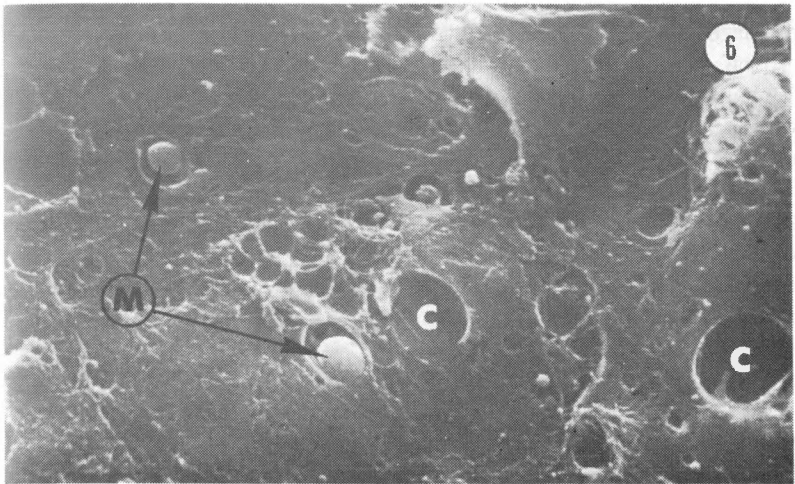
Figure 6.   Arachnoid mater. The outer boundary of the subarachnoid space in this area is cellular and fibrous with macrophages (M) and concavities (C). Notice the connective tissue fibrils in these concavities. (1,000X)

as laminae that approximately parallel the subarachnoid space layer. Between these laminae and among cellular processes, connective tissue fibrils abound.

Arachnoid trabeculae.—Arachnoid trabeculae traversing the subarachnoid space assume a variety of morphological shapes and patterns. Single spindle-shaped trabeculae (Figure 1) exist in certain areas, while in other areas strap-like and spindle-shaped ones are observed together (Figures 15, 16). In the cerebellum, and near the nerve root exits anastomosing sheet-like trabeculae with numerous fenestrations are found. This latter type is observed particularly where the pia and arachnoid are separated by a diminished subarachnoid space. Isolated delicate spindle trabeculae appear only in areas adjacent to regions where pia and arachnoid closely approach each other or come into contact to obliterate the subarachnoid space.

Cellular processes or extensions from both meningeal layers of the subarachnoid space contribute to and are continuous with the trabeculae. From opposing points on the pial and arachnoid surfaces, cellular extensions lift off to help form an arachnoid trabecula. These extensions are often sizeable hillocks that give added surface relief to both the floor and roof of the subarachnoid space. Macrophages are observed lying free on the arachnoid trabeculae.

Subarachnoid macrophages.—The macrophages associated with the subarachnoid space demonstrate extreme pleomorphism. They are mostly free within the subarachnoid space that permits them to assume any shape and move about upon the meningeal linings. They are found on all areas lining the subarachnoid space: pia mater, arachnoid mater, root sheath, arachnoid trabeculae, and adventitia of blood vessels. A general distribution of the macrophages upon the spinal cord pia is shown in Figure 7. There are areas on the spinal pia or nerve root sheaths that have greater concentrations of macrophages or absence of them. There seems to be no pattern to their distribution. In many instances these macrophages form an integral part of the meningeal lining and blend within its surface (Figure 9). The shapes of the macrophages vary from spherical or ovoid to long and slender (Figure 8). Their surface contours reveal variations ranging from entirely smooth to entirely ruffled or wrinkled (Figure 10). The surface of a macrophage may also be smooth in one place and ruffled or blebbed in another (Figures 9, 12). Most of the subarachnoid macrophages have cytoplasmic extensions or processes. Usually these touch the underlying meningeal surface (Figures 11, 12). The cytoplasmic processes vary from short, thick club-like pseudopods to long filamentous-like processes (Figures 11, 12). Occasionally macrophages connected through a cytoplasmic extension are observed (Figure 12).

Exit of nerve roots.—The point of exit of nerve roots from the subarachnoid space has a complicated arrangement of nerve rootlets, blood vessels and arachnoid. Figure 13 shows the point of exit of the fourth cervical nerve on the right side. The dorsal root is composed of three main rootlets. The ventral root is
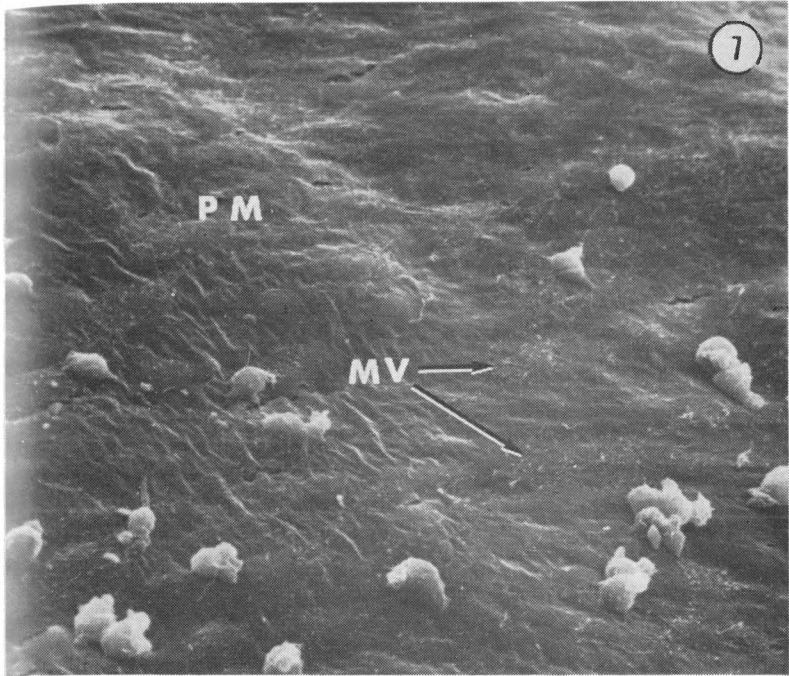
Figure 7.   Macrophages upon surface of spinal pia mater. The surface of the cellular pia mater (PM) is relatively smooth with patches of small microvilli (MV) evident (Figure 11). The macrophage population resting upon the pial surface shows the number and variety of these cells. (650X)
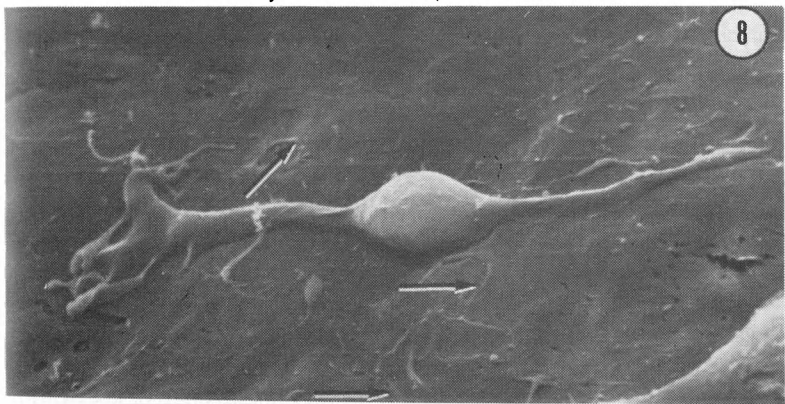
Figure 8.   Macrophages upon surface of spinal pia mater. Two long processes extend from a central dilation. The central dilation probably contains the nucleus. Flat overlapping pial cells are shown at the arrows. (2,200X)
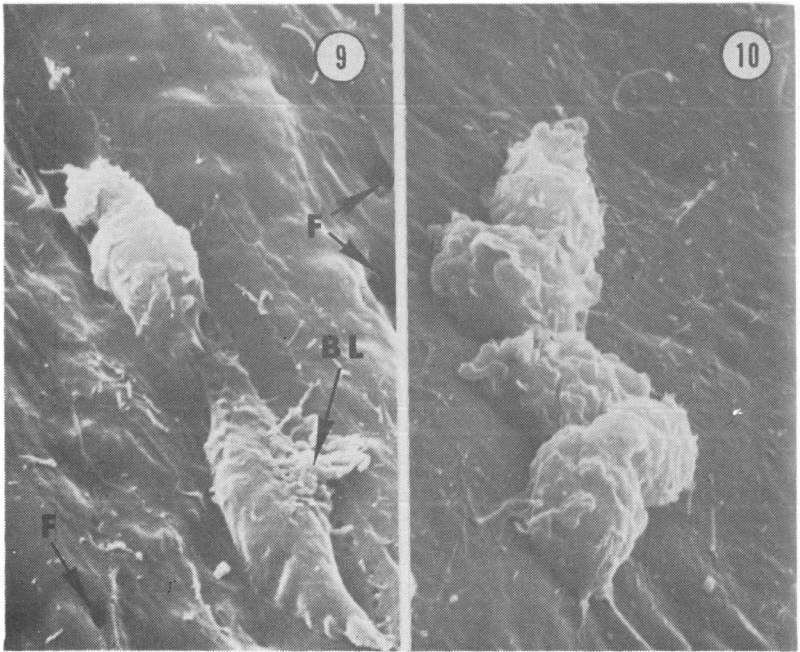
Figure 9.   Surface of nerve root sheath. Two macrophages are shown blending within the surface of the root sheath. The surface of the lower macrophage has both smooth and blebbing (BL) areas. Small fenestrations (F) exist in the root sheath surface. (2,300X)

Figure 10.   Macrophages upon surface of spinal pia mater.   Clusters of macrophages are frequently observed. These macrophages have ruffled surfaces and few cytoplasmic extensions. (2,200X)

composed of four main rootlets, the most caudal of these being subdivided into smaller units. A large blood vessel accompanies the ventral root through the exit. The adventitia of the vessel and the root sheath appear indistinguishable in this view.   The arachnoid is associated with the exiting roots in sheets containing fenestrations and numerous trabeculae. A modified attachment to the arachnoid resembling the attachments of the denticulate ligament runs between the dorsal and ventral roots (Figure 13). Figure 14 illustrates a somewhat similar but less complicated nerve exit. Figure 15 is the ventral exit at the level of C_5 on the right side. An artery and vein pass through the exit with two rootlets. Several trabeculae and a fenestrated arachnoid sheet are seen adjacent to the exit. Figure 15 shows nerve rootlets near their exit.   The dura-arachnoid lies under the rootlets.   Arachnoid trabeculae travel through the subarachnoid space from rootlet to rootlet and from the dura-arachnoid to the rootlets.   Part of the
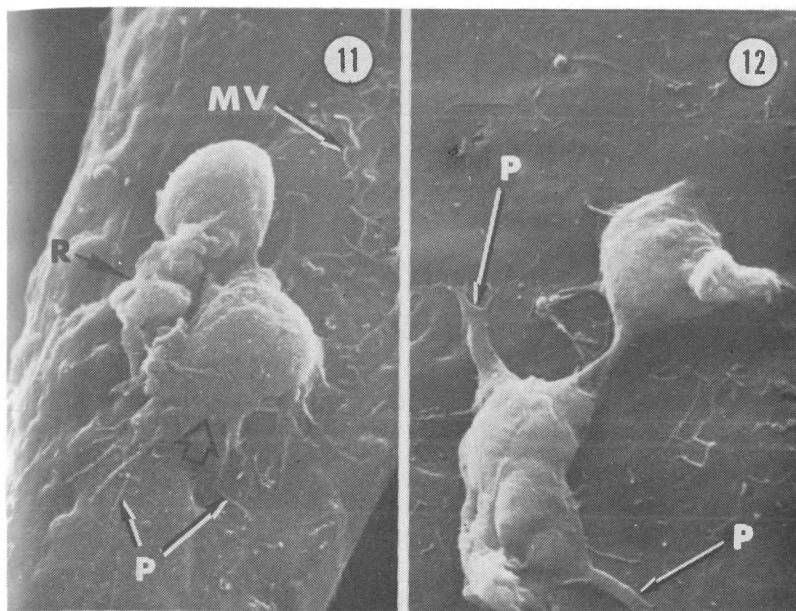
Figure 11.   Macrophages upon adventitia of small vessel. This macrophage is multilobed with mostly smooth surfaces. Some ruffling of the surface (R) is evident.   Continuity exists between the surface of the adventitia and the macrophage (large arrow). Filamentous-like processes (P) touch the underlying adventitia. Microvilli (MV) extend from the adventitia cells. (2,300X)

Figure 12.   Macrophages upon surface of spinal pia mater. Two macrophages are connected through a cytoplasmic process. Their surfaces are mostly smooth. Thick processes (P) touch the underlying meningeal surface. (2,400X)

arachnoid appears as a sheet with numerous fenestrations through it. Figure 16 shows arachnoid trabeculae attaching to the surface of a nerve root.   The trabeculae appear to become continuous with the outer layers of the root sheath.

RESULTS AND DISCUSSION

Previous studies of the subarachnoid space and its leptomeningeal linings employed light and transmission electron microscopy (TEM). Reported herein is a scanning electron microscopic general description of the surface contours of the subarachnoid space.   A more specific objective of this study was to obtain basic scanning data of the leptomeninges in this area.   In our laboratory, scanning electron microscopy is currently being employed to further expand the description of the leptomeninges by concentrating on regional variations in the central nervous system.
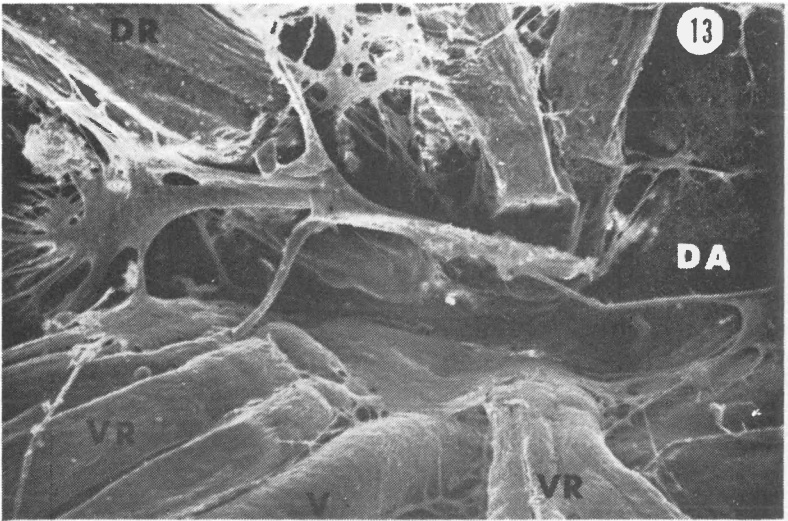
Figure 13. Nerve exit viewed from within the subarachnoid space. Dorsal nerve rootlets (DR) and ventral nerve rootlets (VR) approach the dura-arachnoid (DA) here nearly invisible through foreshortening. A blood vessel (V) accompanies the ventral root. (55X)
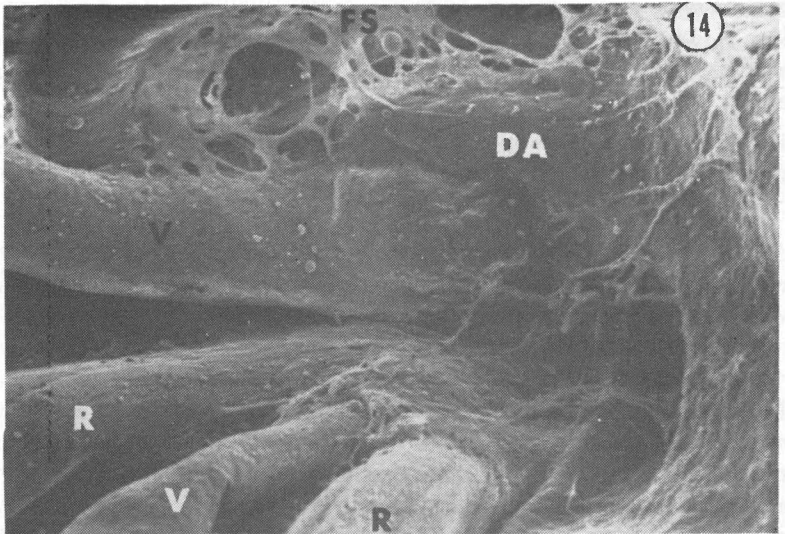
Figure 14. Nerve exit. Nerve rootlets (R) and blood vessels (V) leave the subarachnoid space through the exit. Arachnoid extends from the dura-arachnoid (DA) as a fenestrated sheet (FS) to the adventitia of a blood vessel. (130X)
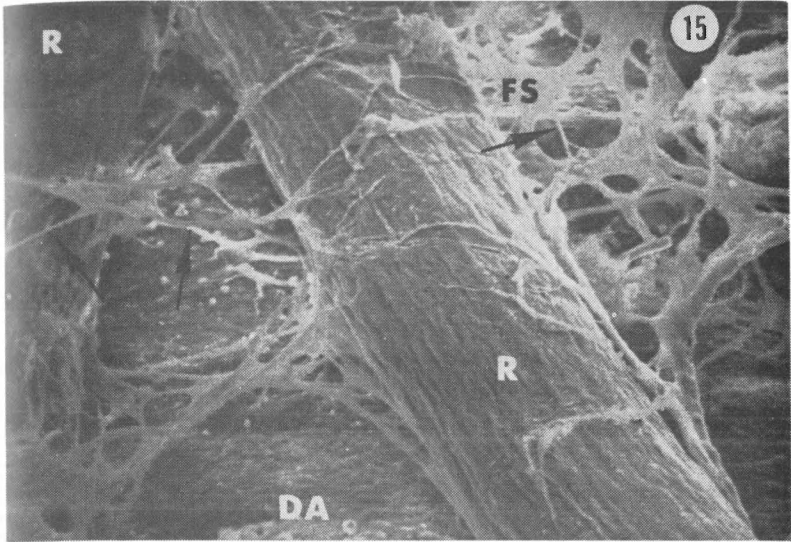
Figure 15. Nerve rootlet (R) passing through the subarachnoid space. Dura-arachnoid (DA) lies beneath the rootlet. A fenestrated sheet of arachnoid (FS) traverses the subarachnoid space. Arachnoid trabeculae (arrows) are also seen. (140X)
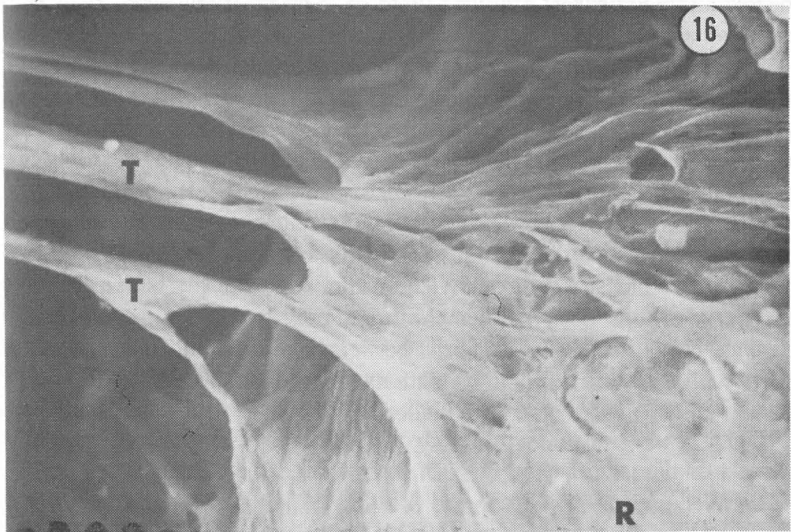
Figure 16. Arachnoid trabeculae (T). The trabeculae pass through the subarachnoid space and attach to the surface of a nerve root (R). The trabeculae appear to be continuous with the outer layers of the root sheath. (750X)

