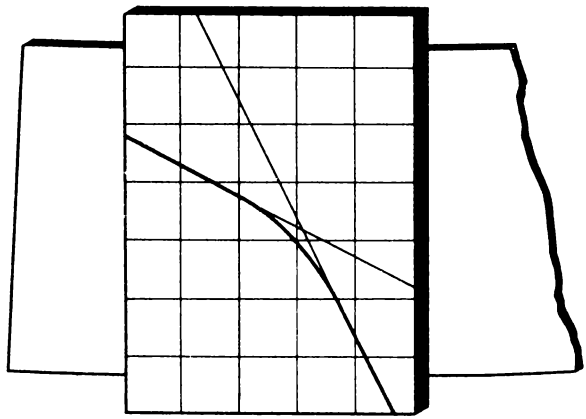
PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

ABSTRACTS



66TH ANNUAL MEETING

April 25 - 27, 1974

North Dakota State University

Fargo, North Dakota

Volume 28 • Part I • April, 1974

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NORTH DAKOTA ACADEMY OF SCIENCE

(Official State Academy: founded December, 1908)

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The Requirement of LM Cells for Biotin and the Effect of Varying Biotin Levels During Growth on Biotin Transport. Joy K. Anderson and James R. Waller. Dept. of Micro., Univ. of North Dakota, Grand Forks, ND.

It has been difficult to assess the biotin requirements of mammalian cells, since most tissue culture media contain high levels of the vitamin. We have developed a method to produce peptone which is biotin free, as determined by microbiological assay. Minimal essential medium (MEM) supplemented with a 0.5% biotin-free peptone was used as a test system to control the biotin level. When LM cells were placed into biotin-free medium we were able to demonstrate a biotin requirement within 2 weeks. Further studies indicated that these cells required approximately 4 X 10^{-9} M biotin (ca.1 ng/ml) for optimal growth. Previous work had shown that biotin transport in LM cells grown in medium 199 (10 ng biotin/ml) occurred by simple diffusion. Cells were grown with graded amounts of biotin (0, 0.1, 10 and 1000 ng/ml) for 8 days and were then used in transport studies. All cells transported comparable amounts of the vitamin. Transport was not dependent on temperature or on the presence of an energy source. Thus biotin uptake in cells grown in any level of biotin seemed to occur by simple diffusion and this uptake did not seem to be under metabolic control.

GEOLOGIC INPUT FOR PLANNING IN CASS AND CLAY COUNTIES, NORTH DAKOTA AND MINNESOTA. B. Michael Arndt and Stephen R. Moran. North Dakota Geological Survey and Dept. of Geology, Univ. N. Dak., Grand Forks, ND 58201.

The NDGS has provided, at the request of the Fargo-Moorhead Metropolitan Council of Governments, a report on geologic data for land-use planning in Cass and Clay Counties. The report is divided into a summary of the land, mineral, and groundwater resources and a series of interpretive maps dealing with general construction conditions and three types of waste disposal.

Aspects of the physical environment of Cass and Clay Counties include: (1) surface earth materials and their distribution, (2) mineral resources, (3) groundwater resources, (4) potential of pollution of these groundwater resources, (5) flooding hazard, and (6) agricultural suitability of the soils.

Bearing capacity, shear strength, compactibility, slope stability, water-table depth, and the existence of special conditions were used to evaluate general construction conditions in the two-county area. Sanitary landfill suitability ratings were made on the basis of permeability of the earth materials, water-table depth, availability of suitable cover material, and ease of excavation and compaction when wet or frozen. Permeability of the earth materials, depth to water table, flooding hazard, and presence of organic soils were the criteria used to evaluate suitability for sewage lagoons. Permeability of the earth materials, depth to water table, and flooding hazard were the criteria used to evaluate suitability for septic tanks. The potential for pollution of groundwater was considered in evaluating the suitability of sites for waste disposal facilities.

COMPOSITION AND VEGETATION RESPONSE TO MANAGEMENT TREATMENTS IN THE SHEYENNE GRASSLANDS. W. T. Barker, H. Goetz and D. O. Erickson, Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak.

Fertilization, burning and mowing treatments have been carried out and studied during 1971, 1972 and 1973 in relation to a three-pasture deferred rotation system of grazing. Six fertilization treatments (33#N, 48#P, 33#N + 48#P, 67#N, 67#N + 48#P and 100#N (expressed in actual lbs. of elemental N and P per acre)) were used. Spring (April 20) and Fall (October 15) burning treatments were made. Mowing was done on June 1, July 1 and Aug. 1. Grazed and ungrazed areas in all treatments and controls were compared on the basis of standing crop (above ground yield) at the end of the growing season. In addition, available soil moisture was determined on a weekly basis and the pH, available $-NO_3$, extractable P and extractable K of the soil was determined on a biweekly basis during the growing season. The management practices of fertilization, burning and mowing in the Sheyenne Grasslands increase utilization of the grassland forage by livestock.

SHEYENNE BASIN STUDY-AN ECOLOGICAL APPROACH TO LAND USE PLANNING. S. D. Bexell* and D. R. Scoby. Depts. of Architecture and Botany, North Dakota State University, Fargo, N. Dak. 58102.

Land use planning is an analyzing process taking a holistic approach to all aspects of land usage. The Lower Sheyenne Basin was selected for this study because of its diversity, critical areas (sand hills), and projected development. Basic land use problems were first identified, then a comprehensive procedure was formulated. The goal of the study was to analyze land use policies within the constraints of the basin's environmental system. A secondary data collecting investigation included as many of the physical, biological and socio-economic aspects of the basin as time and availability of data permitted. Based on ecological restraints the land use pattern for the Sheyenne Basin was summarized as: productive agricultural land in the Red River Valley; a sand hills area acting as a major aquifer recharge source; the area above the river's banks has some potential for development of uses other than agriculture and grazing, preservation of wetland area in the Northwest portion of the basin should be a management goal, and the gallery forest on the flood plain should be restricted to recreational use.

PRIMARY PRODUCTION IN CULTURALLY ENRICHED LAKE SALLIE, MINNESOTA FOLLOWING WEED HARVEST. D. F. Brakke and J. K. Neel. Dept. of Biology, Univ. N. Dak., Grand Forks, N. Dak. 58201

Phytoplankton photosynthesis and respiration increased from 1972 to 1973, both being greatest in August. Maximum gross primary production was 780 mg C/m^3/hr. Production increase in 1973 was greatest at 0.5 meters. Considerable daily and seasonal variation was found at all depths. Photosynthesis was often inhibited by high light intensities. Maximum production moved to or near surface and greatest efficiency occurred (max., 34.98 mg C/m^3/hr/ly/hr) when bloom conditions developed and incident radiation declined. Supported by USEPA (Grant R800490).

A TECHNIQUE FOR MEASURING OXYGEN CONSUMPTION IN SCUBA DIVERS. D. M. A. Brennan, W. W. Bolonchuk, and S. J. Brumleve. Dept. Physiol. & Pharm., Sch. of Med., and Dept. Health, Phys. Ed. & Rec., Univ. No. Dak., Grand Forks, ND 58201.

To design and test a technique for measuring oxygen consumption (VO_2) the subjects, trained SCUBA divers, breathed gas mixtures at appropriate O_2 concentrations (16%, 21%, 29%) in a diluent gas (N_2) at depth (1 ATA, 1.3 ATA) using standard open-circuit SCUBA. The gas bubbles from one expiration were collected underwater by displacement into a plastic bag. Plastic syringes (50 cc) with 3-way stopcocks were used to obtain an aliquot of the expired tidal volume 4 times/15 min test period (T_0, T_5, T_{10}, T_{15}). The gas samples, stored in syringes packed in ice water to inhibit diffusion, were analyzed for O_2 concentration (%) by gas chromatography. Gas volumes used from the SCUBA were calculated for each time interval from calibrated, submersible, pressure gauges. Using the Haldane transformation as a basis for determining VO_2, we were able to demonstrate a reliable technique for measuring the VO_2 of SCUBA divers usable at any depth for any length of time. (Supported in part by ONR Contract No. N00014-68-A-0499 and NIH Grant No. 1 T01 HL05939-01A1.)

DETERMINATION OF TRACE RESIDUES OF 2,4-D AND 2,4-DICHLOROPHENOL IN RED POTATOES. D. Bristol, L. Cook and M. Koterba. Dept. of Biochem., Residue Research Lab., N. Dak. State Univ., Fargo, N. Dak. 58102.

Two methods were developed to determine residues of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DCP (2,4-dichlorophenol) in red potatoes. Total residues were determined after hydrolysis of macerated potato tissue in 2N sulfuric acid whereas free residues were determined without an initial hydrolysis step. Residues were extracted with organic solvent, separated by liquid chromatography on alumina and determined by gas chromatography utilizing an electrolytic conductivity detector. The total residue procedure was used to quantitate 2,4-D and 2,4-DCP down to 0.02 and 0.002 ppm, respectively, with overall average recoveries of greater than 80%. Dissipation studies showed that 2,4-D residues decrease while 2,4-DCP residues increase in whole treated tubers stored at 38°F and that 2,4-D is stable while 2,4-DCP dissipates slowly during frozen storage of chopped potato samples. The cooperation of Dr. D. C. Nelson, Dept. of Hort., N. Dak. State Univ. and Dow Chemical U.S.A. in conducting this work is gratefully acknowledged.

DETERMINATION OF TOXAPHENE RESIDUES IN SUNFLOWERS. D. Bristol, D. D. Hintz and J. T. Schulz., Dept. of Biochem., Residue Research Lab. and Dept. of Entomology, N. Dak. State Univ., Fargo, N. Dak. 58102.

The insecticide Toxaphene was used in the summer of 1973 to control infestations of thistle caterpillar (Vanessa cardui) and sunflower beetle (Zygogramma exclamationis) in commercial sunflowers grown in North Dakota and Minnesota. The resulting residue level was studied and data collected in support of a petition for registration of Toxaphene for this use. The analytical method employed in the residue analysis of sunflower seed samples utilized hexane extraction, fuming sulfuric acid-Celite column cleanup, dehydrochlorination and electron capture gas chromatographic detection of chemically modified Toxaphene. Recovery experiments carried out over a range of 5 to 0.05 ppm averaged > 80%. No residues of Toxaphene greater than 0.05 ppm were detected in treated seed samples.

A STUDY OF CHANGING STUDENT ATTITUDES AS REVEALED THROUGH AN ANALYSIS OF THE EDITORIALS OF THE DAKOTA STUDENT. J. L. Coffin. Dept. of Anthropology, Univ. N. Dak., Grand Forks, N. Dak. 58201

In order to analyze the nature of student attitudes during several decades, the editorials of the Dakota Student were used to ascertain whether what is now being said on the University of North Dakota campus is in any way different from what was being said by students in previous eras. Five issues from each of the Fall and Spring semesters from 1943 to 1973 were used and the editorials were divided into 8 categories, namely athletic program, academic program, student behavior, student activities, politics, student values, institutional ceremonies and traditions, and miscellaneous. Each category was plotted onto matrices which divided them into five groups: those at the campus, local, state, national, and international levels; and, as to whether they were supportive or critical of traditional behaviors. Finally, each category was analyzed in 10 year periods. The sum total of editorial analysis would indicate that there is a new student on the campus if such a student can be defined as one who is increasingly more mature, more academically oriented, more committed to social issues, and one who desires greater participation in institutional affairs.