The SEM of soft biological tissues is considered still to be in the developmental stage, but preparatory techniques and methods (Boyde and Wood, 1969; Kimoto and Russ, 1969; Luce, 1970) referred to earlier in this report produced very acceptable tissue samples. Examination of the fine structure (with transmission electron microscopy) of some of the tissue samples prepared for scanning electron microscopy revealed excellent preservation.

The presence of arachnoid trabeculae throughout the subarachnoid space is well documented (Truex and Carpenter, 1969; Frederickson and Haller, 1971). However, light and transmission electron microscopy reveal trabeculae in a single plane and their actual extent can only be realized through serial sections using these methods. Scanning electron microscopy provides the investigator with an apparent but revealing additional dimension of depth as shown in Figure 13. In most areas of the subarachnoid space, arachnoid trabeculae arise from the pia as relatively uncomplicated pillars. The region of the nerve root that exits in the cervical region is characterized by a greater abundance of these trabeculae (Figure 15). Also, the arachnoid membrane in this area gives rise to broad fenestrated sheets. These may extend between dorsal and ventral nerve roots or may be draped between adjacent nerve rootlets. The sheets may attach directly to the outer layers of the root sheaths or may be attached by typical arachnoid trabeculae. Blood vessels that are found in the vicinity of the nerve exits have similar associations with the arachnoid. This study reveals a much more complicated arrangement of the arachnoid at cervical nerve exits than might be suspected with light and transmission electron microscopy.

This study also confirms that the pia mater, which formally was considered a complete cellular barrier between the subarachnoid space and the central nervous system, has fenestrations. Such discontinuities observed in the cerebral and cerebellar cortices and on the spinal cord agree with fine structural findings for certain gross areas of the central nervous system (Frederickson and Haller, 1971; Morse and Low, 1972; Nelson et al., 1962; Ramsey, 1965; Rovainen et al., 1971). Ramsey reported central nervous tissue extensions (cerebral cortex) into the subarachnoid space through fenestrations on the pial surface, but in our study, projections from neither the brain nor the cord were observed. The presence of connective tissue fibrils between pial cells and the pial surface also agrees with fine structural interpretations (Waggener and Beggs, 1967, Ross and Burkel, 1971; Frederickson and Haller, 1971).

Light and transmission electron microscopy have previously shown subarachnoid macrophages in both normal and experimental studies (Essick, 1920; Wislocki, 1932; Pease and Schultz, 1958; Nelson et al., 1962; Klika, 1967; Frederickson and Haller, 1971; Morse and Low, 1972). However, the great numbers of macrophages seen in this SEM study could not be ascertained from light or transmission electron microscopy. Also, the extreme variation in gross morphology of these cells could not be shown well prior to an SEM study.

From investigations of others there is good evidence showing that many of the macrophages develop from the leptomeningeal cells. Studies of inflammation in the subarachnoid space have shown that the leptomeningeal cells respond to challenging conditions (Essick, 1920; Waggener and Beggs, 1967; Nelson et al., 1961). TEM studies have shown cells with characteristics intermediate between those of leptomeningeal cells and the macrophages (Frederickson and Haller, 1971). The present study with SEM agrees with these findings, showing many examples of macrophages appearing to rise out of the meningeal surface and in various degrees of morphological transformation.

The areas lining the subarachnoid space are explored herein by SEM for the first time. SEM provides a three-dimensional image of the surface of a tissue sample and enables the authors to describe aspects not before observed. The extent and shape of intact leptomeningeal cells, the complex arrangements that vessels and nerve rootlets form, and the extent and sizes of meningeal fenestrations are all revealed more clearly with SEM. Close examination with SEM of the surface contours of the leptomeningeal membranes bounding the subarachnoid space confirms and adds new information to previous light and transmission electron microscopic studies.

ACKNOWLEDGMENTS

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INHIBITION OF AMINOTRANSFERASES BY ASCORBIC ACID IN THE FROG *RANA HEXADACTYLA*

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ABSTRACT

The effect of different concentrations of ascorbic acid on aspartate and alanine aminotransferases was studied in different tissues of the frog *Rana hexadactyla*. The aspartate aminotransferase activity was greater than the alanine aminotransferase activity in all of the tissues studied. In general, both the aminotransferases were inhibited by ascorbic acid in all of the tissues except muscle, the extent of inhibition being different from tissue to tissue.

INTRODUCTION

There are several reports on changes of enzyme activity by administration of ascorbic acid both in vitro and in vivo (Manson, 1967:386; Subbarami Reddi, 1969; Nakashima et al., 1972). However, the mechanism of interaction has not yet been clearly elucidated. In the present investigation we report the in vitro effects of different concentrations of ascorbic acid on aspartate and alanine aminotransferases in various tissues of the frog *Rana hexadactyla*, in order to delineate whether these tissues respond similarly to ascorbic acid.

MATERIALS AND METHODS

The central nervous system (CNS) of the frog *Rana hexadactyla* was destroyed by pithing and tissues of the brain, heart, liver, kidney and muscle were quickly excised with least injury. They were washed thoroughly in amphibian Ringer's medium (Cavanaugh, 1956) and kept for 15 min to recover from shock effects. They were homogenized in 0.25 M sucrose and the homogenate was centrifuged at 2000 x g for 15 min. The supernatant was used for the enzyme determination.

Aspartate aminotransferase (AAT: EC 2.6.1.1) and alanine aminotransferase (AlAT: EC 2.6.1.2) activities were assayed by the colorimetric methods

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of Bergmeyer and Bernt (1965:837, 846). The reaction mixture contained in a total of 2.0 ml:100 μ moles of phosphate buffer at pH 7.4, 100 μ moles of L-aspartic acid (substrate for AAT) or 50 μ moles of DL-alanine (substrate for A1AT), 2 μ moles of α-ketoglutarate and 0.20 ml of tissue supernatant. The experimental tubes received different concentrations of ascorbic acid. After incubation at 37°C the amounts of oxaloacetate or pyruvate formed from the substrates were measured on a Bausch and Lomb colorimeter at 545 nm. The enzyme activity is expressed as μ moles of sodium pyruvate per milligram protein per hour.

Protein content was estimated by the method of Lowry et al. (1951) using bovine albumin as standard. All operations were done at 45° C.

RESULTS

The AAT activity was found to be highest in the heart followed by brain, muscle, liver and kidney (Table 1). All concentrations of ascorbic acid inhibited the brain and muscle enzyme significantly. However, the heart enzyme was unaffected at lower concentrations, but significantly inhibited at higher concentrations (75 and 100 μ moles). Addition of 10 μ moles of ascorbic acid did not affect liver and kidney enzyme. However, the extent of inhibition varied from tissue to tissue.

Table 1.   Effect of ascorbic acid on aspartate aminotransferase activity.

| Tissue | Control | μ moles of ascorbic acid | | | | |
		10	25	50	75	100
Heart	5.26[A] ± 0.83	4.96 ± 0.48	4.76 ± 0.21	4.40 ± 0.27	4.25 ± 0.17	3.96 ± 0.57
Brain	3.83 ± 0.15	2.93 ± 0.15	2.62 ± 0.15	1.83 ± 0.56	1.87 ± 0.31	1.79 ± 0.06
Muscle	3.46 ± 0.39	2.31 ± 0.01	2.27 ± 0.28	1.67 ± 0.48	1.59 ± 0.46	2.22 ± 0.35
Liver	2.83 ± 0.31	2.68 ± 0.53	1.96 ± 0.27	1.83 ± 0.26	1.77 ± 0.37	1.76 ± 0.37
Kidney	2.76 ± 0.17	2.11 ± 0.56	1.97 ± 0.47	1.99 ± 0.59	1.88 ± 0.55	1.77 ± 0.74

[A] Activity is expressed as μ moles of sodium pyruvate/mg protein/h. Each value is an average of four observations with one standard deviation.

Table 2.   Effect of ascorbic acid on alanine aminotransferase activity.

Tissue	Control	μ moles of ascorbic acid				
		10	25	50	75	100
Kidney	2.30[A]	1.93	1.54	1.42	0.91	1.09
	±	±	±	±	±	±
	.045	0.21	0.38	0.60	0.24	0.008
Heart	1.74	1.58	1.07	0.34	0.48	0.48
	±	±	±	±	±	±
	0.33	0.15	0.20	0.03	0.07	0.07
Brain	1.10	0.75	0.78	0.78	0.45	0.50
	±	±	±	±	±	±
	0.14	0.04	0.07	0.07	0.07	0.00
Liver	0.99	1.15	0.70	0.42	0.49	0.37
	±	±	±	±	±	±
	0.19	0.21	0.22	0.10	0.12	0.05
Muscle	0.47	0.51	0.41	0.39	0.38	0.39
	±	±	±	±	±	±
	0.03	0.06	0.07	0.08	0.08	0.10

[A]Activity is expressed as μ moles of sodium pyruvate/mg protein/g. Each value is an average of four observations with one standard deviation.

The maximum inhibition of the enzyme by ascorbic acid was observed in the liver extract and the minimum in the heart extract. In general, the inhibition gradually increased as the concentration of ascorbic acid was increased.

The AlAT activity decreased in tissues in the following order: kidney, heart, brain, liver, and muscle (Table 2). Like the AAT activity, the AlAT activity was also inhibited by ascorbic acid in all of the tissue extracts except in the muscle where the enzyme activity was not altered even in 100 μ moles of ascorbic acid. Higher concentrations of ascorbic acid (50-100 μ moles) elicited 30-70% inhibition of the enzyme in all of the tissue extracts except in the muscle. Of all the tissues, the heart enzyme was inhibited most by ascorbic acid.

DISCUSSION

It is evident from Tables 1 and 2 that the AAT activity is several fold greater than the AlAT activity in all tissues of the frog, suggesting the possible predominance of aspartate metabolism over alanine metabolism. It seems probable that aspartate is more actively metabolized in the heart and brain than in other tissues. In the kidney, the importance of metabolic role of aspartate and alanine seems to be almost equal as is evidenced by similar activities of the two aminotran-

sferases. The interaction of ascorbic acid with the enzyme varies from tissue to tissue as in the muscle. Sincs ascorbic acid inhibits the aminotransferase activity, it is likely that the kinetics of these enzymes are also altered. Such studies are under investigation.

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EFFECTS OF STARVATION ON AMINOTRANSFERASES IN THE FROG *RANA HEXADACTYLA*

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ABSTRACT

Effects of starvation un the levels of aspartate and alanine aminotransferases were investigated in frog *(Rana hexadactyla)* tissues. Activity of both the aminotransferases increased significantly on starvation. Such an increase was maximum in liver tissue followed by muscle and brain tissue. Protein content in brain tissue remained unaltered whereas a decrease was observed in liver and

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muscle tissue during the entire period of starvation. Blood and liver glucose concentration was not changed in the initial periods of starvation. In comparison to protein degradation, reduction in the blood and liver glucose was greater indicating the participation of these aminotransferases in gluconeogenesis during starvation.

INTRODUCTION

Starvation is an extreme nutritional condition during which a number of metabolic parameters are drastically altered. It has been reported that several enzyme activities are increased or decreased during starvation (Freedland and Szepesi, 1971:103-140). Extensive investigations have been carried out in the rat as compared to other vertebrates. Since total free amino acid levels are changed during starvation in the frog, it is of interest to study the enzymes associated with its metabolism. It is well established that the transamination reaction is an important pathway in amino acid metabolism. Of particular importance are aspartate and alanine aminotransferases, whose products form the key intermediates of other metabolic pathways such as carbohydrates (Knox and Greengard, 1965:247-313). Thus these aminotransferases function at the junction of carbohydrate and protein metabolism. Since the frog *Rana hexadactyla* seems to undergo a natural starvation, it was felt pertinent to study the pattern of aspartate and alanine aminotransferases in the frog subjected to starvation under laboratory conditions.

MATERIALS AND METHODS

Individuals of the frog *Rana hexadactyla* were collected from ponds around Tirupati, India and were fed earthworms and cockroaches. After three days of feeding, they were starved for 12 days. At an interval of every two days, four to five frogs were killed by pithing tissues from the brain, gastrocnemius muscle and liver were quickly excised. They were kept in amphibian Ringer's medium (Cavanaugh, 1956) for about 10 min to recover from shock effects. They were then homogenized in 0.25 M sucrose and the homogenate was centrifuged at 2000 x g for 15 min and the supernatant was used for the enzyme assay.

Aspartate aminotransferase (AAT:EC 2.6.1.1) and alanine aminotransferase (A1AT:EC 2.6.1.2) activities were determined by the colorimetric methods of Bergmeyer and Bernt (1965:837,846). The reaction mixture contained in a total volume of 2 ml was: 100 μ moles of potassium phosphate buffer at pH 7.4, 100 μ moles of L-Aspartic acid or 50 μ moles of DL-alanine, 2 μ moles of α-ketoglutarate, and 0.2 ml of the enzyme material. The amounts of oxaloacetate or pyruvate formed after incubation were measured at 545 nm with a Bausch and Lomb colorimeter. The enzyme activity is expressed as μ moles of sodium pyruvate per mg protein per g. The protein content was estimated by the method of Lowry et al. (1951). Bovine albumin served as the standard. Blood glucose was determined colorimetrically by the method of Mendel et al. (1954). Liver glucose was estimated following the procedure of Kemp and Kits Van Heijningen (1954).

All the observations were subjected to statistical validity by calculating the Student's significance test. The probability levels above 0.05 were considered nonsignificant.

RESULTS

As shown in Figure 1, the aspartate aminotransferase activity was highest in the brain followed by muscle and liver. In the brain, the enzyme activity was significantly elevated from the sixth day of starvation, whereas in the muscle and liver it was increased from the fourth day of starvation. The maximum increase of enzyme activity was observed in the liver followed by the muscle and brain.

The alanine aminotransferase activity is depicted in Figure 2. Unlike the aspartate aminotransferase activity, the alanine aminotransferase activity was
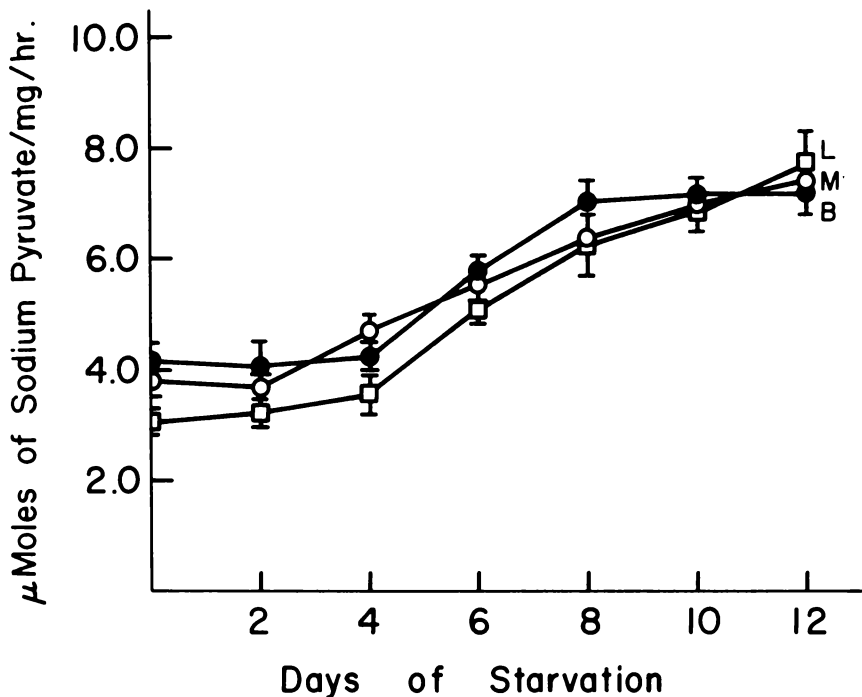
Figure 1. Aspartate aminotransferase activity in the brain (B), liver (L) and muscle (M) of normal controls and starved frogs. Each point represents an average of four observations with one standard deviation.

almost the same in the brain and liver with lowest activity in the muscle. On starvation, the brain enzyme was unaffected until the eighth day, but increased significantly during the rest of the starvation period. In the muscle also the enzyme activity remained unaltered until the sixth day, followed by a gradual in-
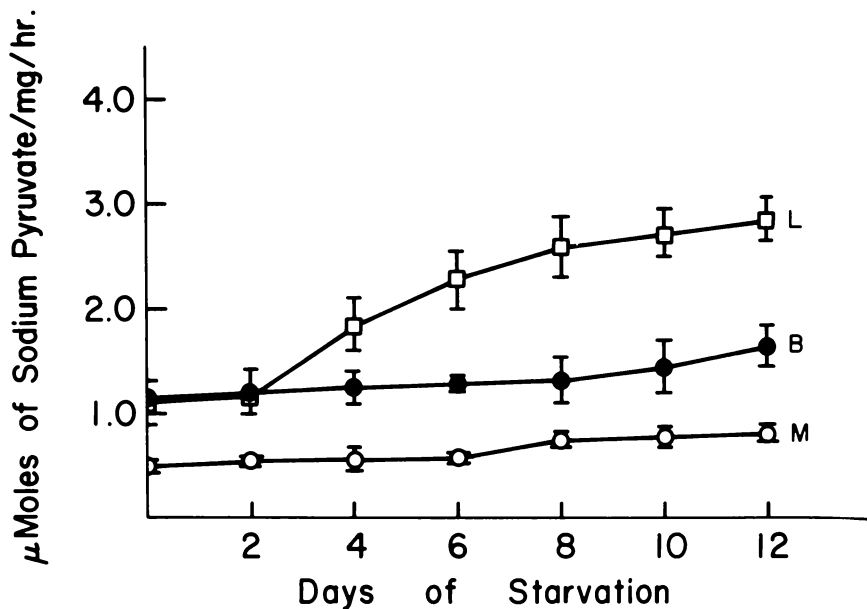
Figure 2. Effect of starvation on alanine aminotransferase activity in the brain (B), liver (L) and muscle (M). Each point represents an average of four observations with one standard deviation.

crease during the other days of starvation. In the liver, a significant increase in the enzyme activity was observed from the fourth day of starvation.

The protein content in normal controls and starved frogs is shown in Figure 3. In the brain, the protein content was unaltered on all days of starvation, whereas in muscle and liver it decreased with the days of starvation. The maximum decrease was observed in the liver rather than in the muscle. Since protein content in the liver was found to be decreased by about 55%, it was felt essential to study the concentration of other organic compounds such as glucose, which is an immediately available energy source during starvation in most animals. The glucose content in liver and blood is given in Table 1. There was no change in blood glucose level until the sixth day of starvation. In the liver, no change in glucose concentration was observed until the fourth day of starvation. Following these days there was a gradual reduction in glucose concentration both in the blood and liver.

DISCUSSION

The predominance of AAT activity over A1AT activity in all tissues is in conformity with the previous findings in other animals (Subbarami Reddi and Swami, 1970; Subbarami Reddi, 1973; Subbarami Reddi and Swami, 1974).
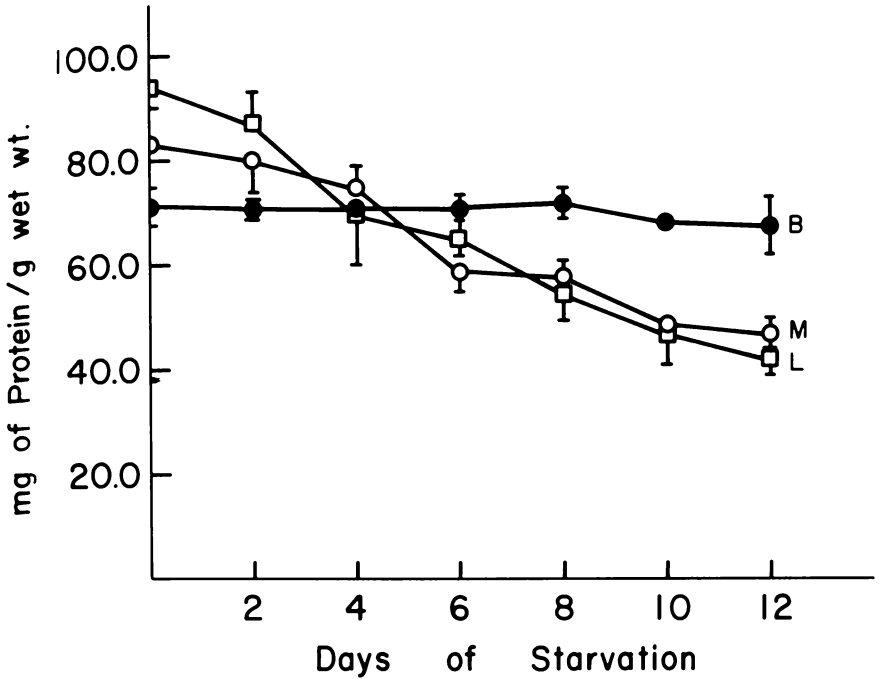
Figure 3. Protein content in the brain (B), liver (L) and muscle (M) of normal controls and starved frogs. Each point represents an average of four observations with one standard deviation.

Table 1. Glucose concentration in the blood and liver of the frog during starvation.

Tissue	Control	Days of starvation					
		2	4	6	8	10	12
Blood	61.50	61.00[A]	55 50	53.00	48 50	45 74	42 50
(mg/100 ml)	±	±	±	±	±	±	±
	7.55	4.16	5 51	2.58	4.43	4 03	3 82
		NS	NS	NS	P<0.05	P<0 01	P<0 001
Liver	28.75	29 50	25.00	23.00	20 75	19 25	18 25
(mg/g wet wt)	±	±	±	±	±	±	±
	2 99	5.00	4 76	2.58	2 50	2 50	4 03
		NS	NS	P<0.05	P<0.001	P<0 001	P<0 001

[A] ich observation represents an average of four values with one standard deviation. NS denotes a value nonsignificant at the 0.05 probability level.

suggesting that the metabolism of aspartate is more prevalent than the metabolism of alanine. Amongst tissues, the brain showed the highest AAT activity indicating that aspartate is actively metabolized to yield glutamate, which is a neurohumor. Since glutamate and aspartate are interconvertible and the former seems to be the only amino acid that is actively metabolized in the brain (Harper, 1972), it may be suggested that the highest AAT activity is associated with the active metabolism of glutamate and aspartate. It seems probable that these dicarboxylic amino acids are of similar importance in the muscle also. However, in the liver glutamate or aspartate metabolism appears to be less important as is evidenced by the lowest enzyme activity. Since the A1AT activity was found to be similar in both the brain and liver, it appears that the metabolism of alanine is the same in these tissues. But in the muscle the metabolism of alanine may be less significant when compared to the brain and liver.

On starvation, all of the tissues showed an increase in the AAT activity. No starvation effects were observed during the initial periods of starvation. Since the concentration of free amino acids increases during starvation in the frog (Subbarami Reddi, unpublished observations), it is presumed that more of the free amino acids may have been available for stimulated catalysis by the enzyme. In order to substantiate these results, the protein content was determined during the days of starvation. It was found that the protein concentration was decreased in the liver and muscle with increasing starvation. However, in the brain the protein content was unaltered on all days of starvation. The reason for such an increase in enzyme activity irrespective of protein breakdown is unknown.

As for the AAT activity, the A1AT activity was also stimulated during starvation in all of the tissues studied. These results support the previous findings of other investigators (Brin and McKee, 1956; Rosen et al., 1959; Waldorf et al. 1963) who reported a severalfold increase in the hepatic A1AT activity in the starved rat. Since these aminotransferases are known to contribute some of the strategic substances such as α-ketoglutarate, pyruvate, oxaloacetate and glutamate for various synthetic reactions (Knox and Greengard, 1965) it was thought essential to investigate whether these enzymes are involved in gluconeogenesis during starvation. Consequently, the glucose concentration was measured in the liver and blood. During the initial periods of starvation glucose levels were not changed in the liver or blood. In comparison to protein degradation, the reduction in blood and liver glucose concentration was low. Hence, it may be suggested that the products of these aminotransferases may stimulate gluconeogenesis during the initial periods of starvation. Also it may be suggested that in the frog *Rana hexadactyla* the utilization of proteins rather than glucose is primary for survival during starvation.

ACKNOWLEDGMENTS

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A COMPATIBILITY-LINKED, HAPLO-LETHAL FACTOR IN RACE 1, *USTILAGO NIGRA* (TAPKE)

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INTRODUCTION

Ustilago nigra (Tapke, 1932), the causal organism of semi-loose smut of barley, *Hordeum vulgare* L., infects barley plants during the seedling stage and produces sori in the inflorescence and occasionally in the flag leaf.

Details of the meiotic process in *U. nigra* are unknown. However, Fischer and Holton (1957) reported that in a similar species, *Ustilago avenae* (Pers.) Rostr., the diploid nucleus migrated into the promycelium during teliospore germination. After meiosis I, a cross wall immediately formed between the daughter nuclei and they migrated toward different ends of the promycelium. These two nuclei each underwent meiosis II, and cross walls developed between daughter nuclei to form a four-celled promycelium, each cell being haploid and uninucleate. The spore acted as the fourth cell when only three cells were visible in the promycelium. Each haploid cell of the promycelium then produced a haploid sporidium by budding if the teliospore was germinated on enriched nutrient media.

Occasionally, haploid sporidia separated from the promycelium will not continue to bud on nutrietn media. These sporidia are called haplo-lethals. Cases of haplo-lethal deficiencies have been reported for *U. bullata, U. kolleri, U. nuda* and *Tilletia foetida* (Fischer, 1940; Hoffman, 1973; Holton, 1951; Grasso, 1955; Nielsen, 1968). In most of these cases the deficiency was closely associated with mating type. This study was undertaken to determine the inheritance pattern of the haplo-lethal factor occurring naturally in race 1, *U. nigra.*

MATERIALS AND METHODS

The least virulent race of *U. nigra*, race 1, was selected for selfing studies. Race 1 is highly virulent on only one differential barley variety, Odessa (C.I. 934). One hundred eighty teliospores of race 1 were germinated on 2% potato dextrose agar (PDA). Spores placed on PDA and incubated at 20°C required 12 h to develop a promycelium and four sporidia. The four sporidia were then removed in order from the promycelium and numbered. The sporidium from the promycelial cell farthest from the teliospore was always numbered 1, the next closest was numbered 2, and so on with the sporidium from the promycelial cell closest to the teliospore being numbered 4. The sporidia were removed from the promycelium to separate areas on the same PDA plate and allowed to form colonies. These

haploid colonies were transferred to V-8 juice agar (Tuite, 1969). Sporidia that failed to divide beyond the third to fifth mitotic division were stained with corophosphine O (Jensen and Kiesling, 1971) and examined using ultraviolet microscopy to determine their nuclear condition.

Mating type for each colony was determined by a modified Bauch test (Bauch, 1922). A carrot agar, consisting of 200 ml of commercially canned carrot juice, 800 ml distilled water, 20 g agar and 3 g $CaCO_3$, was used as a medium. Mating type in *U. nigra* is controlled by a single factor pair, and two plus and two minus sporidia are formed when a teliospore germinates. Two sporidia of opposite mating type must fuse to form a dikaryon before infection can occur. Haplonts of known plus or minus mating types from race 3, *U. nigra* were Bauch tested with each haplont of race 1 to determine mating type of the sporidial cultures. A control mating between the two known mating types from race 3 was made on each petri plate.

Infection studies were carried out using the susceptible barley cultivar, Odessa, to verify the results of the Bauch tests. All dikaryotic and monokaryotic lines from the 180 teliospores of race 1 used for tetrad analysis were applied to the coleoptiles of Odessa seedlings via the paint brush technique (Jensen, 1971). The inoculated seeds were grown to maturity under 16-h day lengths at temperatures of $22 \pm 2°C$ in the greenhouse. If smutted heads developed on the inoculated seedlings, the two sporidial lines were confirmed as being compatible. If no smutted heads developed, the lines were either incompatible or monokaryotic.

Sporidia that failed to divide beyond the third to fifth mitotic division were transferred from the PDA plates to plates of complete medium (Beadle and Tatum, 1945 and Holliday, 1961). The plates were incubated at 20°C for several days to determine if further growth occurred. A single sporidium from each of several thousand germinating teliospores was randomly isolated, and 2,000 sporidial lines which grew normally were maintained. Each of these 2,000 sporidial lines was Bauch tested, and random samples of these matings were tested on Odessa for virulence.

RESULTS AND DISCUSSION

While performing tetrad analyses of race 1 teliospores, it was noted that two of the four sporidia always failed to form colonies beyond the fifth mitotic division. Staining these haplo-lethal sporidia with corophosphine 0 demonstrated that they contained a single nucleus. All of the sporidial colonies which continued to divide had the same mating type and gave a positive Bauch test only when mated with a culture of race 3+, *U. nigra*. The matings which gave a positive Bauch test also infected Odessa barley whereas the unmated sporidial cultures and the negative Bauch test matings did not.

The ratio of plus, haplo-lethal to minus, normal sporidia per teliospore was always 1:1. Segregation for the compatibility and haplo-lethal factors occurred in

both meiotic divisions, but the plus mating type and haplo-lethal factor were always associated. Six sporidial segregation groups are possible with two compatibility factors and four sporidia (Table 1). The order of the sporidial genotypes from the distal end of the promycelium to the teliospore will be determined by the meiotic division in which segregation occurs and the occurrence of cross overs. Cross overs were detected in 121 of the 180 teliospores analyzed as evidenced by the genotype and location on the promycelium of each sporidium in a tetrad. Since plus mating type and haplo-lethal were always associated, it was impossible to detect two-, three-, or four-strand double cross overs. However, the number of teliospores classified in each segregation group was approximately equal (Table 1), which indicated that either double cross overs had little effect upon the distribution of sporidial genotypes or they occurred with equal frequency.

Table 1. Chi square test for goodness of fit for hypothesized 1:1 of 1:1:1:1 ratio of sporidial segregation groups in germinating teliospores of race 1, *Ustilago nigra*.