STUDENT PROJECT IN RENAL SURGERY: PHASE II - EXPERIENCE WITH AUTOGRAFTS. D. Cooper, D. Shabert, M. Montgomery, R. Bowman and M. Koponen. Division of Science, Minot State College, Minot, North Dakota 58701.

The overall goal of the project is the study of the effect of artificial perfusion solutions on the kidney, and the development of a solution suitable for preservation.

Autografts have been attempted in two ways: bilateral nephrectomy with unilateral autograft and unilateral nephrectomy with unilateral autograft. Bilateral nephrectomies were unsuccessful. One unilaterally nephrectomized animal survived until removal of the contralateral kidney. Refinements in surgical anaesthetic and laboratory techniques have been suggested.

Perfusion techniques are under development. A balanced salt solution has been selected for testing, a perfusion chamber has been constructed and histological preparations from a perfused kidney have been examined.

THE EFFECTS OF A LOW LEVEL OF DIETARY CADMIUM IN RATS. J.J. Doyle, R.A. Bernhoft, L.A. Rubin, K.P. Vo-Khactu, A.J. Raymond, and H.H. Sandstead. University of North Dakota and USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

The effects of 5 μg Cd/ml of drinking water on some biochemical and physiological parameters in male and female rats were studied. After 205 days, cadmium had no significant effect on weight gain, feed intake or feed efficiency. However, it depressed the water intake of the males relative to the controls. Total cadmium intake in the cadmium treated males and females was 29.0 and 32.1 mg respectively. The erythrocyte counts and hematocrits of the males were significantly ($p < 0.05$) increased at 114 days by cadmium but the reverse was true in the females. There were no significant differences in WBC counts or hemoglobin between treatments. In vivo retention of Na^{24} was greater in the cadmium treated males than in the controls at 161 days. In vivo retention of K^{42} was significantly greater in the cadmium treated males than in the controls at 189 days but the opposite was true in the females. No significant differences in systolic blood pressure or serum cholesterol were found between treatments at 171 or 212 days respectively.

MINERALOGY OF THE CLAY-SIZE FRACTION OF THE TONGUE RIVER AND SENTINEL BUTTE FORMATIONS NEAR MEDORA, NORTH DAKOTA. Richard Emanuel and Arthur F. Jacob. Dept. of Geology, Univ. of N. Dak., 58201.

X-ray diffractograms of over 80 samples of the Tongue River Formation and 7 samples of the Sentinel Butte Formation indicate that the principle minerals of the clay-size fraction of both formations are mica, montmorillonite-group minerals, halloysite, chlorite, quartz, dolomite, calcite, and feldspar. The 14-15A montmorillonite peak is much higher in samples of the Sentinel Butte Formation, than in samples of the Tongue River Formation, and it is always higher than either the 10A mica peak or the 7.13A haloysite peak. In Tongue River samples, these 3 peaks are of more nearly equal height and the 10A mica peak is commonly higher than the 14-15A montmorillonite peak. The 3.34A quartz peak and the 2.88A dolomite peak are generally higher in Tongue River samples than in Sentinel Butte samples.

Attempts to correlate clay mineralogy with depositional environments in the Tongue River Formation were not successful.

CHEMICAL COMPOSITION OF COMPOSITE AND SELECTED RANGE GRASSES. D.O. Erickson, W.T. Barker, C.N. Haugse and M.L. Buchanan. Dept. of Animal Science, NDSU, Fargo, N.D.

Composite samples of low, mid and upland range and ten of the most common and abundant range grass species found in the Sheyenne Grasslands were sampled bi-weekly from June to October. Chemical fractions which relate to nutritional value were analyzed to determine the variations due to season and species. Correlations were used to determine the relationships of the chemical fractions to each other and digestibilities. Protein, phosphorus, magnesium and potassium were inversely related to season in the composite range and in all of the species except the protein in Stipa comata and Poa pratensis. Protein levels dropped to 3 and 4% in October in some species but did not get below 6.5% in the composite range. In most of the species the fibrous fractions of fiber, lignin and cell wall increased as the season progressed and this was reflected in decreased digestibilities. The mineral contents varied considerably among species and season of the year. Phosphorus levels of many of the species and the composite range decreased as the season progressed and do not meet the requirements of ruminants in any stage of their reproductive cycle. Calcium levels were more consistent with season and among species.

FORAMINIFERIDS FROM THE CANNONBALL FORMATION (PALEOCENE, DANIAN) AND THEIR PALEOENVIRONMENTAL SIGNIFICANCE: GRANT, MORTON AND OLIVER COUNTIES, NORTH DAKOTA. W. E. Fenner. Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58201

In June and July, 1972, 240 samples (of which 55 contained microfossils) were collected from six measured sections in the Cannonball Formation (Paleocene, Danian) in Grant, Morton and Oliver Counties, North Dakota. Twenty-six species of benthonic foraminiferids were identified from these samples: 6 textulariines, 2 miliolines and 18 rotaliines. No planktonic foraminiferids were found. The fauna is characterized by a predominance of individuals of textulariines, especially the lituolids. Although the Cannonball Formation is characterized by an alternating sequence of sandstones and mudstones, the foraminiferid fauna was restricted to the mudstone facies in the upper and upper-middle part of the formation. Two characteristic assemblages based on dominant families and genera are recognized, the lituolid and nodosariid assemblages. The two assemblages and the occurrence of the microfauna in the mudstone facies, suggests nearshore, shallow (less than 100 to 200 m deep), protected environments such as shallow bays behind barrier islands or offshore bars. The dominance of textulariines in the sediments is indicative of lower than normal marine salinity.

METABOLIC ASPECTS OF PLANT COMPETITION. D. S. Galitz. Bot. Dept., N. Dak. State Univ., Fargo, N. Dak. 58102.

Comparative evaluations of major metabolic processes have been informative in explaining the varied development of plants growing under competitive conditions. C.C. Black, et al (Weed Sci. 17:338-344, 1969) have pointed out the importance of the relationship between C-3 and C-4 pathways of carbon assimilation, photorespiration and water requirements in competition. Our studies of reduction and assimilation of nitrate nitrogen in several different species indicate that the responsiveness of the enzyme nitrate reductase (NR)(NADH: nitrate oxidoreductase, EC 1.6.6.1) to changes in light, nitrate availability and temperature may serve as another valid factor in determining the competitive potential of a species. Measurements of endogenous levels of NR show that both light and nitrate are required for in vivo development of the enzyme though not for nitrate ion accumulation in the tissue. The level of extractable NR activity, within limits, was a direct consequence of nitrate ion concentration and light intensity. Data on leaf tissue nitrate, soluble protein, dry matter and enzyme activity reflect these interactions. The relative potential of different species to reduce nitrate and the influence of the light-nitrate interaction on the actual measurable nitrate reducing capacity under experimental conditions is discussed with the aid of a model.

DETERMINATION OF THERMAL CONDUCTIVITY OF ROCK CHIP SAMPLES BY THE DIVIDED BAR METHOD. Paul Gourley Physics Dept., Univ. of N. Dak., Grand Forks, N. Dak. 58201

As a part of a joint effort supported by both the UND Physics Department and the North Dakota Geological Survey for the determination of geothermal heat flow values at western N.D. sites, the thermal conductivities of 45 rock chip samples were measured at 40°C. The samples were selected from the drillings of 3 oil holes and consisted predomintely of shales and limestones. The divided bar method was employed for measurements and the simple volume fraction model for multicomponent systems was assumed for the unknown stack element. The measured thermal conductivity values range from 4 to 12 mcal.cm/cm^{2}sec.C°. Results were reproducible to within ±5%.

Supported in part by UND Faculty Research Fund.

STUDIES OF STRUCTURE AND MOTION IN HYDRAZINE COMPOUNDS BY NMR. Paul Gourley and J. W. Harrell, Jr. Physics Dept., Univ. of N. Dak., Grand Forks, N. Dak. 58201

The spin-lattice relaxation time, T_1, of the protons in hydrazinium hydrogen oxylate, $N_2H_5HC_2O_4$, has been measured over the temperature range -100°C to 200°C by the pulse technique at frequencies of 45MHz and 25MHz. These measurements are an extension of earlier measurements made at 30MHz over a limited temperature range (N. Dak. Acad. Sci, Proc. 25(I):13). From 60°C to 200°C $\ln T_1$ varies linearly with -1/T while from 60°C to -50°C a 1/T linear dependence is observed. The slopes of these lines yield an activation energy of 0.37 eV. A minimum in the $\ln T_1$ vs. 1/T curve is evident at 67°C. A reported phase transition near -100°C was also investigated. In order to further investigate the internal motion and structure of $N_2H_5HC_2O_4$, deuteron magnetic resonance spectra were obtained from single crystals grown from a solution of $N_2H_5HC_2O_4$ in D_2O. The results for $N_2H_5HC_2O_4$ have been compared with results obtained by other workers on other hydrazine compounds.

TRACE ANALYSIS OF ORGANOMERCURY COMPOUNDS -CONDITIONS FOR WET OXIDATION IN THE DETERMINATION OF TOTAL MERCURY. Patrick Graven and D. Bristol. Dept. of Biochem., Residue Research Lab., No. Dak. State Univ., Fargo, N. Dak. 58102.

In developing an analytical method for determination of trace levels of mercury in biological samples, the recovery of mercury in the form of mercuric chloride and methyl mercuric chloride was seen to be dependent upon the conditions of the acid digestion step (J. Assoc. Offic. Anal. Chem., 56:378, 1973). We investigated the response of several mercury compounds to various temperatures and heating periods of wet digestion in a 2:1 H_2SO_4:HNO_3 mixture. Graphical plots of the per cent recovery versus the temperature have shown that ethylmercuric chloride and merthiolate are more easily oxidized to the mercuric ion than methylmercuric chloride, phenylmercuric hydroxide, and phenylmercuric acetate. This information is useful in determining the optimum conditions for analysis of the mercury content in environmental samples containing mercury in an unknown form.

ENERGY VALUES OF ALFALFA, BARLEY AND OATS IN RATION FOR SHEEP. Olafur Gudmundsson, W.E. Dinusson, D.O. Erickson and C.N. Haugse. Dept. of An. Sci., NDSU, Fargo, N.D.