Meiotic division	Sporidial Segregation group[A]	Observed	Expected	Chi square	P
I	1,2	29	29.5	0.00847	
I	3,4	30	29.5	0.00847	
		59		0.01694	0.75-0.90
II	1,3	29	30.25	0.05165	
II	1,4	32	30.25	0.10124	
II	2,3	31	30.25	0.01909	
II	2,4	29	30.25	0.05165	
		121		0.22363	0.95-0.99

[A]Sporidia with minus compatibility and normal growth factors

Table 2. Number of sporidia in parental or recombinant classes of sporidial segregation groups from tetrad analyses of teliospores of race 1, *Ustilago nigra*.

		Classes	
	Total spores	Parental	Recombinant
Meiosis I	236	236	0
Meiosis II	484	242	242

The close association of the plus mating type and haplo-lethal factors was demonstrated further when all 2,000 randomly isolated sporidia, which grew normally, had the minus compatibility factor. Since only one sporidium was selected from each teliospore in this random population and at least half of the sporidia selected did not grow, these 2,000 sporidia were selected from a population of about 4,000 teliospores. No recombinants of the compatibility and haplo-lethal factors were found. The linked haplo-lethal and compatibility factors were estimated to be at least 33.6 map units from the centromere (Table 2) and no more than 0.05 map units apart.

The haplo-lethal sporidia did not grow on the complete media tested which indicated that the expression of the haplo-lethal gene remains unknown, but it is not expressed as an inability to synthesize one or more amino acids.

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STUDIES ON CORN LEAF SURFACE BACTERIAL FLORA[1]

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ABSTRACT

The population size, species diversity, and seasonal variation of the bacterial microflora on the leaves of North Dakota-grown corn (*Zea mays* L.) were examined. The influence of an herbicide, 2,2-dichloropropionic acid (dalapon), on this flora was investigated. Leaf surfaces were sprayed with dalapon at a rate of 1.43 kg/ha. Upper leaf surface cultures were made by the Rodac plate method before and after dalapon treatment. Samples were taken using two bacteriological media, trypticase-soy agar and Beijerincks' nitrogen-free medium. Aerobic, heterotrophic bacteria were unevenly distributed within the area sampled on the leaf surfaces. A range of 1 - 1000 colonies/cm² was observed on the Rodac plates with an overall summer average of 38 colonies/cm². Qualitative and seasonal changes in the bacterial flora were evident throughout the summer and the predominating flora were chromogenic gram positive rods. Dalapon treatment significantly altered the numbers of bacteria present on the leaf surfaces.

INTRODUCTION

The environment of plant leaf surfaces is called the phyllosphere. Here, algae, bacteria, fungi, and insects interact with the plant and with each other to form a microbial community that may be unique to each plant and each environment. The most extensive studies of the phyllosphere have been conducted by Ruinen (1961, 1970) in Africa, and by Leben (1961), Leben and Daft (1964), and Leben et al. (1968) in Ohio. There is no published study on phyllosphere organisms in North Dakota.

The purpose of this study was to isolate, identify, and enumerate the aerobic, heterotrophic bacterial flora of corn upper leaf surfaces; to observe changes in this flora over the growing season; and to determine whether the flora is modified by a surface-applied herbicide.

MATERIALS AND METHODS

The methods used were designed to recover the aerobic, heterotrophic bacteria to the exclusion of other types of microflora that may be present. Impression

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cultures of upper leaf surfaces of corn (*Zea mays* L., experimental strain B569) were made in the summer of 1972 on 6.0-cm-diameter replicate organism detection and counting plates (Rodac R). Corn was selected because it has leaves large enough for the sampling technique. Media used in Rodac plates were trypticase-soy agar containing 1 g/1 yeast extract and 50 mg/1 cyclohexamide, and Beijerincks' nitrogen-free medium (Aaronson, 1970) for the isolation of *Azotobacter*.

Individual corn leaves were sprayed by hand atomizer three times over the 1972 growing season with 1.43 kg/ha 2,2-dichloropropionic acid (dalapon), supplied as the 85% active ingredient field formulation with wetting agent. This herbicide was chosen because its degradation by microorganisms has been studied extensively (Kearney et al., 1965) and because the properties of the formulated material lend themselves to this method of spray application. One leaf on each of ten separate plants was sprayed with the aqueous filter-sterilized dalapon field formulation. One leaf on each of ten different plants was sprayed with sterile water to serve us controls.

Total leaf surface area in an experimental corn plot at North Dakota State University was estimated by measuring total leaf surface area of a 9-plant subplot, and the quantity of herbicide applied per unit area was calculated on this basis. The upper surface area of each leaf to be treated was determined the day before spraying. The rate of dalapon applied per unit area of plot remained constant over the summer. Since the leaf surface area increased two-fold, due to plant growth, the rate of dalapon applied to individual leaves decreased proportionately.

Cultures were taken the day before spraying, one day after spraying, and one week after spraying, unless otherwise indicated. In July, the largest fully expanded leaf was sprayed and bacterial samples were taken with Rodac plates. In August, this leaf and one younger leaf of the same plant were treated in the same fashion. In September, the two previous leaves and one younger leaf were treated likewise. Plates were incubated for 48 h at 28°C and counted at magnifications of 7 to 15 X.

Additional sets of cultures were made by pressing three trypticasesoy Rodac plates, one immediately after the other, on the same area to determine the proportion of flora that was removed from the leaf surface by one impression plate.

Bacteria were identified according to Bergey's Manual (Breed et al., 1957). Climatological data were obtained from the North Dakota State University Agroclimatic Station.

RESULTS

Table 1 shows the density of bacterial colonies that were recovered by the Rodac method on trypticase-soy agar medium. These results indicate a relatively constant population of aerobic, heterotrophic bacteria from early July to September.

Figure 1 is a frequency distribution polygon where the results from all control leaves were combined for all sampling dates. The range of colony densities was 1 - 1000 per cm². This distribution is skewed to the right and has no zero class. Also, the standard deviation increased with the arithmetic mean (Table 1). For these reasons a logarithmic transformation was performed on the data so that a t-test of significance could be applied (Sokal and Rholf, 1969).

There was a rise in bacterial numbers on July 10 and August 23 on both control and treated leaves. However, the rise in bacterial numbers on control leaves was significantly greater than that of dalapon-treated leaves. Although there was no increase in bacterial numbers in September, dalapon-treated leaves had significantly fewer bacteria after spraying than the controls.

There was a rise in bacterial numbers on July 10 and August 23 on both control and treated leaves. However, the rise in bacterial numbers on control leaves *throbacter* with pale orange colonies. Other chromogenic *Arthrobacter*, coryneform rods and *Bacillus* were present. By July 27 the previously dominant, orange *Arthrobacter* sp. had become a minor floral component and was absent in August. After late July, *Bacillus polymyxa* was a characteristic part of the flora. Also present were other *Bacillus, Streptomyces*, chromogenic *Arthrobacter*, and

Table 1. Arithmetic and harmonic means of bacterial colonies/cm², recovered from the same position on the same leaves of field-grown corn, sprayed with 1.43 kg/ha dalapon or with water as a control, during the summer of 1972, by the Rodac method, using trypticase-soy agar.[A]

Date sampled	Date sprayed	Control		Treated	
		Arithmetic mean	Harmonic mean	Arithmetic mean	Harmonic mean
7-8	7-9	52± 67	18.2	32± 34	15.7
7-10		89± 70	70.3	110±158	47.5[D]
7-18		31± 37	17.8	25± 37	14.2
8-9		25± 27	17.4	34± 51	23.8
	8-14[B]				
8-15		74± 60	54.7	37± 46	25.4[D]
8-23		132±128	91.3	73± 63	51.5[D]
9-21		43± 25	36.6	46± 50	35.0
	9-22				
9-23		52± 73	35.8	23± 22	17.5
9-30[C]		—		no sample	—

[A]Data are the average for 10 leaves.
[B]Spraying delayed 5 days due to excessive rain.
[C]Leaves dead by this date.
[D]Significantly different from the controls at the 0.05 level.
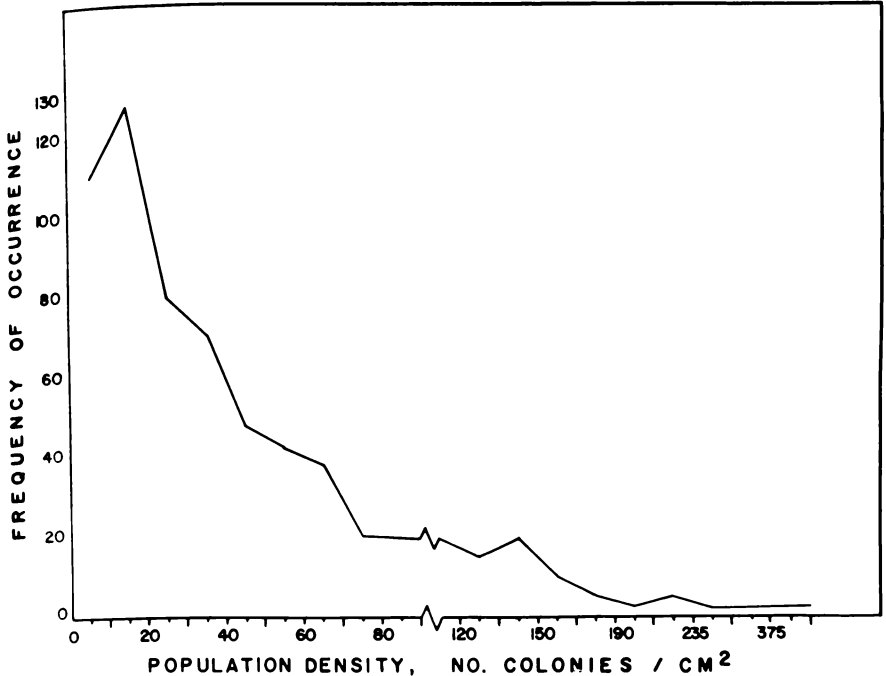
Figure 1. Frequency distribution polygon obtained by combining the numbers of bacterial colonies on trypticase-soy Rodac plates from all control upper leaf surfaces of North Dakota field-grown corn for all sampling dates in 1972.

coryneform rods. No single bacterial species was dominant after late July. In August, *Bacillus pumilus* and *Bacillus circulans* were characteristic members of the flora. *B. polymyxa*, chromogenic *Arthrobacter*, other coryneform rods, and *Nocardia* were also present. In September, the genus *Bacillus* predominated, with the species *B. polymyxa*, *B. lichiniformis*, *B. circulans* (two strains), *B. pulvifaciens*, and *B. pumilus*. Other *Bacillus* spp. were present but have not been identified. A few chromogenic *Arthrobacter* and other coryneform rods were also found. The additional leaves sampled in August and September showed the same numbers and kinds of bacteria in the same ranges of density.

Two general patterns of results were obtained when three trypticase-soy Rodac plates were placed, one after the other, on the same leaf location. The results were as follows: (1) successive plates recovered the same numbers and kinds of bacteria, or (2) the second or third plate recovered bacteria in much greater abundance that were of a different type than the preceding plates (Figure 2).

Azotobacter spp. were found on all corn leaves at each collection.
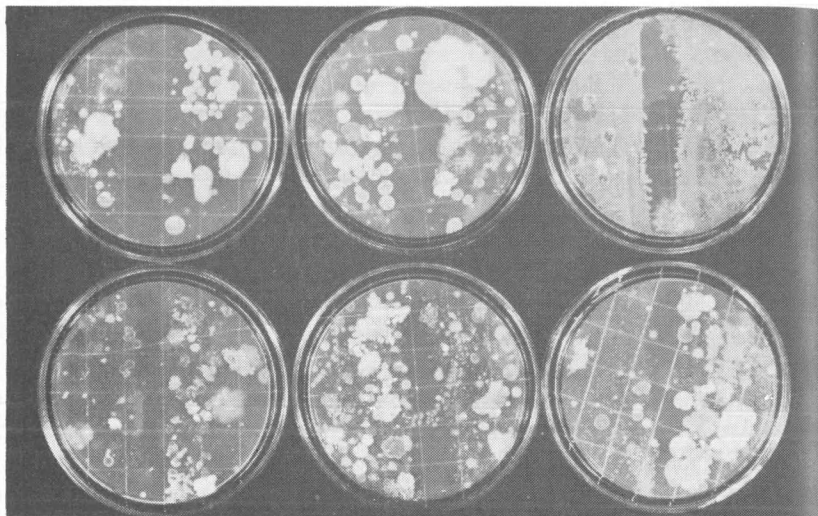
Figure 2. Bacteria recovered from trypticase-soy Rodac plates applied serially to the same corn leaf surface, July 27, 1972. Left to right: first (1), second (2), and third (3) plates taken. Top row: (1)$\bar{x}$=11 colonies/cm^2, (2) 20 colonies/cm^1, (3) 1000 colonies/cm^2. Bottom row: (1) $\bar{x}$ = 99 colonies/cm^2, (2) 104 colonies/cm^2, (3) 113 colonies/cm^2.

DISCUSSION

When the numerical results of this study were combined over the entire season, we found the distribution did not follow a normal pattern, but was skewed. The bacteria present are dependent on the occurrence of other organisms or microcolonies, and on the habitat. The species of plant on which the bacteria reside, the relationships between the leaf and light, temperature, wind, relative humidity, rainfall, insects, and other factors all play a part in the micro-environment in which the bacteria exist. Such factors as the presence or absence of leaf hairs, waxes on the leaf surface, chemical composition or physical relief of the leaf surface (Martin and Juniper, 1970), and secretions of organic or inorganic components by the plant, all influence the ability of the microflora to become established.

Arthobacter spp. are known to secrete amino acids (Mulder and Atheunisse, 1963) and *B. polymyxa* and other *Bacillus* spp. secrete antibiotics (Davis et al., 1967). Stimulation or inhibition of bacteria in the vicinity of these organisms could occur.

Pure cultures of *Arthrobacter, Bacillus,* and *Nocardia* isolated from soil are capable of metabolizing dalapon under laboratory conditions (Kaufman, 1964). Since the main product of metabolism found in soil was pyruvate, bacterial num-

bers might be expected to increase on leaf surfaces after treatment with dalapon. However, the reverse was found in this study.

Dalapon-treated leaves had significantly fewer numbers of bacteria than controls one day after each spraying. This suggests that dalapon suppresses the growth of some of the bacteria. Dalapon is known to be an inhibitor of panthothenic acid production (van Oorschot and Hilton, 1963) and pyruvate utilization in some microorganisms (Redemann and Meikle, 1955).

All of the bacteria found on corn leaves were gram positive with the exception of *Azotobacter*.

Arthrobacter was the dominant genus on the surfaces of young leaves. One strain was found only when corn was growing rapidly. As the leaves matured, bacilli were found with increasing frequency and became the dominant organisms when the leaves were scenescent. Therefore, a succession of aerobic, heterotrophic gram-positive bacteria occurred on upper leaf surfaces of North Dakota field grown corn in 1972.

Leben and Daft (1964) found that the resident flora on cucumber (*Cucumis sativus* L.) leaves consisted predominantly of gram-negative rods. Stout (1960) reported that *Bacillus* spp. were almost totally absent from the bacterial flora of herbiage from New Zealand. Ruinen (1970) indicated that the dominant organisms found on the laminae of *Tripsacum laxum* Nash. in West Africa were gram-negative rods including those which fix nitrogen, with yeasts and coryneform rods also present. She also noted that nitrogen-fixing forms predominate on young plant parts and are replaced by bacteria which prefer or require bound nitrogen. Soybean (*Glycine max* L.) leaf surfaces in North Dakota supported a gram-negative bacterial flora in July of 1971 and only fungi in August (Mullins, unpublished data). Concurrent sampling of sugarbeet (*Beta vulgaris* L.) leaf surfaces revealed a predominantly gram-negative bacterial flora. *Azotobacter* was the only gram-negative component in the predominantly gram-positive bacterial flora of corn leaves in the summer of 1971. This indicates that the surface flora of plant leaves may be characteristic for each plant species and may change with the seasons. The flora recovered in this study is ideally suited to the alternate humid and dry climate of North Dakota. *Bacillus* spores are very resistant to heat, X-rays, dessiccation, anf ultraviolet light (Mandelstam and McQuillen, 1968). Under adverse conditions the coryneform rods of *Arthrobacter* are transformed into cocci, which are resistant to dessiccation (Mulder, 1964).

The Rodac impression plate does not recover all the bacteria from the leaf surface but does given an estimate of the flora. Beech and Davenport (1971) applied 25 successive impression plates to apple leaves and found that yeasts were still recovered on the twenty-fifth plate. Many bacteria probably occur as microcolonies on the leaf surface and only a few are recovered each time, or they may be stratified. The lowermost layer of bacteria could obtain nutrients directly from the leaf surface

exudates (Kunert and Lippert, 1972). Stomata, trichomes, convoluted epidermis and wax are all present on upper corn leaf surfaces (Davis, 1971; Martin and Juniper, 1970), and may affect the retention of organisms when plates are pressed onto the leaf. Leben et al. (1968) found the greatest numbers of bacteria on soybean buds in the depressions over the junctures of epidermal cells and on or near the basal cells of trichomes. The drawbacks to the Rodac method are recognized. However, this is the only known surface sampling method which allows a study of the same intact leaf over the growing season.

The population increases found on July 10 and August 23 showed no obvious relationship to rainfall, relative humidity, or the appearance of pollen. It appeared that bacterial population density increased with temperature, but insufficient data were available to substantiate this.

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EFFECT OF DRYING CONDITIONS ON OIL CONTENT OF SUNFLOWER (HELIANTHUS ANNUUS L.) SEEDS AS DETERMINED BY WIDE-LINE NUCLEAR MAGNETIC RESONANCE (NMR)

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INTRODUCTION

The application of wide-line NMR for determination of oil content in seeds has provided new opportunities for plant breeders (Alexander et al., 1967). The procedure allows for oil determination on a large number of samples, is rapid, accurate, and non-destructive. However, the NMR measurement does not distinguish between liquid water and liquid oil within the seed (Conway and Earle, 1963). Consequently, the moisture in the seed must be removed by drying in order to obtain an accurate estimate of the oil content. This study was undertaken to establish optimum drying methods for analysis of sunflower seed in our laboratory.

MATERIALS AND METHODS

Seed samples were analyzed by a Newport NMR Analyzer. The Newport NMR Analyzer[2] contains a radio-frequency (R - F) coil located in a permanent magnet with a steady field value of 640 gauss. The R-F level for the analysis was set at 300 μA. Samples were placed inside the coil and subjected to both steady and rapidly varying fields produced by the modulation coils on the magnet (Mansfield, 1971). Supplementary modulation was used for all determinations since it greatly reduces saturation effects of the sample. When adjusted properly, there is absorption of the radio-frequency energy by the hydrogen nuclei in a liquid matrix within the sample.

The radio-frequency signal is received by the coil, detected, amplified, and integrated for either 8.2s, 33s, or 2 min 11s. The integrated value appears on a digital voltmeter located on the front of the NMR console.

Standardization of the NMR analyzer was accomplished with a sample of sunflower seed of known oil content. The oil content of the calibration sample was determined by Soxhlet extraction of three replicated seed samples for 24 h with petroleum ether.

The oil content was calculated by using the dry weight, MNR reading, and a calibration constant as follows:

% oil = (NMR reading/weight)/constant.

The calibration constant was determined from sunflower seed samples of known oil content as shown below:

constant = (NMR reading/weight)/known oil content.

For sunflowers, the constant had a value of 0.6341.

Although the linearity of NMR response and amount of oil has been demonstrated for a large number of oilseeds (Conway and Earle, 1963), it was checked with sunflower seed samples of known oil content (by extraction). The number of NMR units per gram of oil in the seed was found to be 63.30 ± 1.09 over the 30^* - 50^* oil content range. For the average sized sample, this is equivalent to a standard deviation of only $\pm 0.02\%$ oil.

Three random samples of sunflower (*Helianthus annuus* L.) seed, varieties Mingren and Peredovik, were dried in beakers in a laboratory oven at 130°C for one hour. Each seed sample was sealed in an Erlenmeyer flask to prevent absorption of moisture, allowed to equilibrate to 25°C, weighed and placed in the NMR magnet for analysis. This procedure of drying and analyzing was repeated four times. The same procedure was followed with sunflower seed dried at 60°C for three consecutive 24-h periods.

The rate of moisture adsorption of dried seeds of each variety was determined by weighing each sample at regular intervals during storage at room conditions (approximately 25% relative humidity and 25°C) in open beakers and in sealed plastic bags. Differences between storage treatments were tested for significance with the Student's t-test.

RESULTS AND DISCUSSION

Table 1 shows that moisture content and NMR reading for both varieties were significantly affected by the one hour of drying at 130°C. For both varieties, additional drying reduced the dry weight but did not significantly change the NMR reading. Since there was more variability in the NMR reading than in the weight of determination, the number of NMR units per gram of seed was not significantly affected.

When the two varieties of sunflower seed were dried at 60°C to retain seed viability, the NMR reading and moisture content decreased significantly after the 24-h treatment (Table 2). Additional drying reduced the dry weight, but did not significantly affect the NMR reading or NMR units per gram of seed.

Drying sunflower seed for one hour at 130°C or 24 h at 60°C provided an accurate estimate of the oil content and additional drying beyond these times was unnecessary. The precision of the NMR read-out with the 2 min 11 s integration period was $\pm 0.1\%$ (49.6% oil for Peredovik).

The results of the moisture adsorption study indicated that seeds stored in

Table 1.   Effect of drying on Peredovik and Mingren sunflower seed at 130°C.

Variety	Time (h)	Sample[A] [*] wt. (g)	Moisture content (%)	NMR units[A]	NMR units/g[A]
Peredovik	0	14.14[A]	15.66[A]	531[A]	37.53[A]
	1	12.30[B]	0.58[B]	387[B]	31.48[B]
	2	12.25[C]	0.21[C]	389[C]	31.73[C]
	3	12.24[C]	0.11[C]	387[B]	31.60[B]
	4	12.22[D]	——	388[B]	31.71[B]
Mingren	0	13.66[A]	16.60[A]	254[A]	18.60[A]
	1	11.78[B]	0.58[B]	180[B]	15.31[B]
	2	11.74[C]	0.18[C]	181[B]	15.42[B]
	3	11.73[C]	0.11[C]	182[B]	15.49[B]
	4	11.71[C]	——	180[B]	15.34[B]

[A]Values with different superscripts are significantly different from values above at the 1% level.

Table 2.   Effect of drying on Peredovik and Mingren sunflower seed at 60°C.

Variety	Time (h)	Sample[A] wt. (g)	Moisture content (%)	NMR units[A]	NMR units/g[A]
Peredovik	0	14.05[A]	10.89[A]	510[A]	36.27[A]
	24	12.72[B]	0.39[B]	401[B]	31.50[B]
	48	12.72[B]	0.36[B]	402[B]	31.61[B]
	72	12.67[C]	——	398[B]	31.41[B]
Mingren	0	10.30[A]	13.09[A]	229[A]	22.20[A]
	24	9.16[B]	0.56[B]	149[B]	16.30[B]
	48	9.16[B]	0.56[B]	149[B]	16.30[B]
	72	9.11[C]	——	148[B]	16.25[B]

[A]Values with different superscripts are significantly different from values above at the 1% level.

plastic bags adsorb moisture from the atmosphere even though the bags were sealed (Figures 1 and 2). Peredovik seed stored for a period of eight days in open beakers and in sealed plastic bags under atmospheric conditions adsorbed approximately 1.39% and 0.71% moisture and then stabilized (Figure 1). Mingren seed stored in open beakers and in sealed plastic bags under the same conditions adsorbed approximately 1.88* and 0.90* moisture in eight days (Figure 2). The sharp maximum in the moisture content curve at day 11 for the seed stored in
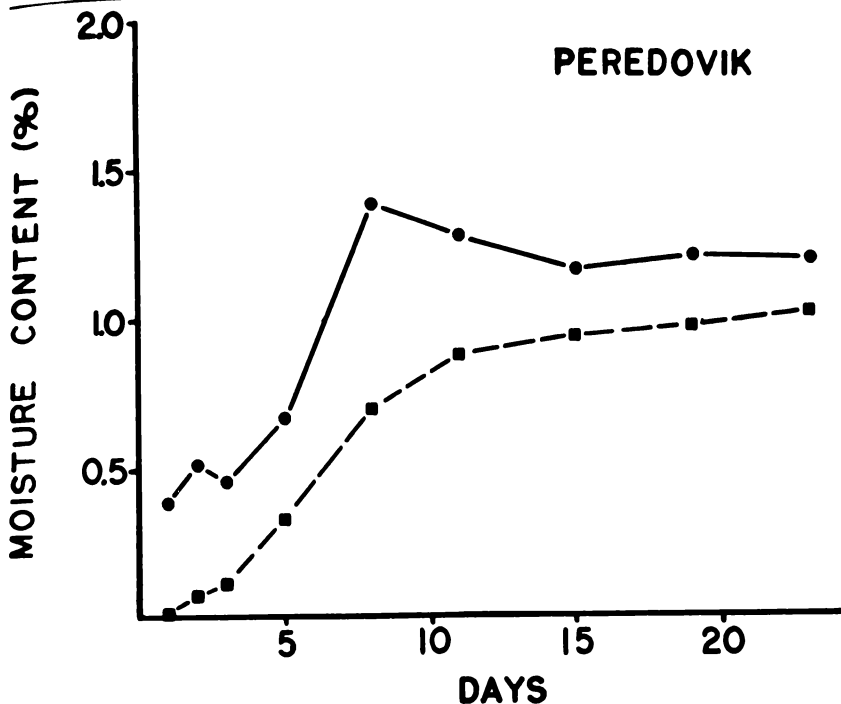
Figure 1.   Adsorption of moisture by Peredovik sunflower seed for three weeks. Seed stored in plastic bags = - - - - - - - - - - - - - - - -; Seed stored in open beakers = ————————————————.

open beakers was not apparent for the seed stored in plastic bags. This increase in moisture content corresponded with an increase in relative humidity at that time. Mingren seed adsorbed more moisture than Peredovik seed under both conditions. This is probably due to the larger surface area of the hull of the Mingren seed.

The study demonstrates the importance of proper seed handling after drying in order to obtain an accurate oil content value. If a relative ranking of seed samples by oil content is all that is needed, it is not necessary to dry the samples provided that they have a uniform moisture content.

To determine the optimum conditions for analyzing 10,000 seed samples per year, we considered six operations in our total system. These included cataloging the samples, drying, weighing, NMR analysis, calculation of oil content and the reporting of results. We found that the critical step for ths overall operation was the time required for the drying of the seed. Drying times of one hour at 130°C or 24 h at 60°C provided for accurate NMR analysis and the most efficient operation of the total analytical process.
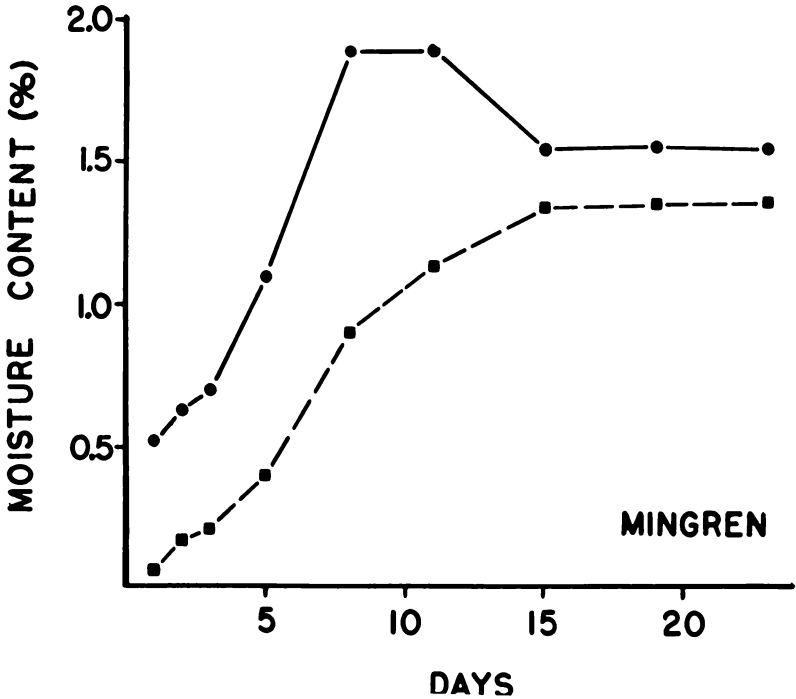
Figure 2.   Adsorption of moisture by Mingren sunflower seed for three weeks. Seed stored in plastic bags = -; Seed stored in open beakers = ————————————————.

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EFFECT OF PLANT EXTRACTS ON GERMINATION OF WILD OATS (*AVENA FATUA* L.)

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ABSTRACT

Water extracts of wild North Dakota plants were evaluated for physiological activity on the germination of wild oat *(Avena fatua* L.) seed. Extract derived from 1 g dry weight of plant tissue in 5 ml distilled water was added to a filter paper in petri dishes. Each extract treatment included 10 seeds placed in a petri dish with three replications. Treatments were maintained in a seed germinator at 20 C and evaluated for germination and seedling development over a 14-day period.

Water extracts of *Asclepias ovalifolia* leaves, stems, roots and flowers, *A. syriaca* stems, *A. incarnata* leaves and flowers, and *A. verticillata* flowers suppressed normal germination up through five days, after which some showed coleoptile elongation. By 13 days branched adventitious roots were evident but normal radical growth was inhibited. Water extracts of *A. verticillata* leaves, stems and roots, *A. incarnata* stems and roots, *A. verticillata* leaves, *Delphinium virescens* leaves and *Xanthium italicum* leaves were physiologically inactive.

INTRODUCTION

Wild oats (*Avena fatua L.*) continues to cause a major economic loss in North Dakota agriculture, costing an estimated $35 million annually. Use of artificially synthesized herbicides are presently coming under increasing criticism for their potentially harmful ecological side effects. Alternative control measures, other than cultural, involve the utilization of biological systems acting within the normal dynamics of the ecosystem and may be classified into categories of selective pathogenic agents, controlled insect infestation, and plant allelopathy.

The pathogenic approach to pest control appears promising in the case of control of the insect family Noctuidae, which includes cutworms and corn earworms (Agriculture Research Staff, 1972). Goeden (1971) suggested the feasibility of using insects for control of silver nightshade (*Solanum elaeagnifolium* Cab.) and cited literature on other reported potential insect control agents.

Plant competition and plant allelopathy have been described collectively as phytotoxic interaction interference among plants (Bell and Koeppe, 1972). Allelopathic compounds, compared to chemically synthesized pesticides, may be more desirable because naturally occurring secondary metabolic products may be more easily biodegraded (Agrichemical Age Staff, 1971). Allelopathy has been studied either as living plant to plant interactions (LP/PI) or as plant extract to plant interactions (PE/PI). Several reviews emphasizing LP/PI systems include those by Bell and Loeppe (1972), Bonner (1950), Borner (1960), Garb (1961), Risser (1969), Rovira (1969), Whittaker and Feeny (1971), and Woods (1960).

Muller (1966) stated that a plant is essentially a leaky system. Bonner (1950) concluded it is a reasonable assumption that plants excrete secondary products similar to those existing within the plant.

Biological activity of plants has generally been assessed using extracted plant material. Farnsworth (1966) reviewed biological and phytochemical screening of plants. Mattson (1972) summarized the reported insecticidal, bacteriological, and pharmacological activities of the 49 species he studied, three of which were included in this study.

Leath et al. (1972), using extraction techniques similar to ours, studied respnses of alfalfa (*Medicago sativa L.*) pathogens to extracted saponins and other secondary product fractions from alfalfa. They found the extracts were capable of increasing or suppressing colony growth in most of the 18 pathogens studied and concluded that saponins have a potential as a source of disease resistance. The biological activity of saponins in plant extracts can be modified by interactions with other components of the extract like tannins (Cheeke, 1971).

Gilbert, Menzies and Griebel (1969) found the volatile fraction of the water extract of alfalfa could induce qualitative and quantitative changes in growth of soil microflora. Selleck (1972) studied the effects of extracts of small everlasting (*Antennaria microphylla* Rydb.), field pussytoes (*A. neglecta* Greene), and leafy spurge (*Euphorbia esula* L.) on seed germination and subsequent seedling development of several species and found decreases in germination percentage, increases and decreases in coleoptile elongation, and suppressed radicle elongation. Helgeson and Konzak (1950) observed decreased elongation of the radicle and coleoptile in wheat (*Triticum aestivum* L.) and flax (*Linum usitatissimum* L.) caused by extracts of field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* (L.) Scop.). Le Tourneau, Failes, and Heggeness (1956) and Le Tourneau and Heggeness (1957) found 24 species inhibiting germination and root and shoot growth. Evenari (1949) reviewed data on germination-inhibiting plants and stated that germination and growth inhibitions are nearly always associated. Other PE/PI studies include those by Ohman and Kom-Haley (1968) and Muller et al. (1969).