In a change over design experiment with yearling ewes, energy values in feed, pH and volatile fatty acids (VFA) concentrations and ratios in the rumen were determined. The rations were pelleted alfalfa, barley or oats. Chromic oxide paper was used as a marker and total intake of feed and water were recorded. Feed, feces and rumen fluid samples were collected. The gross, digestible and metabolizable energy (Mcal/kg) were respectively 4.20, 1.92, 1.59 for alfalfa; 4.29, 32.0, 2.63 for barley; and 4.42, 2.76, 1.58 for oats. The system developed by Rattray et al. (J. Anim. Sci., 36:115, 1973) was used to calculate the net energy for maintenance (NEm), net energy for gain (NEgw) and net energy for gain on wool free basis (NEg). The values (Mcal/kg) were respectively 0.85, 0.39, 0.33 for alfalfa; 1.68, 1.00, 0.97 for barley; and 1.39, 0.79, 0.75 for oats. Feeding alfalfa resulted in higher ($P<.05$) C_2 concentration and lower ($P<.05$) C_3 and total VFAs concentration in the rumen fluid than feeding grain. The C_4 level was significantly ($P<.05$) different for the three rations with barley > oats > alfalfa. The C_2/C_3 ratio averaged 4.58, 1.53 and 1.29 respectively for the alfalfa, barley and oats with all values significantly ($P<.05$) different.

MULTIFUNCTIONAL GLUCOSE-6-PHOSPHATASE-PHOSPHOTRANSFERASE: EFFECT OF MEMBRANE INTEGRITY ON CATALYTIC BEHAVIOR. Hans M. Gunderson and Robert C. Nordlie, Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak. 58201

We reported earlier (Biochem. Biophys. Res. Comm., 52, 601-607, 1973) that both synthetic (carbamyl-P: glucose phosphotransferase; I) and conventional hydrolytic (II) activity of the above enzyme are found in liver nuclei. Maximal activity of I and II can be observed with an intact membrane (nucleus). Destruction of membrane integrity as occurs with isolated nuclear membrane, endoplasmic reticulum, and plasma membrane constrains I and II. This constraint can be relieved by detergents or other disruptive treatments. Ki values for Pi and ATP for intact nuclei closely match those for detergent treated fragmented membrane preparations. Electron micrographs of preparations from a variety of species show the nuclei to be intact and substantially free of contamination. Other studies include substrate specificity, pH effects, Km values, and modifications by various detergents and other factors. Involvement of activities of the enzyme in insulin independent glucose transport is suggested on the basis of its critical location in cellular membranes. Control of I and II through subtle, membrane mediated conformational changes is postulated. (Supported by NIH grant AM07141 and Amer. Diab. Assn.)

Practical Isolation Procedure for Esterification by the Catalytic Dehydrator, V.J. Gutenkunst and V.I. Stenberg. Dept. of Chem., Univ. N. Dak., Grand Forks, N. Dak. 58201

A practical product isolation procedure has been developed for the "Catalytic Dehydrator" method of synthesizing esters. (J. Org. Chem., 36, 2548, 1971). The "Catalytic Dehydrator" is a combination of a sulfonated polystyrene copolymer and drying agent ($CaSO_4$). It promotes equilibrium reactions which have water as one of the products. The following acids were used: benzoic, oxalic, trichloroacetic, undecenoic, phenylacetic, fluoroacetic and 1-naphthaleneacetic. The reaction time was six hours and the reactions were done in a separatory funnel. The optimum procedure is draining the separatory funnel containing the reaction mixture, washing of the solids with methanol and the eluents purified by two sets of distillations. The first distillation removes the excess methanol, and the second vacuum distillation purifies the ester. Yield analysis was carried out by glpc of the reaction products after a six-hour reaction time. Possible loss of the product due to retention of the esters on the "Catalytic Dehydrator" was studied by presoaking the acid polymer or both the acid polymer and drying agent with methanol but no effect on the results was observed. Quantitative ester yields were obtained with undecenoic, phenylacetic and trifluoroacetic acids.

TRANSIENT CIRCULATORY CONTROL IN CHLORALOSE-ANESTHETIZED CATS. R. E. Hammond, Dept. Physiol. & Pharmacol., Univ. N. Dak., Grand Forks, ND 58201 and E. W. House, Dept. Biol., Idaho St. Univ., Pocatello, ID 83201.

The degree of cardiovascular reflex depression present during alpha-chloralose anesthesia has long been a subject of controversy (A. J. P. 210:854, 1966). In this experiment, systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were measured from the left common carotid artery in adult cats before and after administration of alpha-chloralose (80 mg/kg, i.v.). Postural changes were used to evaluate cardiovascular reflex activity. In anesthetized animals (head down), SBP, MAP and HR increased significantly above controls ($P < .05$). In anesthetized cats (head up), SBP and MAP were significantly lower than controls while HR was significantly higher than controls ($P < .05$). In unanesthetized cats (head up and head down), SBP, MAP and HR remained essentially unchanged. Chloralose-anesthetized cats showed evidence of cardiovascular reflex depression via baroreceptor hypofunction and diminished vasomotor activity.

THE TOXIC EFFECTS OF SWAB MATERIALS ON A HERPES VIRUS. B. R. Hanson and I. A. Schipper. Dept. of Vet. Sci., N. Dak. State Univ., Fargo, N. Dak. 58102.

Recovery of infectious bovine rhinotracheitis (IBR) virus from calcium alginate wool was compared with virus recovery from cotton swabs. The adsorptive and elution properties of cotton and calcium alginate wool swab materials were studied by direct exposure of the swab materials to IBR virus in buffered tissue culture medium at 4 C. Calcium alginate wool was found to be virucidal to IBR virus. This was apparent after exposure of the virus to calcium alginate wool for only 2 h. Sonication of the calcium alginate wool-virus suspension and use of buffered tissue culture fluids at pH range of 5.5 to 8.0 did not elute the virus. Freezing and thawing decreased the virus titer. The cotton swabs did not significantly lower the titer of the virus when exposed to the virus for a maximum of 48 h. From this study it can be concluded that cotton is superior to calcium alginate wool for optimum recovery of IBR virus in diagnostic virology. Diagnostic swab specimens should not be frozen, but maintained at 4 C until used for inoculation on tissue cultures.

THE INTESTINAL PISCINE ACANTHOCEPHALA FROM MCMURDO SOUND, ANTARCTICA. Harry L. Holloway, Jr. and James A. Spence.

Leiper and Atkinson (1915) described *Echinorhynchus campbelli*, *E. rennicki* and *E. debenhami* from cods. Johnston and Best (1937) synonymized *E. rennicki* with *E. campbelli* and transferred the concept and *E. debenhami* to *Leptorhynchoides* Kostylew, 1924. Although some morphological features of the worms do not agree with the definition of the genus. They also suggested that *L. debenhami* may be identical with *Echinorhynchus megarhynchus* Linstow, 1892. However, Sinzar (1959) transferred *E. megarhynchus* to *Aspersentis* Van Cleave, 1929. *L. debenhami* does not conform to the characteristics of *Aspersentis*. This study is based on the comparative analyses of entire & sectioned worms; 52 collected during the Austral summer of 1964-65, from cods and eel pouts; 21 collected during the Austral winter of 1965-66 from cods; and 14 specimens of the three original species from the British Museum of Natural History. We concur in the actions of Johnston and Best (1937) and further suggest that the definition of *L.debenhami* is based on immature worms strikingly similar to *L. campbelli*. Subsequently, in addition to formally acting on this similarity, lectotypes of *L. campbelli* will be designated. The one genus and three species of intestinal spiny headed worms formerly recognized appear to represent only one species. Supported by N.S.F. Grants GA 146, 228 and 495.

GLUCOSE SYNTHESIS IN THE ISOLATED PERFUSED RABBIT LIVER. C.A. Huibregtse and P.D. Ray. Dept. of Biochemistry, Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak., 58201.

The intracellular distribution of phosphoenolpyruvate carboxykinase in rabbit liver plus available information regarding the process of gluconeogenesis as it occurs in the rabbit suggests that this species may provide a good model of glucose formation as it occurs in humans. In order to investigate further the process of glucose formation in rabbits, we have adapted the technique of perfusing isolated rabbit livers with rabbit red blood cells suspended in oxygenated Krebs-Ringer-HCO_3^- buffer; thus we can study liver processes *per se* in the absence of external influences. Following determination of glucose utilization by the rabbit red blood cells in the perfusate, we determined that livers isolated from 48-hour fasted rabbits synthesize glucose at a rate of 0.15 μmoles/min/g liver in the absence of exogenous substrate. We subsequently established the ability of such livers to synthesize glucose in the presence of 10 mM lactate, alanine or glycerol to be about 0.44, 0.21 and 0.37 μmoles glucose/min/g liver respectively. Establishment of these basic rates of glucose formation from such substrates has allowed us to investigate various means of regulating gluconeogenesis in rabbits and to compare the regulatory mechanisms observed to those noted in other species. (Supported by NIH AM 12705 and by the American Diabetes Assoc.)

STRATIGRAPHY AND DEPOSITIONAL ENVIRONMENTS, HUSKY LIGNITE MINE, DICKINSON, NORTH DAKOTA. Arthur F. Jacob. Dept. of Geology, Univ. of N.Dak., Grand Forks, N.Dak. 58201.

In the Husky Lignite Mine near Dickinson, North Dakota, there are sand beds that are about 10 feet thick, have erosional bases, and become finer-grained from bottom to top. They contain high-angle cross strata in the lower part and mixed low-angle, straight, cross strata and small-scale, curved sets of cross strata in the upper part. They are interpreted as deposits of point bars in high-sinuosity streams. Results of Cotter's (1971) method of analysis indicate that the streams that deposited the sand beds were approximately 10 feet deep, 60 feet wide, had sinuosities of about 2, mean annual discharges of about 150 cubic feet per second, meander lengths of about 700 feet, drainage areas of about 200 square miles, stream lengths of about 30 miles, and channel slopes of about 3 feet per mile.

Yellow-brown clayey and sandy silt beds that contain climbing-ripple cross strata are interpreted as deposits of natural levees. Lignite beds and gray beds of clay and silty clay that have a high organic content, distorted stratification and iron-sulfide nodules are interpreted as deposits of flood basins. Beds of evenly laminated clay and silty clay are interpreted as deposits of lakes and ponds on the alluvial plain.

ZINC AND COPPER ANALYSIS OF EDIBLE FATS AND OILS. R.A. Jacob and L.M. Klevay. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

Accurate and sensitive analysis for zinc and copper in foods is required by the association of metabolism of these metals with the epidemiology of coronary heart disease (CHD) (Klevay, Am. J. Clin. Nutr. 26: 1060, 1973). Fats and oils which have also been implicated in the epidemiology of CHD have been particularly difficult to analyze for trace amounts of the metals. Extraction of the oils with hydrochloric acid ethylenediaminetetraacetic acid (EDTA) solution followed by atomic absorption analysis results in recoveries of 98% and 96% and reproducibilities of $\pm$ 0.001 µg/g and $\pm$ 0.004 µg/g for copper and zinc, respectively, for samples with metal concentrations of 0.06 µg/g. The method is more sensitive and accurate than the direct method (Official, J. Amer. Oil Chem. Soc. 49: 432A, 1972) and more rapid than the char-ashing method of Evans, et al. (J. Amer. Oil Chem. Soc. 48: 840, 1971). It seems apparent that the extraction technique could be used to preconcentrate other metals from fats and oils.