Five species of *Asclepias* have been reported biologically active and phytochemically positive as follows:

A. incarnata L.—This plant has been shown to suppress growth of mammalian tumors and to have caused mammalian central nervous system, bacteriological, and insect developmental reactions. Phytochemical studies have detected alkaloids (Mattson, 1972), flavonoids, saponins, and tannins (Wollmann, Schermeister and Schmiess, 1972).

A. ovalifolia, Dcne.—Phytochemical studies have detected alkaloids and saponins (Sinha et al., 1967).

A. speciosa Torr.—Insecticidal and physiological insect activities and suppression of tumor growth have been reported. Alkaloids and saponins have been detected (Mattson, 1972).

A. syriaca L.—Several types of mammalian pharmacological activities have been reported from this plant. It has been found to be insecticidal, to influence insect development and to affect tumor growth. Phytochemically, the plant has been reported to contain saponins, tannins, alkaloids, flavonoids, and steroids (Mattson, 1972).

A. verticillata L.—Bacteriological activity (Nickell, 1959), toxicity to animals (Muenscher, 1948) and suppression of tumor growth have been reported from this plant (Abbott et al., 1966). Historical reports of the use of this plant as a cancer treatment have been made (Hartwell, 1967). Phytochemical studies have detected alkaloids, flavonoids and tannins (Wollmann et al., 1972).

The purpose of this study was to investigate the effect of plant extracts from wild North Dakota plants, not previously evaluated, on the germination of wild oats.

MATERIALS AND METHODS

Plant materials used in this study were collected by the Department of Pharmacognosy, North Dakota State University, from naturally occurring populations throughout North Dakota. Identification of plant specimens was verified by Dr. O. A. Stevens and voucher specimens were retained by the Department of Pharmacognosy. Species used in this study were *Asclepias incarnata*, *Asclepias ovalifolia*, *Asclepias pumila*, *Asclepias speciosa*, *Asclepias syriaca*, *Asclepias verticillata*, *Delphinum virescens*, and *Xanthium italicum*. Fresh plant material at the flowering stage was separated into the various plant parts and both air-dried and freeze-dried samples were prepared. Material was ground to a number 40-sized powder.

Twenty-four grams of each powdered sample were consecutively extracted with petroleum ether, 95% ethyl alcohol, and distilled water. Only water extracts were used in this study and these were reconstituted to a concentration of 1.0 g equivalents of dry tissue per 1.0 ml water.

Wild oat seed used in this study was taken from bulks collected at Fargo, North Dakota in 1967 and 1972. The 1967 seed had nearly 100% germination. The 1972 seed had less than 50% germination and can be considered more dormant

than the 1967 seed. Wild oat seeds vary widely in color; however, only grey-colored seeds were used in this study.

Extract derived from 1 g dry weight of plant material was diluted with 5 ml distilled water and applied to 9-cm petri dishes containing two filter papers each. Ten wild oat seeds were distributed in each petri dish and placed on trays in a Model 18 Stults seed germinator at 20 C. Three replications of extract treatments and distilled water checks were made for both 1967 and 1972 seed.

Conductivity was measured on an Industrial Instruments, Inc. conductivity bridge and compared with a known concentration of a potassium chloride solution. Replicated pH readings were recorded.

Daily observations were made and measurements were recorded at seven and 13 days. Data collected include percentage germination, time to germination, shoot or coleoptile lengths, root lengths, root growth habit, and germination sequence variations.

RESULTS

Results indicate air-dried plant part water extracts of *Asclepias* species were active in delaying or preventing radicle elongation independent of coleoptile development, and in causing abnormal root development on wild oat seedlings.

Table 1 summarizes the effects of the plant extract treatment that were active in affecting germination and in some cases those causing subsequent anomalous seedling development. The one-week data are estimated observational data and represent only radicle and coleoptile development. The 13-day data represent the measured average length of the primary and first node adventitious roots (those arising below the level of divergence of the coleoptile), and thus some data may appear inconsistent but the inconsistency results from averaging the lengths over all roots.

The experiment was designed to detect extracts effects on germination and therefore inactive plant extracts allowed the normal germination sequence. Seedling development after germination in some of the unreactive plant extract treatments was also reduced.

Initial pH readings made on the distilled water checks and the diluted extract treatments were all found to range from 5.5 to 6.0. The pH of all treatments, including the non-reactive ones, became more basic within two days after initiating the treatments and ranged between 6.5 and 9.0. The pH of only two extracts increased to more than 8.0. There was no apparent relationship between the pH change and the effects of the treatments on germination.

Conductivity readings of the diluted extracts, which in this case is by no means a measure of osmolarity, ranged near and averaged close to an equivalent 100 ppmw potassium chloride solution. No relationship between the conductivity readings and the inhibitory effects detected on germination was apparent.

The results of the *Asclepias* plant parts found reactive in affecting germination

Table 1. Average effects of active aqueous extracts of *Asclepias* species plant parts on shoot-root ratios, germination percentage, and root and germination anomalies.

Asclepias species	Plant part	Ratio of coleoptile to radicle (cm-7 days) 1967 Seed	1972 Seed	Ratio of shoot to root (cm-13 days) 1967 Seed	1972 Seed	Percent germination 1967 Seed	1972 Seed	Reaction code [B]
incarnata	Leaf	5/0	4/0	16/2	12/3	77	95	1-(3 days), 2, 6
	Flower	5/0	3/0	17/4	16/4	67	73	1-(3 days), 3, 5
ovalifolia	Leaf	2/0	4/0	8/1	10/2	17	43	1-(7 days), 4, 6, 7-(coleoptile reaction)
	Stem	5/0	6/0	15/3	14/2	67	73	1-(4 days), 4, 5
	Root	5/0	4/0	16/3	9/2	70	83	1-(4 days), 4, 6, 7-(branched)
	Flower	5/0.3	4/0.3	15/2	14/2	87	87	3
speciosa	Leaf	0/0	0/0	0/0	4/0	0	7	1-(12 days), 2, 7-(development of coleoptile)
	Root	6/0.8	6/0.8	10/2	10/2	87	80	4, 5
	Rhizome	8/0	8/0	12/0	10/0	70	57	1, 2, 6
	Flower	0/0	0/0	0/0	0/0	0	0	1-(complete inhibition)
syriaca	Stem	0/0	0/0	0/0	4/0.2	0	10	1, 2, 6, 7-(germination)
	Leaf [A]	8/3	8/10	17/5	17/5	94	75	8-(branched)
verticillata	Flower	4/0	4/0	16/2	17/4	93	67	4
	Leaf	3/1.5	4/1.5	16/2	13/2	90	67	4, 5
control	- -	7/8	7/8	13/10	10/12	94	46	

[A] Average of freeze-dried and air dried treatments. All other treatments are air-dried only.
[B] Reaction codes:
1 Delay or prevention of coleoptile and radicle development.
2 Delayed germination followed by normal coleoptile development only.
3 Delayed germination followed by normal coleoptile development and at least partial development of primary and first node adventitious roots.
4 Normal coleoptile development, radicle absent, delayed, or undeveloped.
5 Crown adventitious roots present in addition to at least some primary and first node adventitious roots.
6 Crown adventitious root development only.
7 Reaction in 1972 seed only (reaction specified in parenthesis).
8 Reaction in 1967 seed only (reaction specified in parenthesis).

are given in Table 1. All discussion relates to Table 1 and all data are from air dried plant materials unless otherwise specified.

A. incarnata.—Leaf and flower extract treatments prevented normal germination and resulted in seedling development that consisted of delayed shoot elongation and a root system that consisted only of secondary adventitious roots. Seedlings developed only crown adventitious roots (those arising above the level of divergence of the coleoptile) on the leaf extract treatments. Flower extract treatments, in addition to the crown adventitious roots, developed first node adventitious roots, but no primary roots.

A. ovalifolia.—Leaf, stem, root and flower extract treatments delayed germination, and coleoptile elongation occurred prior to observable root development. The leaf extract treatments delayed germination seven days and the percentage of seeds where coleoptile elongation eventually occurred was greater in the 1972 seed. Primary root elongation occurred only in the flower extract treatments. Crown adventitious roots developed early and uniformly in all treatments.

Abnormal branching of developing secondary roots occurred in the stem and root extract treatments. The branching was exclusive to the 1972 seed in the root extract treatments.

A. speciosa.—Leaf and flower extracts inhibited germination through seven days. Coleoptile elongation occurred only in the 1972 seed treated with leaf extract. Rhizomes delayed germination and completely inhibited root growth except for crown adventitious roots. Three and five crown adventitious roots arose in these seedlings rather than in the normal pair. Root extracts caused roots to be abnormal and branched.

A. syriaca.—Stem extract treatments completely inhibited germination in the 1967 seed. Coleoptile elongation and crown adventitious roots developed after nine days in the 1972 seed; however, primary root elongation did not occur. Leaf extract treatments caused abnormal branching of the roots on the 1967 seedlings.

A. verticillata.—Flower extract treatments caused coleoptile elongation to occur prior to primary root elongation. After 10 days, normal primary and secondary root development occurred. Leaf extracts caused excessive branching and abnormal crown adventitious roots.

Plant part extracts which were inactive on wild oat germination include *Delphinium virescens* Nutt. leaf, *Asclepias incarnata* stem and root, *A. pumila* (A. Gray) Vail. leaf and fruit, *A. syriaca* root (air-dried and freeze-dried) and flower, *A. verticillata* stem and root, and *Xanthium italicum* Moretti leaf.

DISCUSSION

Avery (1930) described normal germination of oats (*Avena sativa* L.) as consisting of the following sequence of events: (1) the coleorhiza bursts through the pericarp and is soon penetrated by the primary root which grows rapidly downward, (2) the coleoptile, enclosing the growing point and embryonic leaves, begins elongating, (3) two adventitious roots are initiated just below the level of divergence of the coleoptile and (4) about ten or more days after germination, a third pair of roots may appear above the level of divergence of the coleoptile. Extract treatments (Table 1) caused deviations from this sequence, and these appear to be characteristic of *Asclepias* species extract effects.

Defective root development has been observed resulting from PE/PI. Le Tourneau and Heggeness (1957) reported abnormal root growth caused by leafy spurge and quackgrass (*Agropyron repens* L.). Leaf and stem extracts of leafy spurge depressed radicle elongation of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) but stimulated coleoptile elongation (Becker, Guyot, and Heim, 1951). Extracts of soil in which leafy spurge and small everlasting were grown inhibited radicle elongation but not germination or coleoptile elongation in smooth brome (*Bromus inermis* Leyss.) (Selleck, 1972). Muller et al. (1968) found that whiteleaf sage (*Salvia leucophylla* Greene) leaves produce volatile terpenes that reduced root development of wild oats and other herbs as a result of an

inhibition of oxygen uptake. It was further reported that cell division and vertical elongation of wild oats was inhibited by this sage (Muller and Hauge, 1967). Retig, Holm and Struckmeyer (1972) reported that all four weed species they studied in LP/PI systems caused at least microscopically detectable abnormal anatomical root development in cabbage (*Brassica oleracea* L.) and tomato (*Lycopersicum esculentum* Mill.). Retig and Holm (1971) concluded that compounds from the extracts were taken up by the roots. Helgeson and Konzak (1950) observed clubbing and massing of the root growth in flax seeds treated with field bindweed and Canada thistle extracts. These abnormalities appeared similar to those caused by 2,4-D treatment. Abnormal root development in treated wheat was also found. Ohman and Kommedahl (1964) observed abnormal root development in alfalfa using quackgrass extracts.

Lund, Pearson, and Buchanam (1970) found reduced root growth in soybeans caused by treatments of trifluralin. Root disfunction in honeyvine milkweed (*Ampelamus albidus* (Nutt.) Britt.) has resulted from 2,4-D treatment and was thought to be caused by increased cellulase activity (Coble and Slife, 1971). Schooler, Bell and Nalewaja (1972) observed abnormal root growth in foxtail barley (*Hordeum jubatum* L.) caused by Siduron and concluded the apparent susceptability was genetically controlled.

Data from *Asclepias ovalifolia* leaf, root and flower, *A. verticillata* stem, and *A. speciosa* and *A. syriaca* leaf treatments indicate a differential germination response in the 1967 'non-dormant' and the 1972 'dormant' seeds. Branching reactions and unsequential coleoptile radicle emergence suggests a physiological response. Data obtained does not allow a comprehensive evaluation of the physiological mechanism.

Atwood (1914) concluded that after-ripening in wild oats is independent of shell coat effect, the exclusion of water, the water content of the seed at harvest, and light. Oxygen supplied to the wild oat embryo or increased embryo acid content, accompanied by increased water-absorbing power of the embryo, may account for the differential germination. Johnson (1935) came to similar conclusions and he further felt that stimulation of germination percentage as a result of freezing was based on an increased permeability to oxygen. Cumming and Hay (1958), however, concluded that daylight inhibits germination of partially dormant seeds on the soil surface. Hay (1967) reported the possible dormancy mechanisms might include enzyme blockage in food reserves conversion, or the absence or tying up of some co-enzyme or key metabolite. Black (1959) found two chemical inhibitors using chromatographic techniques. These were readily diffusible in water and suppressed by increasing oxygen concentrations. It appears from Johnson's work (1935) that dormancy in wild oats might be controlled genetically by one or more dominant genes. He found also some stimulation in germination percentage resulted from treatments of ether, low concentrations of sodium thiocynate, and potassium nitrite, which he felt resulted from the selec-

tive uptake of NO^{-3} ions. Isensee, Jones and Turner (1971), however, observed concentration dependent on total accumulation of Picloram in oats as opposed to selective uptake in beans.

Growth regulator studies have shown (Holm and Miller, 1972) that abcissic acid at 10^{-4} M concentration completely inhibits wild oat germination, but that thiourea, kinetin, 2-chloroethylpnosphonic acid, and naphthalene acetic acid had no effect.

Le Tourneau and Heggeness (1957) observed that the pH of the spurge extracts became alkaline during germination. The addition of KH_2PO_4 buffer solution did not alter the effects of the extracts. Optimum pH for germination of the five species studied by Evetts and Burnside (1972) was found to be between 4.0 and 8.0. Only two treatments in this study diverged from this range. Le Tourneau et al. (1956) discounted pH and osmotic pressures equivalent to 4.5 atmospheres in 0.1 M glucose solution as causes of abnormal germination and root growth in extract-treated seeds. Evenari (1949) stated that pH or osmotic pressure could not account for the inhibitory effects of the tested extracts.

Evetts and Burnside (1972), studying crop and weed species, found no differences in germination or coleoptile length from seeds germinated in petri dishes containing solutions with less than 5,000 ppmw NaCl. Radicle elongation on the average was not affected by concentrations less than 2,500 ppmw. Concentrations of 10,000 ppmw NaCl did not cause effects comparable to those observed in this study.

SUMMARY

Wild oat seeds were germinated on petri dishes containing water extracts of *Asclepias* species plant parts. Germination reactions were observed in five of the species tested. The active plants and the reactions caused are summarized below.

1. *A. speciosa* leaf and *A. syriaca* stem extract completely inhibited germination in the ''less dormant'' 1967 seed. *A. speciosa* flower extract inhibited germination in both 1967 and 1972 seed.

2. *A. incarnata* leaf, *A. ovalifolia* leaf, stem and root, *A. speciosa* leaf and rhizome, *A. syriaca* stem, and *A. verticillata* leaf and flower extracts altered the normal sequence of germination events and caused coleoptiles to elongate before the primary root elongated.

3. *A. syriaca* leaf, *A. verticillata* leaf, *A. ovalifolia* root, and *A. speciosa* root extracts caused abnormal branching in the wild oat root systems.

4. Delayed germination was observed in seed treated with *A. incarnata* leaf and flower, *A. ovalifolia* leaf, stem, root and flower, *A. speciosa* leaf and rhizome, and *A. syriaca* stem extracts.

The apparent biological activity of various *Asclepias* species plant part extracts affecting wild oat germination and seedling development suggests additional research is needed to identify the active principle of these extracts affecting wild

oat germination process. An understanding of the active principle(s) has a potential for developing chemicals for wild oat control.

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A STUDY OF FREE AND PROTEIN AMINO ACIDS OF TWO *COPRINUS* SPECIES AT DIFFERENT STAGES OF. DEVELOPMENT

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ABSTRACT

The amino acid and total protein content of the fruiting bodies of two closely related species of Basidiomycetes, *Coprinus comatus* (shaggymane) and *Coprinus atramentarius* (inky cap), was determined at different stages of maturity. The amino acids were qualitatively identified using paper chromatography and quantitatively determined as individual amino acids using a 'Technicon' amino acid analyzer and as total protein by the Kjeldahl method. The variation of total and individual amino acid content at different stages of maturity may be useful in distinguishing between the two species chemotaxonomically and in the investigation of the reported toxicity of *C. atramentarius* when ingested with an alcoholic beverage. All protein amino acids identified were found in both species. However, the occurrence of nonprotein amino acids and the total protein content varied markedly ir ɔlant parts and stages of maturity.