EFFECT OF ETHANOL ON INTESTINAL LYMPH IN THE RAT. F.A. Jacobs and T.W. Winter. Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N.D. 58201

Experiments were carried out to ascertain the effect of ethanol on lymph flow and protein transport into intestinal lymph of Sprague-Dawley rats weighing 250-400 g. Eighteen hours after cannulation of the mesenteric lymph duct animals were perorally fed a fatty test meal consisting of either, 1) bovine cream (12.5% butterfat) 7 vol. blended with 3 vol. 0.85% NaCl; or 2) 10% corn oil emulsified in 0.2% aq. deoxycholate mixed 7:3 with 0.85% NaCl. Lymph samples were collected for 4 hours and then a second "meal" was fed with 95% ethanol substituted for the saline as in the first meal. Protein concentration was determined colorimetrically and protein and lipoprotein constituents determined by strip and disc gel electrophoresis. Changes induced by ethanol resulted in: 1) an increase in the flow rate of lymph from five to ten fold; 2) a corresponding increase in the movement of protein; 3) changes in the electrophoretic distribution of lipoprotein. It was also observed that lymph flow was inhibited when deoxycholate was used. Supported in part by NIH Research Grant No. MH 19235 ALC).

AMMONIA-NITROGEN EXCRETION BY DAPHNIA. T.R. Jacobsen and G.W. Comita. Dept. of Zoology, North Dakota State University, Fargo, N. Dakota 58102

Ammonia-nitrogen excretion by Daphnia was measured during the period, May 30 - October 8, 1973 from organisms collected from Silver Lake, Minn. Four to ten organisms were incubated in 15 ml tubes containing 0.45 u filtered lake water for 0.4 to 1.0 days with similar water in the controls. The method of Solorzano (1969) was used for the determination of ammonia. Ammonia nitrogen excretion for the period varied from an initial value of 0.260 on 30 May to a peak of 0.483 ug-N animal^{-1} day^{-1} on the 12 of June; then continually decreased to 0.046 ug-N animal^{-1} day^{-1} on October 1. Ammonia-nitrogen excretion mg^{-1} dry body wt varied from an initial high of 16.8 ug-N mg^{-1} dry wt day^{-1} on 30 May, then decreased continually to a low value of 2.0 ug-N mg^{-1} dry wt day^{-1} on October 1. Concurrent Chlamydomonas reinhardii-fed Daphnia from the same populations showed similar results. The relationship between ammonia-nitrogen excretion by Daphnia and lake temperature was investigated.

AFFINITY CHROMATOGRAPHY OF A SUGAR-UNSPECIFIC NUCLEASE FROM TOBACCO CELL CULTURES. A. M. Janski* and A. E. Oleson. Dept. of Biochem., NDSU, Fargo, N. Dak. 58102.

A multifunctional phosphohydrolase (EC 3.1.4.9) possessing RNase, DNase and 3'-nucleotidase activities is present in the extracellular medium of cell cultures of Nicotiana tabacum. This enzyme was partially purified by chromatography with phosphocellulose, DEAE-cellulose and Sephadex G-75. Nucleotides bearing a 2'-phosphomonoester group competitively inhibited the 3'-nucleotidase activity of the phosphohydrolase. The enzyme exhibited much greater affinity for substrate (3'-AMP) and two inhibitors (2'-AMP and $NADP^+$) at pH 5.5 than at pH 7.0. The value of the K_i for $NADP^+$ was 0.009 mM at pH 5.5 and 0.26 mM at pH 7.0. Periodate-oxidized $NADP^+$ was coupled to agarose-adipate hydrazide by the procedure of R. Lamed, et al. (Biochim. Biophys. Acta 304:231, 1973). The partially purified preparation of sugar-unspecific nuclease readily adsorbed to $NADP^+$-agarose at pH 5.5-6.0. The enzyme was eluted with dilute buffer at pH 7.0.

TRANSITORY VESTIBULAR DYSFUNCTION FOLLOWING DIRECT APPLICATION OF LIDOCAINE (XYLOCAINE) TO THE MIDDLE EAR IN GUINEA PIGS. C. B. Jensen and B. De Boer. Dept. Physiol. & Pharm. Univ. No. Dak., Grand Forks, ND 58201.

Eye movements were electronically recorded from guinea pigs following unilateral application of 1% Xylocaine to the external auditory canal. Nystagmus and impaired equilibrium were observed in one animal. Autopsy revealed a ruptured tympanic membrane on the treated side. Unilateral application of Xylocaine to the middle ear resulted in deviation toward the treated side, and nystagmus toward the opposite side within 45 min. Recovery occurred within four hours. Bilateral application of Xylocaine to the middle ear resulted in decreased righting ability; nystagmus was weak or absent. Fenestration of the treated labyrinth produced no significant alterations. Fenestration of the contralateral labyrinth depressed nystagmus significantly. Xylocaine does not penetrate the tympanic membrane but presumably enters the internal ear if access to the middle ear is permitted. Nystagmus results from an imbalance in the tonic impulses from the left and right labyrinths. (Supported in part by ONR Contract No. N00014-68-A-0499 and NIH Training Grant No. 1 T01 HL05939-01A1.)

DIFFERENTIAL EFFECTS OF Mg^{2+} ON VARIOUS ACTIVITIES OF GLUCOSE-6-PHOSPHATASE: SOME OBSERVATIONS AND METABOLIC DIRECTIVE IMPLICATIONS. W. T. Johnson, W. Colilla, and R. C. Nordlie. Biochem. Dept., Med. Sch., Univ. N. Dak., Grand Forks, ND 58201

The effects of added Mg^{2+} on various hydrolytic and synthetic activities of hepatic D-glucose-6-phosphate phosphohydrolase have been investigated using four different types of enzyme preparations. Supplemental Mg^{2+} was found to exert little (<5%) or no stimulatory effect on either glucose-6-P or mannose-6-P phosphohydrolase activities. However, phosphotransferase activities were either unaffected (mannose-6-P:glucose phosphotransferase), modestly inhibited (carbamyl-P:glucose phosphotransferase), or extensively inhibited when PPi, CTP, ATP, GTP, or ADP was the phosphoryl donor. Moreover, no stimulation of ATP:glucose phosphotransferase activity by supplemental Mg^{2+} was observed at pH 7.0, but Mg^{2+} did ameliorate the inhibition by ATP of glucose-6-P phosphohydrolase activity. From various kinetic observations and a noted direct, positive correlation between the binding constants of various phosphate substrates with Mg^{2+} and observed extents of inhibition by Mg^{2+} of enzymic activity, the observed inhibition probably involves chelation of Mg^{2+} with phosphate substrates which diminishes their ability to bind to the enzyme. These studies also suggest that Mg^{2+} may function as an activity-discriminant metabolic director. Support: USPHS Grant AM-07141

PRODUCTION AND NUTRIENT CYCLING ON TWO SLOPES IN A NATIVE FOREST ECOSYSTEM. *Keith T. Killingbeck* and *Mohan K. Wali*, Dept. of Biol., Univ. of N. D., Grand Forks, N. D. 58201.

An intensive study on aspects of productivity and nutrient cycling is being carried out at the Forest River Biology Area in northeastern North Dakota. Study sites, located on opposing slopes of a stream-cut ravine, bear a dominant vegetation of *Quercus macrocarpa, Fraxinus pennsylvanica* var. *subintegerrima* and *Tilia americana*. The mean coverage value for the tree stratum of the east slope is 82.1% while the corresponding value for the west slope is 96.2%. For the shrub stratum, the mean coverage values for east and west slopes are 82.1 and 86.7%, respectively. Although soils on both slopes are predominantly sandy clay loams, the east slopes have a higher mean pH (7.7) and electrical conductivity (70.8 μmhos/cm) as compared to west slopes (6.7 and 51.6 μmhos/cm, respectively). Mean litter production for the east slope was 2.8 m.tons/ha while for west slopes, the mean was 3.4 m.tons/ha. Throughfall was 11% higher on the west slopes. Measurements of rainfall, stemflow, throughfall, and litterfall are being monitored regularly. Lysimeters have been installed to determine quantitatively the chemical nature of percolating waters through the profile. *Supported by UND Faculty Research Grant No. 4522.*

TYPE AND REFERENCE SECTIONS FOR A NEW MEMBER OF THE FOX HILLS FM., CRETACEOUS, S.-CENTRAL N. DAK. M.C. Klett and J.M. Erickson. Dept. of Geol. and Geog., St. Lawrence Univ., Canton, N.Y. 13617

Field study in Emmons and Sioux Cos., N. Dak., has revealed a new stratigraphic unit of the Fox Hills Fm., herein named the Linton Member. It consists of a gray-green to cream, fine-grained, subangular, moderately to poorly sorted, well indurated, siliceous sandstone. Cross strata and flat bedding are present locally, the unit being generally structureless. Analyses indicate a range of lithologic constituents which place the rock between feldspathic arenite and subgraywacke. Vertical, preserved roots, Equisetum, and Ophiomorpha also characterize the unit. Wood fragments are abundant in upper beds. The member is exposed in central and southwestern Emmons and eastern Sioux Cos., N. Dak., eastern Corson Co. and southward in S. Dak. Exposure is generally limited to butte caps. Thickness ranges from 0.5 to 6 m. A type section is designated in N1/2 sec. 8 and 9, T.132N., R.76W., 1 mi. E. of Linton, N. Dak. Reference sections are located in NE1/4, NW1/4, sec. 28, T.130N., R.78W. Emmons Co. and 11 mi. W. of McLaughlin in northwestern Corson Co., N. and S. Dak. respectively. The Linton Member is described as a channel sand, deposited in and along a major, wide but shallow, southward-flowing estuarine tidal river. (Supported in part by NSF-URP grant GY-10713)

METALLIC ELEMENTS AND THE EPIDEMIOLOGY OF CORONARY HEART DISEASE. L.M. Klevay. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

The widely publicized hypothesis relating the risk of coronary heart disease (CHD) to the amount and type of fat habitually consumed fails to explain many diverse epidemiologic features of the disease. Kahn (Am. J. Clin. Nutr. 23: 879, 1970) has estimated that only 8% of the increase in risk of mortality due to CHD in the U.S. in the last half century can be attributed to changes in the consumption of fat.

Experiments with animals (Klevay, Am. J. Clin. Nutr. 26: 1060, 1973) have shown that an increase in the ingested ratio of the zinc to copper produces a metabolic imbalance resulting in hypercholesterolemia. A similar imbalance may account for the hypercholesterolemia of pregnancy, the lowering of cholesterol in plasma with ethylenediaminetetraacetate, the metabolism of cholesterol in histidinemia, the production of hypercholesterolemia in rabbits by the feeding of histidine and the lowering of cholesterol in serum by the feeding of calcium.

The increased risk of CHD associated with increased consumption of sugar, consumption of soft water, lack of exercise and decreased consumption of vegetable fiber are consonant with a similar imbalance in the metabolism of zinc and copper.

EFFECT OF AN ORAL HYPOGLYCEMIC AGENT ON EMBRYONIC MORTALITY IN RATS. C. Kubik, J. Tilton, C. Haugse and M.L. Buchanan. Dept. of An. Sci., NDSU, Fargo, N.D.

Virgin female rats were used in six trials to determine the effect of the hypoglycemic agent Dymelor (Eli Lilly) on embryonic mortality. Rats were fed the agent for various lengths of time post-breeding at two different dose levels. Blood glucose levels were determined and the glycogen content of various tissues analyzed. Also, the ovulation rate, number of viable embryoes, and detectable resorptions were noted. In trials (I-VI) the percent embryonic mortality, respectively, for the control and treatment groups was C- 19.9, 28.0, 26.4, 34.8, 24.5, 28.5; T- 28.0, 20.1, 16.1, 17.1, 16.4, 14.8, ($P < .01$). In trial IV the muscle glycogen content was higher in the control group ($P < .01$) while the fetal glycogen content was higher in the treatment groups in trials III and IV ($P < .01$) and VI ($P < .05$). The number of detectable resorptions were higher in controls than treatment animals in trials IV-VI ($P < .01$). Data suggests a significant benefit of a hypoglycemic agent, possibly through making available the maternal stores of glycogen to the developing embryo. It appears that no further benefits would be derived from any higher drug level or extended length of feeding beyond 5 mg per day per animal for six days.

USE OF FLUORESCENT ANTIBODY FOR THE RAPID DETECTION OF *SALMONELLA* IN WATER. S. L. Kunkel and M. C. Bromel. Dept. of Bacteriology, N. Dak. State Univ. Fargo, N. Dak. 58102

The fluorescent antibody (FA) technique was used as a means of detecting *Salmonella* in water. Water samples were passed through a membrane filter (pore size 0.45 um), to concentrate the organisms. The entire filter and accompanying organisms were immersed in 15 ml of Selenite Cystine Broth (S-C) (BBL), and incubated at 43° C. After 12 hours incubation, the membrane filter was removed and the S-C was passed through another membrane filter and washed with 50 ml sterile phosphate buffer (pH 7.2). The filter with enriched bacteria was re-enriched in S-C and incubated at 43° C for 8 hours. Smears and touch slides were then made from the broth and filters respectively, and the direct FA procedure was applied with Bacto-FA Salmonella Poly (Difco). Slides were examined by use of an AO Fluorolume microscope. The presence of any rod-shaped, fluorescing bacteria was designated as a positive result. Biochemical and serological confirmation followed. Using the S-C *Salmonella* re-enrichment technique, no cross-reactions with other genera of the *Enterobacteriaceae* were observed. Supported in part by North Dakota Water Resources Research Institute.

DECOMPOSITION AND LEACHING OF METHYL 2-BENZIMIDAZOLE CARBAMATE IN SOIL. H. M. Lacy, I. R. Schultz, and J. R. Fleeker. Dept. of Biochem., N. Dak. State Univ., Fargo, N. Dak. 58102.

The decomposition and movement of methyl 2-benzimidazole carbamate (MBC), a fungicidal metabolite common to both benomyl and thiophanate-methyl, was studied in Fargo silty clay, Barnes sandy loam, and Towner loamy fine sand. When MBC-$^{14}CH_3$ was incubated in soil, 15.6 percent of the applied radioactivity was recovered as $^{14}CO_2$ over a 53-day period. Under the same conditions, using MBC-2-^{14}C (ring-labeled), 0.4 percent of the applied ^{14}C was recovered as $^{14}CO_2$. 2-Aminobenzimidazole was found in soil incubated with MBC. MBC did not move in soil columns on leaching with 18 cm of water, nor did the fungicide affect soil nitrification or respiration at levels of 100 ppm.

SUBMERGED AQUATIC VASCULAR PLANTS OF NORTH DAKOTA.
G. E. Larson and W. T. Barker. Dept. of Botany, N. D. S. U., Fargo, N. Dak. 58102

During 1973, a three year taxonomic study of North Dakota aquatic vascular plants was initiated. This paper presents some interesting notes on the submerged aquatic vascular plants occurring in the state. Species of the following genera are discussed: Potamogeton, Ruppia, Najas, Zannichellia, Elodea, Heteranthera, Ceratophyllum, Ranunculus, Callitriche, Myriophyllum, Hippuris and Utricularia. As a result of fieldwork and herbarium study, additional distribution data and some interesting taxonomic problems have been revealed. Variants in Ruppia were found which have been treated both as separate species, R. maritima L. and R. occidentalis S. Wats., and as varieties of a single species, R. maritima var. rostrata Agardh and R. maritima var. occidentalis (Wats.) Graebn. Our representatives of the Ranunculus aquatilis complex, which consist of R. subrigidus W. B. Drew and R. longirostris Godr., have previously been lumped under R. aquatilis L. (or R. trichophyllus Chaix).

Effects of Aging on Histone Methylases of Rat Brain and Liver.
Catherine T. Lee and John A. Duerre. UND Med. School, Grand Forks, ND 58201.

The methylation of brain and liver histones from rats of different age classes were studied by incubating the nuclei from both organs with S-adenosyl[^{3}H-methyl]methionine at 37° for 30 min. The histones were extracted from chromatin with 0.4 N HCl. The specific activities of brain histones were 142,000 dpm/mg from new borns and 26,000 dpm/mg from 900-day-old animals. The values for liver histones were 65,000 and 28,000 dpm/mg respectively. The labeled histones were subfractionated on Bio-Gel P-10 columns. Histones F_3 and F_{2a1} were the only components found to be highly methylated. The products of methylation were mainly mono- and dimethyllysine. The histone methylases were isolated from the liver and brain nuclei of young and old animals. The enzyme activity was determined by incubating the methylases with histones prepared from brain and liver nuclei from young and old rats in the presence of S-adenosyl[^{3}H-methyl]-methionine. It was found the reduction in the degree of histone methylation was due to the decrease in methylase activities. There were only small differences in the ability of the histones from either the young or old animals to serve as methyl group acceptors. (Supported by NIH Grant 5 R01 NS09725.)

CONSIDERATION OF THE SO_2 GROUND-LEVEL CONCENTRATIONS FROM POWER PLANT OPERATIONS. K. W. Li. Dept. of Mechanical Engineering, North Dakota State University, Fargo, North Dakota 58102.

In the advent of the coal development in North Dakota, the public has become concerned with the possible environmental impacts. One of the environmental problems is a high sulfur dioxide ground-level concentration in the downwind distance from power plant stacks. This paper is intended to present a mathematical model for prediction of SO_2 ground-level concentration. Also revealed in this paper are the relationships between the concentration level and plant design parameters. These include the stack height, stack arrangement, distance between the stacks, and other parameters. One numerical example has been prepared for illustration. Other engineering approaches to the SO_2 controls are briefly discussed.

AN INVESTIGATION OF THE PHYSICAL AND CHEMICAL PARAMETERS OF SHEEP LIVER 6-PHOSPHOGLUCONATE DEHYDROGENASE. K.E. Lorenzen and J.E.D. Dyson, Biochem. Dept., Univ.N.Dak.Med.Sch., Grand Forks, N.Dak. 58201

The second oxidative enzyme of the pentose phosphate pathway, 6-phosphogluconate dehydrogenase, isolated from sheep liver (Dyson, J.E.D., D'Orazio, R.E., and Hanson, W.H. (1973), Arch. Biochem. Biophys. 154, 623) has been studied with respect to its chemical and physical properties. Sedimentation velocity, sedimentation equilibrium, and Sephadex G-200 column chromatography studies demonstrate a mol. wt. of 106,000 daltons for the native enzyme. Treatment of the enzyme with SDS (sodium dodecyl sulfate) and study by disc gel electrophoresis, sedimentation equilibrium and CPG-10-370 (Controlled Pore Glass 10-370 A) indicate a dissociation of the enzyme into two 50,000 mol. wt. species. Upon further treatment of the enzyme with dithiolthreitol, a reduction in molecular weight to 25,000 daltons occurs. Total amino acid analysis following performic acid oxidation, together with titration of the enzyme with p-chloromercuribenzoate, suggests the presence of two cystine residues per 106,000 mol. wt. With the substrates $NADP^+$ and 6-phosphogluconate, binding studies indicate two substrate binding sites per 106,000 mol. wt. Thus these results suggest an enzyme composed of two similar subunits, each containing an active site, each subunit containing two chains held together by a disulfide bridge.

ATTITUDES TOWARD COAL DEVELOPMENT AND POST-GRADUATION PLANS OF HIGH SCHOOL SENIORS IN WESTERN NORTH DAKOTA - A PRELIMINARY REPORT. Clark S. Markell and David Bickel. Experimental Coll. and Cooperative Ed. Progr., Minot State Coll., Minot, ND 58701

A sample that included about 10% of the more than 5,000 high school seniors from 28 western North Dakota counties responded to a questionnaire designed to reveal attitudes toward existing and proposed coal development in the Williston Basin. The post-graduation plans of young adults about to assume a greater role in the manpower resources of the state were ascertained. The envisioned impact of new economic development and the present socio-economic environment may be important factors to young adults in personal decision-making concerning their future and their relationship to the region. Irregardless, these attitudes have immediate importance to government agencies, industrial and educational leaders concerned with economic planning, environmental development, and human resources. Correlations were made between students' attitudes and perceptions about coal development and several personal variables. A multiple regression was also carried out using interest in coal development as a dependent or criterion variable. Attitudinal data collected in counties which have experienced little if any lignite development was compared with counties where development has occurred.

ZINC DEFICIENCY IN THE PRENATAL RAT. J.M. McKenzie, G.J. Fosmire and H.H. Sandstead. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

Offspring of rats fed a zinc-deficient diet during the last trimester of pregnancy show impaired behavioral responses at 6 weeks of age even though they had been fed adequate zinc from delivery (Halas et al., Int. Rev. Neurobiol., Suppl. 2; In Press). This paper describes biochemical findings on fetuses taken by cesarean section on the 22nd day of pregnancy from dams fed the zinc-deficient diet from the 15th day of gestation. The findings are compared to those of fetuses from pair-fed (undernourished) and ad libitum fed control dams which had been given adequate zinc. Zinc-deficient and pair-fed fetuses weighed less than fetuses from ad libitum dams. Placental weight and brain weight of the zinc-deficient fetuses were not affected, while liver weight of the zinc-deficient fetuses was reduced in comparison with the ad libitum control fetuses; pair-fed fetuses had intermediate values. DNA and RNA analyses revealed no striking differences in brain or placental values; liver DNA was reduced in the zinc-deficient fetuses. The incorporation of tritiated thymidine into DNA suggested that biosynthesis of DNA was lower in the brains of the zinc-deficient fetuses, intermediate in the pair-fed controls, and higher in the ad libitum controls.