INTRODUCTION

Information about the chemical components present in the carpophores of wild Basidiomycetes is limited because of the great difficulty in obtaining consistent supplies of plant material at the desired stages of development. Many biochemical studies of higher fungi have been undertaken for the purpose of detecting patterns of chemical constituents in taxonomic groups which would be useful in chemotaxonomy and increasing attention is being given to all nitrogenous constituents (Fowden, 1964:173). Fungi which are known to be (or reported to be) toxic have been the subject of numerous investigations and conflicting reports. Although the fruiting bodies (mushrooms) of *C. comatus* and *C. atramentarius* are generally considered to be edible, a toxic reaction has been reported to occur when *C. atramentarius* is ingested with an alcoholic beverage. It is the purpose of this paper to compare the relative changes in the amino acid and total protein content of these two species of *Coprinus* at different stages of maturity by using three different methods of analysis: Kjeldahl method, paper chromatography, and a 'Technicon' amino acid analyzer.

MATERIALS AND METHODS

Plant material.—Carpophores of *Coprinus atramentarius* and *Coprinus comatus* were collected in Becker County, Minnesota during September and October, 1969. The carpophores were cleaned with a damp towel and divided into three groups: immature, mature, and very mature. Immature specimens were tightly closed, entirely white and showed no indication of sporulation. The mature specimens were slightly expanded and were salmon-pink at the lower margin of the pileus and to approximately one-fourth of the distance to the top of the pileus. The very mature specimens were salmon-pink from one-half to three-fourths of the distance to the top of the pileus. Autodigestion had begun at the lower margin and up to about one-fourth of the distance to the top. The lower part of the pileus was turning to a black liquid. Each group was further separated into stipe and pileus, freeze dried, and ground to a No. 40 powder.

Determination of total protein content.—The Kjeldahl method (Talbot, 1946:355-358) was used on 1-g samples of the freeze-dried tissues.

Paper chromatography of the amino acids.—One-gram samples of the different tissues were refluxed with 30 ml of 6 N hydrochloric acid for 6 h. The hydrolyzates were then neutralized with solid sodium bicarbonate and filtered. The hydrolyzates, separate standard amino acids (0.1% in water) and a mixture of the reference standards were chromatographed following the descending technique on Whatman No. 1 filter paper using the upper phase of n-butanol/glacial acetic acid/water (4:1:5) as a developer. The spots were revealed with ninhydrin reagent and heating for 10 minutes at 100°C, followed by respraying with 1% cupric nitrate solution (Bode et al., 1952:524).

Amino acid analysis using Technicon auto-analyzer.—The different freeze-dried tissue samples were analyzed by the procedure described by Hamilton (1963:2055). For each analysis, 7.0 ml of 6 N hydrochloric acid was added to a known weight (6.0-11.0 mg) of the dried tissue. The mixture was frozen by immersing the tube in an ethanol-carbon dioxide bath. The tube was then evacuated to 50 microns and sealed. The samples were hydrolyzed for 24 h at 110°C, and dried in a test tube rotary evaporator at 40°C. A known quantity of norleucine as an internal standard in 0.1 N hydrochloric acid was added and the sample was redissolved. An aliquot (2.0-4.0 mg) of each sample was then applied at the top of a 'Chromobeads type B' column. The elution was affected by a series of sodium citrate, sodium chloride, Brij 35, and Versene buffer solutions with increasing pH 2.88-5.00. Identification of the eluted amino acids was carried out by monitoring the absorption of the colors produced with ninhydrin reagent by three photometers set at 440 nm, 570 nm, and 570 nm, with slit widths of 15 mm, 7 mm, and 15 mm. The absorption was recorded on a strip chart recorder with a speed of 6 inches/h.

An analysis of a mixture of standard amino acids was carried out each time there was an alteration of any factor in the system.

The peak areas of the known standards were computed and the ninhydrin positive substances in the samples were quantitatively calculated.

RESULTS AND DISCUSSION

Total protein content was calculated for both species at all stages of maturity by the Kjeldahl method (Table 1). The pileus of *C. atramentarius* contained the highest percentage of protein in the immature stage, whereas the pileus of *C. comatus* contained the highest percentage in the very mature stage. The protein percentage for the stipes of both species decreased from the immature to the mature stage and then increased in the very mature stage to a much higher percentage than that of the immature stage. *C. comatus* showed the greatest range of percentages (19.35%-40.70%) whereas the range for *C. atramentarius* was (18.55%-34.80%). This analysis provides striking evidence of the quantitative variation of constituents relative to plant parts and stages of maturity.

The amino acds in the hydrolyzates of the different parts of *C. atramentarius* and *C. comatus* at the different stages of maturity are given in Table 2. Although both species contained the same amino acids, in most cases the quantity of methionine, valine, tryptophan, alanine, threonine, glycine, and aspartic acid was considerably greater in *C. comatus*. In addition to the identified amino acids, serveral other ninhydrin-positive spots appeared which were not identified.

Quantitative determination of the individual amino acids using the amino acid analyzer method (Table 3) shows a significant percentage of ammonia. This can be explained by the almost complete destruction of tryptamine by the 24-h acid hydrolysis. This amino acid is converted to tryptophan and further to ammonia (Hamilton, 1963:2055). Only a residual peak appeared on the chart for tryptophan. Glutamine is also decomposed, resulting in the production of ammonia.

Table 1. Percentage of protein content of *Coprinus atramentarius* and *C. comatus* at three stages of maturity.[A]

	Immature		Mature		Very mature		Average
	[P]P	S	P	S	P	S	
C. atramentarius	27.15	21.20	25.60	18.55	26.70	34.80	25.60
C. comatus	19.35	22.20	31.35	19.40	33.90	40.70	27.81

[']Average of two determinations calculated on dry weight basis by the Kjeldahl method (Talbot, 1946:355-358).

[P]P=pileus; S=stipe

++=Concentration of standard (0.1%)

Table 2 Amino acids of *Coprinus atramentarius* and *C. comatus* in the hydrolyzate identified by paper chromatography.[A]

AMINO ACIDS	R$_f$ Value	C. atramentarius						C. comatus					
		Immature		Mature		Very mature		Immature		Mature		Very mature	
		P[B]	S	P	S	P	S	P	S	P	S	P	S
Leucine Isoleucine Phenylalanine	0.68	•...	++	++++	++	++	++	+++	+++	++++	+++	+++	++++
Methionine Valine Tryptophan	0.54	+	+	++	+	++	+	+++	++	+	++	+++	+++
Tyrosine	0.44	+	+	+	+	+	++	++	+	-	+	++	+++
Proline	0.44	+	+	++	+	+	+	-	+	+	+	++	+++
Alanine	0.42	+	+	++	++	++	+	+++	+++	+	++	++	++
Threonine	0.40	+	+	++	++	++	++	+++	++	+	++	++	++
Glycine	0.31	+	+	++	+	+	+	++	++	+	++	+++	+++
Histidine	0.29	+	+	+	+	+	+	+	+	+	+	++	++
Lysine Asparagine	0.22	++	+	+++	++	++	+	++	++	+	+	++	++
Aspartic	0.16	++	+	+++	++	+++	++	++++	+++	++	+++	++++	++++
Arginine	0.18	++	+	+++	++	++	+	+	++	+	++	+++	++++

[A]Freeze dried tissues
++=Concentration of standard (0.1%)   [B]P=pileus; S=stipe
-=Absent

Table 3. Percentage of individual amino acids in *Coprinus atramentarius* and *C. comatus*[A] using a Technicon amino acid analyzer.

AMINO ACIDS	C. atramentarius						C. comatus					
	Immature		Mature		Very mature		Immature		Mature		Very mature	
	P[B]	S	P	S	P	S	P	S	P	S	P	S
Lysine	0.61	0.33	1.20	0.36	0.85	0.64	1.06	0.89	0.60	0.73	1.09	0.92
Histidine	0.22	0.12	0.42	0.15	0.37	0.27	.30	0.33	0.22	0.26	0.48	0.46
Ammonia	0.40	0.35	0.55	0.35	0.55	0.54	.52	0.60	0.49	0.51	0.89	0.92
Arginine	0.72	0.34	1.20	0.40	0.94	0.67	1.00	0.95	0.68	0.92	1.17	1.23
Aspartic	1.01	0.51	1.72	0.58	1.38	1.28	1.82	1.58	0.99	1.44	2.13	2.09
Threonine	0.51	0.27	0.86	0.30	0.72	0.68	.90	0.83	0.53	0.73	1.13	1.08
Serine	0.54	0.30	0.99	0.32	0.83	0.78	.84	0.77	0.48	0.69	1.04	1.26
Glutamic	1.57	0.91	3.38	0.98	2.80	2.72	2.69	2.39	.60	2.24	2.80	2.92
Proline	0.48	0.41	0.90	0.28	0.69	0.59	.75	0.65	0.38	0.59	0.84	0.93
Glycine	0.56	0.30	0.89	0.14	0.74	0.67	.97	0.83	0.55	0.72	1.12	1.24
Alanine	0.69	0.36	1.12	0.41	0.89	0.82	1.60	1.42	0.77	1.29	1.36	1.16
.5 Cystine	0.05	0.04	0.13	0.03	0.12	0.08	.10	0.07	0.04	0.05	0.14	0.08
Valine	0.61	0.32	1.02	0.37	0.88	0.69	1.11	0.87	0.60	0.75	1.13	1.28
Methionine	0.15	0.06	0.26	0.08	0.21	0.16	.29	0.25	0.17	0.21	0.31	0.36
Isoleucine	0.50	0.26	0.89	0.30	0.67	0.55	.84	0.75	0.50	0.65	0.94	1.12
Leucine	0.77	0.41	1.48	0.46	1.14	0.91	1.50	1.21	0.80	1.07	1.56	1.97
Tyrosine	0.09	0.12	0.68	0.12	0.53	0.47	1.07	0.69	0.09	0.53	3.07	3.18
Phenylalanine	0.29	0.17	0.86	0.19	0.66	0.73	.87	0.74	1.27	0.58	1.58	1.26
Glucosamine	0.00	0.00	0.48	0.00	0.66	0.18	0.00	0.00	0.00	0.00	0.88	0.00
Citrulline	0.12	0.22	0.00	0.33	0.00	1.24	0.54	0.60	0.36	1.04	1.09	2.76
∝ Amino Butyric Acid	0.09	0.18	0.05	0.00	0.11	0.00	0.11	0.20	0.10	0.07	0.11	0.00
γ Amino Butyric Acid	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	7.58	6.16	18.53	5.80	14.19	14.13	18.36	15.21	10.73	14.56	23.45	25.30

[A]Freeze dried tissues
[B]P=pileus; S=stipe

Small amounts of ammonia may be produced in varying degrees by the decomposition of other acid-labile amino acids.

It is also obvious from the results that the modest percentage of lysine, arginine, glycine, threonine, valine, isoleucine, and leucine in the pileus was similar at each stage of maturity in both species. On the other hand, aspartic and glutamic acid, as to be expected, were found in relatively large amounts. The percentage of glutamic acid may have been substantially increased by the decomposition of glutamine. Like the lysine group mentioned above, the amount of these two amino acids increased in the pileus of *C. atramentarius* up to the mature stage and was then reduced in the very mature stage. In *C. comatus* both amino acids were present in high concentration in the immature tissue, decreased in the mature stage, and increased again in the very mature stage.

The relatively small amount of tyrosine also increases initially to the mature stage and then decreases in the very mature stage of *C. atramentarius*. An entirely different variation in tyrosine content occurs in *C. comatus* where an unusually high concentration is found in the very mature tissues.

Comparing the percentage of amino acids in the pileus of the two species at the mature stage, it is evident that the percentage in *C. atramentarius* is approximately twice that present in *C. comatus*, except in the case of phenylalanine. This marked difference might be sufficient to distinguish between the two species.

Another notable difference between these two species was the usual occurrence of large amounts of all amino acids in the pileus of *C. comatus* at the immature and very mature stages of development, whereas the highest percentage in *C. atramentarius* was in the mature stage.

A comparison of the percentages of the individual amino acids in the stipe tissue of the two species at comparable stages of maturity is similar to that of the pileus except that the percentages are greater at all three stages of maturity for *C. comatus*. This fact may prove to be of value as an additional constant in differentiating between the two species.

In stipe tissue the amino acid composition of the proteins present in both species varied greatly with the stage of maturity. This is to be expected since fungal tissue develops rapidly and is closely associated with a high rate of metabolism.

A few non-protein amino acids appeared in low percentages at some stages of maturity and in some plant parts. Citrulline occurred in both plant parts at all stages of maturity of *C. comatus*, whereas in *C. atramentarius* it was absent from the pileus of the mature and very mature stages. On the other hand, γ amino butyric acid was found in the stipe of the immature stage of *C. atramentarius* only.

These non-protein amino acids may also be useful in chemotaxonomic classification of higher fungi and in the investigation of the reported toxicity of *C. atramentarius*. The total protein percentagescalculated by the Kjeldahl deter-

mination were higher than those obtained by the amino acid analyzer technique. This would be expected because all inorganic nitrogen is included in the Kjeldahl analysis. Other organic nitrogenous compounds and unusual amino acids in low concentration would also contribute to this difference.

Considering the special advantages and the limitations of each of the three methods used in this study, a good correlation of results was obtained. All methods showed an astonishing dissimilarity of metabolic patterns for these two species of *Coprinus* which are so closely related taxonomically. This great difference may prove to be useful in developing chemotaxonomic groups and in the investigation of the reported toxic reaction of *C. atramentarius*.

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THERMODYNAMIC STUDIES OF CHYMOTRYPSIN IN LIGHT AND HEAVY WATER

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ABSTRACT

The acylation kinetics of the chymotrypsin-catalyzed hydrolysis of 2,4-dinitrophenyltrimethylacetate was investigated in light and heavy water at several temperatures. The rate of acylation was 1.4 times faster in light water then in heavy water, but the energy of activation was nearly the same in the case of heavy water. In both solvents the entropy of activation was negative, which shows that the system becomes more orderly when acylation occurs. In heavy water, the entropy change was more negative, which accounts for the decrease in rate. The entropy change associated with complex formation is positive. These results cannot

be explained in terms of structural change alone, but must be examined using solvation. It appears that during complex formation the system releases solvent but binds solvent when acylation occurs.

INTRODUCTION

Solvent isotope effects have been widely used to characterize chemical reactions (Robertson, 1967; Gold, 1969). For α-chymotrypsin, the isotopic solvents light and heavy water have been employed to distinguish among the several proposed mechanisms. An appreciable effect has been reported for both acylation and deacylation kinetics (Bender and Hamilton, 1962; Bender et al., 1964), and the results have been used to rule out any mechanism in which the active site acts only as a nucleophile. It was concluded, however, that both acylation and deacylation involve proton transfer.

More recently, the mechanism of chymotrypsin-catalyzed hydrolysis has been termed a charge relay process via an imidazoyl group (Blow and Steitz, 1970). However, work employing mixtures of light and heavy water (Pollock et al., 1973) indicates that this type of mechanism might not be entirely valid.

All chemical reactions possess a free energy of activation, which is thermodynamically comprised of an entropy and enthalpy factor. For the most part, solvent isotope effects have been considered to be caused by a variation in the enthalpy (energy) of activation due to differences in zero point energies (Robertson, 1967). This is no longer believed to be entirely true (Tseng and Stewart, 1971), as both enthalpy and entropy are isotopically dependent.

The prime purpose of this work is to investigate the solvent isotope effect on the acylation kinetics of chymotrypsin using 2,4-dinitrophenyltrimethylacetate (DNPTMA). In order to determine the solvent isotope effect (sie) on both the entropy and enthalpy of activation, the system was studied at several temperatures. Previous studies have been restricted to a single temperature. Our results should be particularly useful in establishing the role that solvent plays in enzyme catalysis.