PHYSIOLOGICAL RESPONSES OF IMMATURE STAGES OF THE HOUSEFLY TO ALCOHOLS, ALKYNES AND FATTY ACIDS. F. W. Mosha and M. S. Quraishi. Dept. of Entomology, N. Dak. State Univ., Fargo, ND 58102

We have discussed (J. Econ. Entomol., 64:787, 1971 and Can. Ent., 104:1505, 1972) the selective responses of various developmental stages of house flies to saturated and unsaturated fatty acids and their esters. This paper deals with the effects of nonanols (1-,2-,4-,5-), octynes (1-,2-,3-,4-,) and octynoic acids (3-,4-,5-,6-) on immature stages of house flies by topical application and food treatment. Some of these chemicals were found to possess selective action on the developing wing and on the emergence of the adult. Melanized and unmelanized puparia responded differently to some of the chemicals tested. In general, melanized puparia were more resistant to the toxic effects of 1-,2- and 3-octynes and 5- and 6-octynoic acids. In the cases of nonanols and other chemicals these differences were not so marked.

CHARACTERIZATION OF PURINE NUCLEOSIDE ORTHOPHOSPHATE RIBOSYLTRANSFERASE FROM BOVINE THYROID. Thomas P. Moyer and Allan G. Fischer, Biochemistry Department, North Dakota State University, Fargo, North Dakota 58102.

Establishment of the existence of xanthine oxidase (E. C. 1. 2. 3. 2) in bovine thyroid as a possible source of H_2O_2 necessary for iodination of thyroxine has led to the study of purine metabolism in the gland. A purine nucleoside orthophosphate ribosyltransferase (E. C. 2. 4. 2. 1) has been partially purified from the cytosol. The enzyme is specific for purine nucleosides, with the specificity being pH dependent. The K_m's and K_i's will be discussed for various substrates to establish which of the many possible purine routes is most probable. Phosphoribosyl transferase (E. C. 2. 4. 2. 8) and ribohydrolase (E. C. 3. 2. 2. 1) catabolic activities were not found in the same preparations.

PHYLLOPLANE BACTERIA ON NORTH DAKOTA-GROWN FIELD CORN. J. S. Mullins and D. G. Davis, USDA, ARS, Metabolism and Radiation Research Laboratory, State University Station, Fargo, N. Dak. 58102, and D. S. Galitz, Department of Botany, NDSU, Fargo, N. Dak. 58102.

The phylloplane bacterial flora of North Dakota-grown corn (Zea mays L.) was studied over the 1972 and 1973 growing seasons by the replicate organism detection and counting (Rodac(R)) plate method and by scanning electron microscopy (SEM).

The Rodac technique gave reproducible results but has definite limitations in estimating the numbers and kinds of bacteria present. SEM micrographs showed the pattern and habit of the bacteria, including cell division. Bacteria isolated were Bacillus spp. and coryneform rods in 1972 and mainly Bacillus polymyxa in 1973.

EFFECT OF NICKEL DEFICIENCY ON HEPATIC PROTEIN SYNTHESIS IN THE RAT. D.R. Myron and F.H. Nielsen. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

In the nickel deficient rat, sucrose density gradients of liver post mitochondrial supernatant fractions (PMS) have been consistent with a decrease in polysomes and an increase in monosomes. To pursue this apparent influence of dietary nickel on hepatic protein synthesis, experiments were conducted to study the rates of liver regeneration and amino acid incorporation following partial hepatectomy in nickel deficient and supplemented control rats. Dietary nickel had no influence on the rate or regain of liver mass. However, the rate of protein synthesis, as measured by ^{14}C-leucine incorporation in vitro by liver PMS, was consistently higher in the nickel deficient rats throughout the period of liver regeneration. Furthermore, sham-operated and unoperated nickel deficient rats also exhibited greater protein synthetic activity than controls. Finally, the PMS from these two groups was separated into polysomal and cell sap fractions, and tested for activity in an amino acid incorporating system. In contrast, the greater activity with either component was derived from the nickel supplemented group. Thus, it appears that a factor promoting amino acid incorporation in the PMS of nickel deficient rats is lost upon further resolution into cell sap and polysomal fractions.

CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF THE MISSOURI RIVER BELOW GARRISON DAM. Joe K. Neel, Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak. 58201

A 2.45 mi river reach approximately 20 miles below Garrison Dam, and shortly downstream from entrance of the Knife River, has discharge and water quality generally controlled by Garrison Reservoir releases. Chemical and plankton data indicate persistence of individual flow paths but some cross channel movement. Nitrogen occurred consistently as NO_3-N but rarely as NH_3- or NO_2-N. Soluble reactive phosphorus was absent for extended periods but reached high concentrations on three dates. Plankton was dominated by diatoms and at times considerably influenced by Knife River inflow. The shifting sand bottom permitted macrobenthos development only in quiet areas behind jetties and in shoal pockets. Macrophytes occurred widely in shallows outside strong current paths. Supported by Basin Electric Power Cooperative.

MOLLUSKS OF THE SHEYENNE RIVER, NORTH DAKOTA: PRESENT AND PAST. R. D. Norby, A. M. Cvancara, and J. B. Van Alstine. Dept. of Geol., Univ. Ill., Urbana, Ill. 61801(RDN), and Dept. of Geol., Univ. N. Dak., Grand Forks, ND 58201

The Sheyenne River in eastern North Dakota was studied for living and fossil mollusks primarily during the summer of 1966; additional data were taken in 1968, 1969, and 1973. Thirty-one stations were sampled for living species, and fossils were collected from exposures at six sites along the middle and lower reaches of the river. Twenty-nine species were living in the river: 10 unionid bivalves (mussels), 6 sphaeriid bivalves (pill clams), and 13 gastropods (4 prosobranch and 9 pulmonate snails). Only two mussels were found in Lake Ashtabula; four snails and two pill clams were generally more frequently found in the lake than elsewhere. Two snails, *Gyraulus circumstriatus* and *Aplexa hypnorum*, were found only in Sheyenne Lake. *Campeloma decisum*, represented by empty shells from a lower station in the river, is newly reported for the state. River discharge appears to primarily affect the occurrence of mollusks. Long periods of no flow restricts mussels and high discharge correlates with greater number of mussel species. Certain pill clams and snails occurred more frequently in flowing water and others in stagnant (non-flowing) water. High turbidity may also limit all groups of mollusks. The fossil fauna generally resembled the living fauna but lacked 12 living species; two mussels (*Quadrula quadrula* and *Proptera alata*) may only occur as fossils. Mollusks inhabited the "ancestral" Sheyenne River that flowed into Lake Agassiz about 13,500 years ago, and the modern Sheyenne at least as early as about 2,500 years ago.

METABOLISM OF THE HERBICIDE PROPHAM (ISOPROPYL CARBANILATE) IN THE CHICKEN AND GOAT: ISOLATION AND IDENTIFICATION OF METABOLITES IN TISSUES AND MILK. G. D. Paulson and A. M. Jacobsen. U. S. Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, N. Dak. 58102

Chickens and a goat were given a single dose (100 mg/kg body wt) of isopropyl carbanilate-[phenyl-^{14}C(U)]. Radiolabeled metabolites in goat milk, goat tissue, and chicken tissue were isolated by solvent extraction and column chromatography and then identified by comparative spectrometry. Goat milk and goat tissue contained the sulfate ester of isopropyl 4-hydroxycarbanilate and two minor unidentified carbon-14-labeled fractions. Chicken tissue contained the sulfate ester of isopropyl 4-hydroxycarbanilate, the glucuronic acid conjugate of isopropyl 4-hydroxycarbanilate, isopropyl carbanilate, and two minor unidentified carbon-14-labeled fractions.

PRELIMINARY INVESTIGATION OF THE INDIAN RESERVE GRANODIORITE PLUTON, SOUTHWESTERN ONTARIO. W. M. Peterson and F. R. Karner. Dept. of Geology, Univ. of N. Dak., Grand Forks, N. Dak. 58201.

The pluton outcrops about one km northeast of Kejick at the north end of Shoal Lake at latitude 49° 38' N and longitude 95° 04' W. The area of exposure is about 6 km^2 and has an elliptical shape about 4.0 km by 1.6 km with the major axis trending E-W. Field study at 60 locations and point-count analysis of 30 thin sections shows that the rock is typically a pink, medium-grained, hypidiomorphic granular, granodiorite with minor oligoclase phenocrysts and scattered, small, greenstone xenoliths. The rock consists of 50% oligoclase, 26% quartz, 13% slightly perthitic microcline, 4% biotite, 3% sericite, 2% epidote and minor opaques, sphene and apatite.

Observations of possible major genetic importance include the following: (1) Microcline abundance ranges from about 5% to 20% and is concentrated in the northwestern part of the exposed area and in the margins. Oligoclase ranges from about 40% to 60% and is concentrated in the core. (2) East-west linear trends of mineral abundances are faintly discernible on contour maps. (3) Oligoclase typically contains two or three, thin, euhedral to subhedral, internal alteration zones marked by a concentration of fine-grained sericite and epidote. Supported by National Science Foundation Undergrad. Res. Participation Grant GY 10699.

Descent of the Rocket of MOLNIYA 2-6 into the Earth's Atmosphere. Franz H. Rathmann, North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota 58102; and Klaus Gunther John, Institut für Geophysik und Meteorologie, Braunschweig, 33, West Germany.

The descent of the rocket of the Soviet satellite MOLNIYA 2-6 into the earth's atmosphere on August 21, 1973, was observed by tens of thousands of amateur astronomers, and chance observers. Some hundred persons on a 300-mile-wide path from El Paso, Texas, to Thunder Bay, Ontario, have sent descriptions of their observations, including one photograph. Most of the observers saw only a single object, with flaming pieces breaking off, but St. Croix Interstate Park observers saw at least twenty separate rocket paths. According to NORAD, the satellite itself has an elliptical path with major axis about 53,100 kilometers, perigee and apogee distances of 415 and 39,956 km. The rocket path observed leads via Iceland, the USSR, Indian Ocean, Antarctica, back to Texas.

Aryl-Isoxazoles and Aryl-Halo Oximes. Franz H. Rathmann and James Knecht, Department of Chemistry, North Dakota State University of Agriculture and Applied Sciences, Fargo, North Dakota 58102.