THEORETICAL

The substrate DNPTMA (Tseng, 1964) reacts with chymotrypsin to form a stable intermediate trimethylacetyl-chymotrypsin. In the process 2,4-dinitrophenol is released. This product has a large extinction coefficient ($E_{355NM} = 1.4 \times 10^4$), which allows the acylation reaction to be conveniently measured without any interference from deacylation. Also, the pKa of 2,4-dinitrophenol is near 4. Thus the product is completely ionized over the pH range normally

employed in enzyme investigations. The rate curves were analyzed by the method of Guggenheim (Stewart and Lee, 1967):

$$\ln|r_2 - r_1| = \text{constant} - kt_1, \quad [1]$$

where r_1 and r_2 are any readings proportional to the concentration of product taken at a fixed time interval (difference between t_2 and t_1); t_1 is the time in seconds corresponding to r_1, and k is given by Stewart and Lee (1967):

$$k = \frac{e_0\, k_2}{e_0 + K_m} \quad [2]$$

The acylation rate constant k_2 is then determined using an inverse plot of equation [2]

$$\frac{1}{k} = \frac{1}{k_2} + \frac{K_m}{k_2}\,\frac{1}{e_0} \quad [3]$$

where e_0 is the initial enzyme concentration and K_m the Michaelis constant

$$K_m = \frac{k_2 + k_{-1}}{k_1} \quad [4]$$

The constants k_1 and k_{-1} are the forward and reverse rate constants for complex formation.

The temperature dependence of k_2 is:

$$k_2 = \frac{kT}{h}\quad \exp\,(\Delta S^*/R)\quad \exp\,(-\Delta H^*/RT) \quad [5]$$

where k is Boltzmann's constant, T is the absolute temperature, h is Planck's constant, R is the gas constant, and ΔS^* and ΔH^* are the entropy and enthalpy of activation.

MATERIALS AND METHODS

Materials.—α-chymotrypsin, 3x crystallized and lyophilized, salt free, was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Solutions were prepared by dissolving a weighted amount of enzyme in cold water and these were stored at 5°C. A molecular weight of 24,800 was used for α-chymotrypsin, and

the concentration in the final reaction mixture was varied from 2.016×10^{-5} to 10.08×10^{-5} M. The substrate 2, 4 dinitrophenyltrimethylacetate (DNPTMA) was synthesized (Tseng, 1964) by reacting 2, 4-dinitrophenol with trimethylacetyl chloride in dry pyridine. Known amounts of recrystallized material were dissolved in isopropanol and diluted by a factor of 100 with deionized, CO_2-free, distilled water. No spontaneous hydrolysis of the DNPTMA was observed but all solutions were used within 48 h after preparation. The final concentration of substrate in the reaction mixture was 1.12×10^{-5} M. Phosphate buffer (0.2 M) was prepared by mixing the appropriate amounts of mono- and dihydrogen phosphate solutions to give pH 8 (Gormori, 1955). The pH of each solution was checked with a Radiometer pH meter. The final concentration of buffer in the reaction mixture was held at 0.025 M. Experiments involving heavy water were performed in the same manner as with light water. The heavy water (99.8% D_2O) was purchased from Merck Chemical Division, St. Louis, Missouri, and was used without further purification. The pD of the heavy buffer was calculated by adding 0.4 units to the pH meter reading. Reactions in light water were carried out at 10, 20, and 30°C, whereas those in heavy water were performed at 20, 25, and 30°.

An appropriate computer program was written to handle all calculations using least square analysis.

Kinetic measurements.—The rates of hydrolyses were recorded using a Beckman DU Spectrometer equipped with a Leeds and Northrup Speedomax G recorder having a chart speed of 6 in per minute. The concentration of 2,4-dinitrophenol released was recorded at 355 mm employing a zero suppression method. Using an arbitrary zero suppression voltage, sufficient radiation was allowed to fall on the photomultiplier detector to give a zero voltage reading. This was accomplished by opening the slit until the recorder voltage could be balanced to zero. Any radiation absorbed by the production of phenol then resulted in a deflection that was proportional to the amount of product. These recordings were plotted according to equation 1, the slope of which was used to determine the rate constant for acylation. In a typical experiment, 0.5 ml of buffer and 0.5 ml of substrate were mixed and placed in one side of a split compartment cell (Pyrocell, Westwood, New Jersey). One ml of the appropriate enzyme solution was placed in the other side of the cell. After zeroing the recorder, the reactants were mixed by inverting the cell several times, but gently to prevent air bubbles. The reaction was then recorded to completion. The procedure was repeated at a fixed substrate concentration (1.12×10^{-5} M) but the enzyme concentration was varied from 2.016×10^{-5} to 10.08×10^{-5} M. The temperature of the cell compartment was held constant by the circulation of water from a refrigerated bath. Runs were carried out at three temperatures in light water and repeated in heavy water.

RESULTS AND DISCUSSION

The rate constants for acylation kinetics in light and heavy water as calculated by a computer using least square error analysis are tabulated in Table 1. A typical Guggenheim plot (equation 1) of rate data is shown in Figure 1. The acylation rate constants (k_2) and the Michaelis constants (K_m) were determined at three temperatures from equation 3 using Lineweaver-Burk plots such as shown in Figure 2. The entropies (ΔS^*) and enthalpies (ΔH^*) of activation are given in Table 2 and these were calculated from inverse temperature plots such as illustrated in Figure 3.

The custom in enzyme kinetics has been to measure the ratio K_H/k_D (rate constants in light and heavy water) at a single temperature and use the magnitude of this ratio to postulate the type of mechanism involved. Simple solvation and nucleophilic processes produce ratios of less than 1.5, whereas proton transfer processes result in ratios greater than 2. Although this approach is useful in establishing an acceptable mechanism, it does not account for the greatly enhanced rates of hydrolysis that occur in the presence of enzymic catalysts. From equation 5 it is obvious that a large ΔS^* or a small ΔH^* will result in a great increase in the rate constant k. It is, therefore, worthwhile to examine these quantities experimentally.

Some difficulties were encountered in obtaining reliable experimental results. This was partly because the excess enzyme method was employed and the sub-

Table 1.   Rate constants for acylation in light and heavy water.

	H_2O		
	$10^{\circ}C$	$20^{\circ}C$	$30^{\circ}C$
k_2 sec^{-1}	0.031	0.048	0.100
K_m X 10^4	0.820	1.30	2.89

	D_2O		
	$20^{\circ}C$	$25^{\circ}C$	$30^{\circ}C$
k_2 sec^{-1}	0.035	0.050	0.070
K_m X 10^4	1.80	2.60	3.53

Table 2. Thermodynamic activation parameters in light and heavy water at 25°C.

Rate Constant	ΔS^* cal M^{-1}		ΔH^* kcal M^{-1}	
	H_2O	D_2O	H_2O	D_2O
k_2	-32.4 ± 3.1	-25.8 ± 0.5	9.34 ± 0.92	11.54 ± 0.14
	-22.4^a		12.56^a	
	-23.0^b		$11.1^{\ b}$	
K_m	-41.1	-36.4	10.24	11.5
	-28.9^a		13.9^a	
$\dfrac{k_2}{K_m}$	8.7	10.6	-0.90	0.04
	6.3^a		-1.34^a	

[a] This value was calculated using rate constants at 20°C and 30°C.
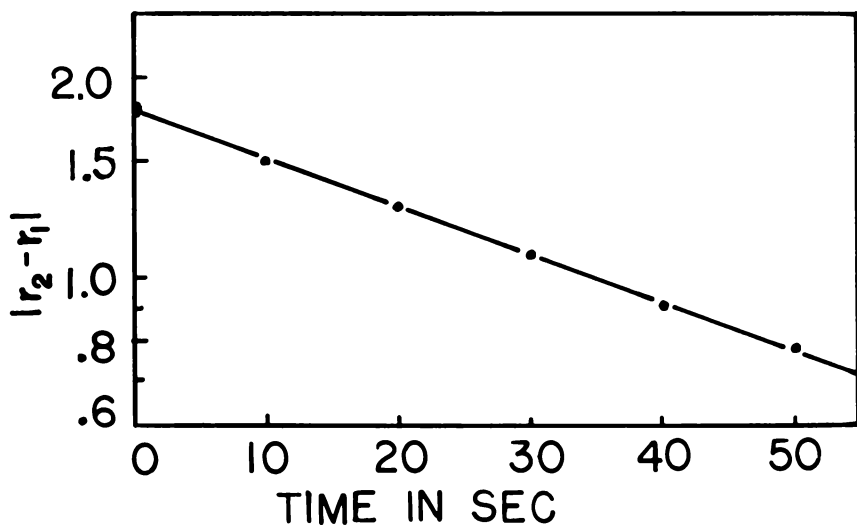[b] Tseng and Stewart (1964).

Figure 1. Typical Guggenheim plot (eq. 1) of rate data. D_2O at 20°C. $E_0 = 1.0 \times 10^{-4}$, and $S_0 = 1.12 \times 10^{-5}$.
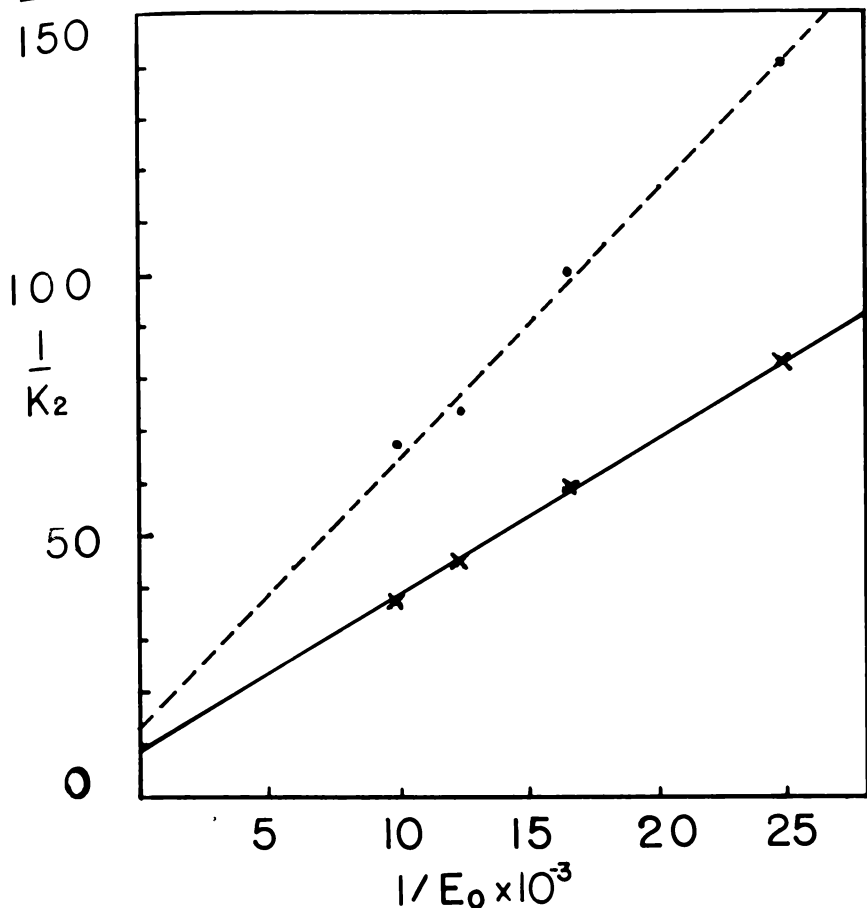
Figure 2.   Lineweaver-Burk plot (eq. 3) of the k values obtained from Guggenheim plots of the rate data. The solid line is for H_2O and dashed line for D_2O at 30°C; $S_0 = 1.12 \times 10^{-5}$ M.

strate concentration had to be large enough to be measurable but small enough to be considerably less than the amount of enzyme used. At low temperature (10°C) the reaction was extemely slow and there was a lack of solubility. As illustrated in Figure 3, the results for D_2O are scattered. This scattering appears to be caused by the result at 10°C. If this result is neglected, then the data in Table 2 are in good agreement with those in the literature (Tseng and Stewart, 1964; Laidler, 1958:202-205).
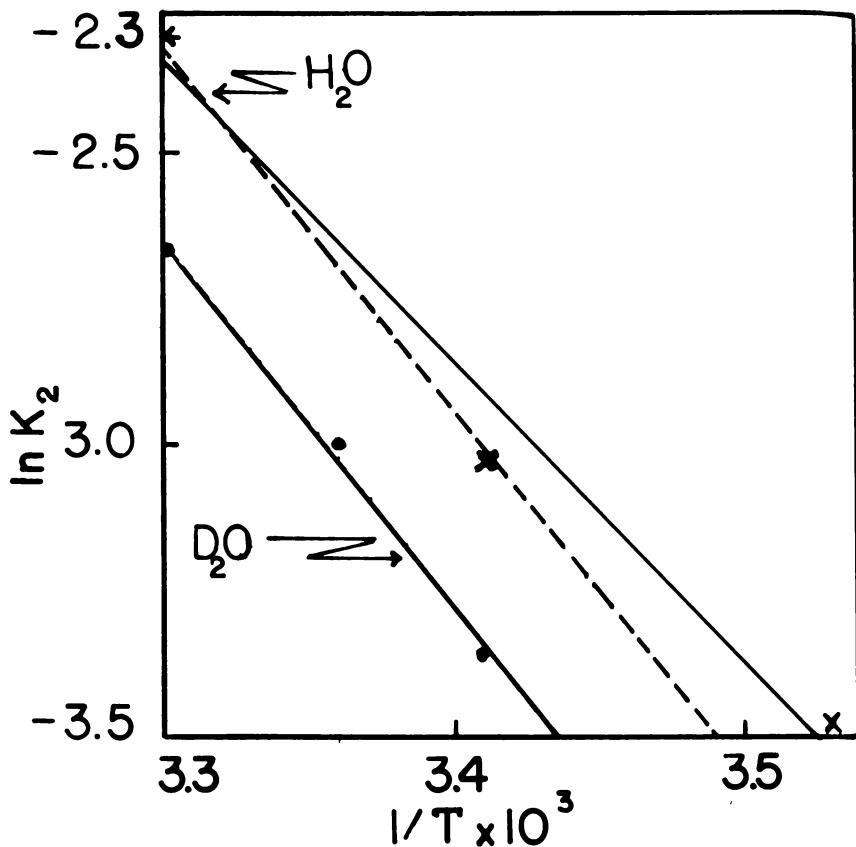
Figure 3. Inverse temperature plot (eq. 5) of the acylation rate constants (k_2) for H_2O and D_2O (●). The dashed line for H_2O is in agreement with the literature (Tseng and Stewart, 1964; Laidler, 1958).

Although the magnitude of ΔH^* is important, this constant does not lend itself to the same detailed interpretation as ΔS^*. The ΔS^* values in Table 2 are negative and compare favorably with published results obtained for chymotrypsin with other substrates (Laidler, 1958:202-205). The results can be interpreted in two ways, both of which lead to negative ΔS^* values. In terms of structure, the rate-controlling step concerns the release of product from the enzymic site. This process is believed to cause a refolding of the enzyme, and therefore, a negative entropy change. Secondly, solvent also plays an important part. If the product is more polar than the reactants, then the transition state will probably involve a

change in polarity; this will bring about the freezing of solvent and a negative entropy change. The influence of solvent is further amplified by the location of the substrate in the hydrophobic cavity of chymotrypsin (Blow and Steitz, 1970). Any product that is released from this cavity must be resolvated; once again the entropy change is negative. This important aspect of catalysis is verified by a more negative $\Delta S^*{}_D = -25.8$ cal mole^{-1} for D_2O, than $\Delta S^*{}_H = -23$ cal mole^{-1} for H_2O. The more negative ΔS^* for the heavier isotopic solvent results from the increase in mass transported over the free energy barrier when an increase in solvation occurs in the transition state (Tseng and Stewart, 1971).

The results for the ΔS^* corresponding to the k_2/k_m ratio are also of interest. In Table 2, these results are positive. This is expected if these constants are related to complex formation (Laidler, 1958). K_m is given by equation 4. If $k_2 > k_{-1}$, $k_2/K_m = k_1$; or if $k_2 < k_{-1}$, $k_2 / K_m = k_2 k_1$. For the latter case the $\Delta S^* + \Delta S^*{}_1 - \Delta S^*{}_{-1}$, since ΔS^* is an exponential term in equation 5. Because the values of ΔH^* for k_2/k_m are near zero and ΔH for $1/K_m$ is exothermic, it would appear that $k_2 < k_{-1}$ and $1/K_m$ is the equilibrium constant for complex formation between enzyme and substrate.

Thus a critical analysis of the results in Table 2 suggests that the chymotrypsin-catalyzed hydrolysis of an ester acquires at least part of its rate enhancement from solvation. When the substrate enters the hydrophobic pocket it is stripped of solvent and ΔS^* (k_2/K_m) is positive, and when product is released it must be resolvated and ΔS^* (k_2) is negative. Also contributing to this is the structural unfolding and refolding of the active site that takes place during catalysis (Laidler, 1958). The entropy changes for this phenomenon are in the same direction as the desolvation and resolution processes.

ACKNOWLEDGMENTS

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