Dimethoxybenzaldehydes were converted to their oximes, chlorinated to the corresponding hydroxamic acid chlorides, and condensed with sodio-acetoacetic ester to yield 3-(dimethoxyphenyl)-5-methyl-isoxazole-4-carboxylic acid esters. The 2,5-dimethoxybenzaldoxime was treated with bromine to yield 2,5-dimethoxybenzald-bromo-oxime. The bromoxime reacted with methyl, ethyl, and tert-butyl alcohol to form the corresponding C-alkyl esters of the 2,5-dimethoxybenzhydroxamic acid. Similarly, the bromoxime reacts with ammonia, aniline, etc., to form the corresponding hydroxamic acid amides, anilides, etc. Several new ester, amide, etc., derivatives of 3-phenyl-5-methyl-isoxazole-4-carboxylic acid were also prepared.

LATE CENOZOIC DRAINAGE EVOLUTION IN NORTHWESTERN McKENZIE COUNTY, NORTH DAKOTA. Nena L. Salomon. Dept. of Geology, Univ. N. Dak., Grand Forks, ND 58201

Several stages in the evolution of the Yellowstone River drainage system are represented within an area of 180 square miles in northwestern McKenzie County, North Dakota. During the earliest stage, the river deposited sandy gravel containing chert and volcanic and plutonic rocks; the upper surface of this gravel is about 270 feet above the present flood plain of the Yellowstone River. Subsequent downcutting was interrupted by a glacial advance that dammed the river, forming a lake in which evenly bedded silt and clay were deposited. As the glacier retreated, a series of overflow channels drained the lake. Drainage was reestablished and gravel derived from local bedrock was deposited in the tributaries of the Yellowstone River. Drainage was again disrupted by two glacial advances. As the last glacier retreated, gravel containing chert and carbonate, volcanic, and plutonic rocks was deposited in a series of meltwater channels. The upper surface of this gravel is about 40 feet above the present flood plain of Charbonneau Creek and is graded to about the level of the lowest terrace along the Yellowstone River. Gravel underlying this terrace contains chert and volcanic, plutonic, and carbonate rocks. Renewed downcutting established the present flood plains of the Yellowstone River and Charbonneau Creek.

Supported in part by the North Dakota Geological Survey.

ARCHAEOLOGICAL INVESTIGATIONS AND INTERPRETATIONS OF A TIPI RING SITE, 32SH205. F. Schneider and P. Treat. Dept. Anthro.-Arch., UND, Grand Forks, N.D. and Dept. Anthro., Univ. Missouri, Columbia, Mo.

Archaeological investigations at a site of 81 tipi rings located in Sheridan County, North Dakota, were conducted as part of the archaeological salvage program in the Garrison Diversion Project. The site is located at the junction of the McClusky Canal and Lonetree Reservoir.

The excavation of four of the rings revealed unexpected results. Previous investigators of tipi ring sites report finding small amounts of cultural material. Our excavations not only found an abundance of cultural material, but this material occurred in a nonrandom distribution indicating activity areas in and around the tipi rings. Also of note is the high frequency of Knife River Flint associated with lithic artifacts. The source of this material is believed derived from prehistoric quarries located west of the Missouri River. The site has two cultural components, Besant(A.D. 100-600) and a Late Prehistoric component.

Supported by U.S. Bureau of Reclamation and contracted through the National Park Service.

COMPARATIVE EFFECTS OF DIETARY VITAMIN A AND p,p'-DDT ON MICROSOMAL ANILINE HYDROXYLASE ACTIVITY AND VITAMIN A IN LIVERS OF YOUNG QUAIL AND DUCKLINGS. Mamduh Sifri, Jerry L. Sell and Kenneth L. Davison. An. Sci. Dept., NDSU & MRRL, USDA, Fargo, ND.

Two experiments were conducted using mallard ducklings and Japanese quail. For one week post hatching, birds were fed a semipurified diet devoid of vitamin A to deplete their vitamin A stores. At eight days of age, the birds were allotted randomly to four dietary treatments: no vitamin A or 6000 I.U. of vitamin A per kg of diet; no p,p'-DDT or 100 ppm of p,p'-DDT arranged in a complete factorial. Experiments were of two weeks duration. Vitamin A concentration in livers of ducklings and quail was highest for those birds fed 6000 I.U. of vitamin A. Birds receiving dietary DDT stored less vitamin A in their livers than did those fed the same diet without DDT. This indicated that DDT affected vitamin A metabolism and/or storage in the liver. The inclusion of vitamin A, DDT, or both in the ration significantly increased microsomal aniline hydroxylase activity in livers of ducklings. However, the same ration treatments decreased the enzyme's activity in quail. This is further evidence of genuine differences among species of the Aves class concerning the effect of p,p'-DDT (N. Dak. Acad. Sci. Proc. 27:33, 1973) on metabolism.

Effect of Ethanol Ingestion on Choline Phosphotransferase and Phosphatidyl Ethanolamine Methyltransferase Activities in Liver Microsomes, David N. Skurdal and W.E. Cornatzer, University of North Dakota, Grand Forks, N.D. 58201

The assay of choline phosphotransferase which catalyzes CDP-choline and α,β-diglyceride and phosphatidyl ethanolamine methyltransferase which catalyzes the methylation of phosphatidyl ethanolamine to form phosphatidyl choline have been determined and expressed as specific activity (nmoles phosphatidyl choline/min/mg protein) in liver microsomes. Female rats were fed a liquid diet for 2 and 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. There was a significant increase in the activity of the choline phosphotransferase in the liver microsomes in the animals fed 5% ethanol for 2 weeks when compared to pair-fed controls. There was a significant increase in the activity of phosphatidyl ethanolamine methyltransferase in the liver microsomes in the animals fed ethanol for 2, 6 and 10 weeks when compared to controls fed sucrose.

PROJECT LIGNITE: CONVENIENCE FUELS FROM NORTHERN GREAT PLAINS PROVINCE LIGNITE. A. M. Souby, Eng. Exp. Sta., Univ. N. Dak., D. E. Severson and W. R. Kube. Dept. of Chem. Eng., Coll. of Eng., Univ. N. Dak., Grand Forks, N. Dak. 58201

The Office of Coal Research has established a project at the University of North Dakota having as its ultimate goal the determination of the feasibility of an integrated plant using solubilization, gasification, carbonization and hydrogenation to produce a variety of upgraded fuel products from North Dakota lignite. Initially, emphasis was placed on the study of the solution-hydrogenation process to produce solvent-refined lignite (SRL). A small continuous process development unit was designed and is being constructed. Batch autoclave tests were conducted to obtain necessary design data. This paper reports some of the initial results from the batch autoclave tests investigating various process variables and operating techniques. (Department of the Interior Office of Coal Research Contract Number 14-32-0001-1224).

A GENETIC ANALYSIS OF ADULT PLANT LEAF RUST RESISTANCE IN CHRIS WHEAT. G. D. Statler, Dept. of Plant Path., NDSU, Fargo, ND.

The inheritance of adult-plant leaf rust resistance was investigated in Chris wheat. Thatcher was used as the susceptible parent in reciprocal crosses with Chris for the genetic analysis. All the F_1 progeny were susceptible to culture 70-1 (race 1) Puccinia recondita f. sp. tritici, indicating that one or more recessive gene(s) conditioned adult plant leaf rust resistance in Chris. F_2 plants from reciprocal crosses segregated approximately three susceptible to one resistant when inoculated with culture 70-1 ($P > .25$). This suggested that resistance in Chris was conditioned by a single recessive gene. Backcross-F_1 plants from reciprocal crosses segregated approximately one resistant to one susceptible when inoculated with 70-1 ($P > .90$) Backcross-F_2 families from susceptible backcross-F_1 plants segregated three susceptible to one resistant and those from resistant backcross-F_1 plants were homozygous resistant. This supported the monogenic recessive gene hypothesis developed on the basis of F_2 data.

DIGESTIBILITY OF COMPOSITE AND SELECTED RANGE SPECIES.
G. Tenesaca, D.O. Erickson, W.T. Barker and C.N. Haugse.
Dept. of Animal Science, NDSU, Fargo, N.D.

In-vitro digestibilities were determined on composite range (low, mid and upland sites) and 10 of the more common species of the Sheyenne Grasslands. Samples (4 replicates) were taken bi-weekly during the grazing season (early June to mid October). The digestibility of the composite range and the individual species varied with the season but were significantly higher in the early summer compared to latter in the fall. The digestibility of the composite samples ranged from 77% (early summer) to 33% (October). Carex lanuginosa digestibility was lower (26% in October) than any other species. The digestibility of Bouteloua gracilis ranged from 84% in early June to a low of 39% in October. Juncus balticus in terms of digestibility was low the entire season ranging from 49% to 31%. The upland range was higher in digestibility in the latter part of the grazing period compared to low and mid sites. Digestibility varies with cool and warm season grasses and also with rainfall during the grazing season.

SURVEY OF SNOWMOBILE USE IN NORTH DAKOTA, K. F. Thompson, D. R. Scoby, and T. McDonald. Depts. of Botany and Soc.-Anthro., North Dakota State University, Fargo, North Dakota 58102.

A snowmobile use questionnaire was mailed to 25% of the registered snowmobile owners in North Dakota in March, 1973. The purpose was to determine snowmobilers' needs regarding facilities and controls, and to identify their attitudes toward the sport.

From 1468 (27.0%) usable returns, the survey revealed these characteristics: The average snowmobile owner is 38 years old, earns more than $10,000 a year, has 2 children and 1.5 snowmobiles. He is probably a farmer, retailer, or business or professional person. His family uses 7 gallons of gasoline weekly, mostly for pleasure riding. They do nearly half of their snowmobiling on weekends, much of it on private land. Almost a third have gone out-of-state to snowmobile, the majority to Minnesota.

Opinion questions dealt with drinking and snowmobiling, establishing minimum age requirements, effects on wildlife, safety hazards, and terrain and trail preferences. Additional comments were encouraged. In cooperation with the North Dakota State Outdoor Recreation Agency.

FACTORS CONTROLLING HEATING VALUES OF LOW-RANK COALS. Francis T. C. Ting. Dept. of Geol., Univ. N. Dak., Grand Forks, ND 58201

Weathering, inherent moisture and mineral matter, oxygen content, and regional increase in the degree of coalification toward the Rocky Mountains, have long been considered to affect the heating values of low-rank coals in North Dakota, Montana, and Wyoming. My studies indicate that petrographic composition and differential recovery in coring operations also play important roles in affecting and in evaluating the heating values of these coals. Liptinitic components (such as resinite and exinite) have higher heating values (16400-17200 Btu/lb) than huminite (12400-12600) and inertinite (11200-11800) on a dry basis. Presence of 5% liptinite could increase the heating value by more than 300 Btu/lb, assuming the average heating value of lignite as 11000 Btu/lb on a dry basis.

Coring is the most commonly used method to obtain a sample for chemical analysis, and cores are usually not fully recoverable. Loss of material is generally caused by the presence of friable layers in the coal that are readily disintegrated and removed by drilling fluid. Drilling during the summers of 1972 and 1973 has substantiated this observation. The friable layers are high in lower-heating value inertinites and mineral matter. Consequently, this biased result ensues a differential concentration of higher heating value components in the cored samples. At a power plant complex near Center, ND, the as-mined coal is consistently lower in heating value than the cored samples drilled before the mine was opened. The differences range between 200 to 400 Btu/lb, and average 300 Btu/lb. Differential concentration of high heating value components may explain this discrepancy. *Supported by NSF (Grant GA-31950).*

QUANTITATIVE AND QUALITATIVE DETERMINATION OF MORPHINE IN URINE. M. Tofano, C. Jensen, and B. De Boer. Dept. Physiol. & Pharm., Univ. No. Dak., Grand Forks, ND 58201.

The procedure described is simple and readily repeated. It is effective for either qualitative or quantitative analysis of morphine. Measured quantities of morphine were added to 10.0 ml urine samples. Saturated Na_2CO_3, was added to bring pH to 8.2. This solution was added to 20 ml of a Benzene-N Butanol solution (1:1) and shaken for 20 min. After extracting the morphine from the solvent layer with 5.0 ml of 0.1 H HCl it was divided into 2.0 ml and 3.0 ml aliquots. For qualitative analysis 2.0 ml was evaporated; redissolved in 1.0 ml absolute methanol and spotted (20 ul) on a Gelman ITLC Type SAFD media. The chromatograms were developed in ethylacetate, absolute methanol and NH_4OH (85:10:5). Spraying sheets with iodoplatinate solution aided detection. RF values of 0.25 were observed. For quantitative analysis, the 3.0 ml aliquot was prepared for spectrophotometry using a modified Nitroso method. Optical density was measured using a Beckman ACTA II using the tungsten lamp set at 444 mu and a slit of 0.15 mm. The method is adequate where small quantities of urine are available. (Supported in part by ONR Contract No. N00014-68-A-0499.)

METHODOLOGY FOR CONCENTRATING INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS FROM WATER. S. R. Tschider, I. A. Schipper, and D. L. Berryhill. Depts. of Vet. Sci., and Bact., N. Dak. State Univ., Fargo, N. Dak. 58102.

Four methods were employed to concentrate Infectious Bovine Rhinotracheitis (IBR) virus from one-liter quantities of distilled water. The membrane concentration procedure was found to be superior to the aluminum hydroxide ($Al(OH)_3$), polyelectrolyte 60 (PE 60), and two-phase systems utilized. Virus recovery rates ranged from 0-6% and 0-18% for the $Al(OH)_3$ and PE 60 procedures respectively, to 24-137% for the membrane concentration procedure. The two-phase dextran sulfate virus concentrate was found to be destructive to bovine kidney cell monolayers, and was therefore unsuitable. Cellulose nitrate membrane filters (0.45 μm pore size) efficiently adsorbed 4×10^4 IBR virus plaque-forming units per liter, and the viruses were recovered by elution with fetal calf serum while undergoing sonic treatment. The average recovery rate was 70.3%. The membrane adsorption method proved to be an inexpensive and efficient method for obtaining 100-fold concentrations of IBR viruses.

Herpesvirus hominis type 2 Latency in Rabbit Kidney Cells: Evidence Indicating a Non-Permissive Infection. J. Varani and J. J. Kelleher. University of North Dakota, Grand Forks, ND.

Incubation of rabbit kidney cells infected with Herpesvirus hominis type 2 at 41C resulted in no detectable production of virus. Following incubation at 41C, cultures were transferred to 37C and spontaneous reactivations of virus occurred in 85% of cultures. Lag periods of 1-45 days were observed after transfer to 37C. More than 24% of the cultures had lag periods of at least 7 days. The rate of thermal inactivation of the virus at 41C, as determined by measuring infectious virus in freeze-thawed cultures was logarithmic. Approximately 99% of the virus was inactivated within 13 hours. If, however, virus was assayed by transferring viable, infected cells to carrier cultures, as much as 1% of the virus could be detected in cells even after 6 days of incubation at 41C. This indicated that virus was present in some form that is not readily susceptible to thermal inactivation. Experiments employing BUDR substantiated this. This drug effectively inhibits virus growth under normal conditions. When virus and inhibitor were added to cultures at the same time and the cells were incubated at 41C inhibition did not occur. These studies indicated that virus exists in a non-replicating, intracellular form in this system. In such a "latent" form virus is resistant to both thermal inactivation and drug inhibition.

SOME CHEMICAL AND BIOLOGIC CHARACTERISTICS OF GROUND WATER IN THE VICINITY OF A SANITARY LANDFILL. J. W. Vennes*, J. K. Neel, G. W. Fossum and M. Arndt. Depts. of Microbiol., Biology, Sanitary Engr., and Geology, Univ. of N. Dak.

A newly constructed sanitary landfill at Langdon, N.D., allowed an excellent research facility for the determination of the effects of disposal of solid wastes on ground waters. Ten observation wells were utilized for observation and sampling. Capability for determination of direction of flow and changes in chemical and biologic constitutents of impact and peripheral waters was established. Determinations for heavy metals, standard chemical water analyses, nutrients, pesticides, radioactivity and microbial numbers are presently being carried out. Monthly samplings have occurred since October, 1973. Results to date have been somewhat variable vis a vis any specific parameter. The methods thus far utilized and the results obtained to date will be presented and discussed.

INTERACTION OF DIFFERENT CYTOPLASMS WITH COMMON WHEAT GENOME ON LEAF TRANSPIRATION. W. E. Vian and M. E. Duysen. Dept. of Agron., and Dept. of Bot., N. Dak. State Univ., Fargo, N. Dak. 58102

Transpiration from the upper leaf surface was measured with a diffusion porometer to observe the interactions of alien cytoplasms and the common wheat genome.

Cytoplasms from Aegilops ovata, Ae. speltoides, Triticum zhukovskyi and T. boeoticum interact with the T. aestivum var. 'Chris' genome reducing the transpiration resistance by 2-3 times. The greatest difference between the cytoplasms and Chris control was on the first leaf and the highest resistance value was for Ae. ovata cytoplasm throughout the life cycle. The transpiration resistance decreased with maturity for all cytoplasms. The flag leaf value for all cytoplasms and the Chris control was similar. The stomata of T. zhukovskyi and T. boeoticum cytoplasms were open at 10:30 am but in all other cytoplasms the stomata were closed. The stomata were closed in all cytoplasms at the 2:30 pm reading.

MINERAL COMPOSITION OF BROMEGRASS AFTER LONG TERM FERTILIZATION. F.R. Vigil and D. W. Meyer. Dept. of Agronomy. N. Dak. State Univ., Fargo, N. Dak. 58102

Forage samples from an old bromegrass (Bromus inermis L.) sod fertilized annually since 1954 at 0, 33, 66, 133, 200 and 266 lbs nitrogen (N)/acre were analyzed to determine if long term fertilization influenced the mineral composition. The brome was harvested two times per year (June and September) in 1972 and 1973. Samples were digested using a perchloric acid solution, diluted and analyzed with either a colorimeter or an atomic absorption spectrometer for phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca) and manganese (Mn). Considerable variation among years, harvests and treatments were observed for all minerals determined. P content tended to increase in 1972, but not in 1973, with increasing N during the first harvest. K content was not influenced by the N level during the first harvest but increased at the 266 lbs N/acre treatment during the second harvest. Ca content increased with increasing N levels during the first harvest. However, Ca content decreased with increasing N levels during the second harvest; Ca content was higher during the second than first harvest. Differences in Mg content were detected, but no discernible trend was evident. Mn content increased significantly at the 266 lbs N/acre but was not influenced by the 66 lbs N/acre.

Regulation of Biotin Transport in Bacteria. James R. Waller, James Deering and J. K. Anderson. Dept. of Microbiology, Univ. of North Dakota, Grand Forks, ND 58201.

Transport and accumulation of the B vitamin biotin by *Lactobacillus plantarum* is controlled by the amount of biotin in the external environment. Marked reduction of transport activity occurred only when biotin was detected free inside the bacterial cells. This occurred when the level of biotin in the growth medium exceeded that required for maximum growth and saturation of internal biotin binding sites. When external biotin exceeded that required for growth, the activity of the uptake system decreased directly with the degree of excess. Vitamin excesses of 2 fold caused a reduction in accumulation capacity of 7%; 4 fold excess: 22%; 6 fold: 57%; 8 fold: 83%. Complete inhibition never occurred; even in a 40 fold biotin excess, the transport system retained 4-5% activity. Bacteria grown in biotin excess, possessing low transport activity, rapidly regained maximum activity when grown with low biotin. Conversely, cells with maximum transport activity rapidly lost activity when grown in excess biotin. If bacterial cells capable of maximum transport were saturated with biotin under non-growing conditions, their ability to take up biotin did not diminish. Thus, inhibition of the transport system required the presence of excess biotin during active growth.

CHEMICAL CHARACTERISTICS OF GROUND WATER IN THE ELK VALLEY AQUIFER. C. A. Wardner Emeritus, Dept. of Chem. U.N.D. and J. K. Neel, Dept. of Biol., U.N.D., Grand Forks, N.D. 58201

The chemical characteristics of the Elk Valley Aquifer is part of a total study involving the Turtle River Basin. This report concerns only the aquifer data. Bulletin 53, N. D. State Water Commission was used to locate desirable wells for sampling in an area above the junction of the North and South Branches of the Turtle River near McCanna N.D. Three wells selected range in depth from 14 to 26 ft.

A summary of data show that: (1) considerable variations in specific ion concentrations occur between wells in the same aquifer; (2) concentration changes occur within the same well over the sampling period and even from one sampling date to the next; (3) NO_3-N varied (.04-2.8 p.p.m.) in Well #1, (1.0-7.0 p.p.m.) in Well #2, (1.8-7.8 p.p.m.) in Well #3 with the higher concentrations occurring in the latter half of the sampling period; (4) seasonal variation in available PO_4^{3-} with none showing late Spring and Summer months. The soil (clay) at ground water level analyzed 6.4 p.p.m. available PO_4^{3-}; 1500 p.p.m. total PO_4^{3-} in the 594 micron size; 3225 p.p.m. total PO_4^{3-} in the 74 micron size, an indication of increased fixation as clay particles approach colloidal size.

STUDIES ON THE NATURE OF RESISTANCE OF WHEAT TO INFECTION BY PUCCINIA RECONDITA F. SP. TRITICI. J. E. Watkins and G. D. Statler, Dept. Plant Path., Col. of Agr.,NDSU, Fargo, ND.

Several biochemical and histological factors were studied in near isogenic wheat lines inoculated with an isolate of Puccinia recondita f. sp. tritici. A comparison of these factors between line Lr3, developing a resistant (0;) infection type, and line Lr2, developing a susceptible (4) infection type, showed both biochemical and histological differences. Changes in total buffer-soluble proteins and total phenols in either line were very similar to those in the healthy checks. Inoculated Lr3 had peroxidase activities 63% greater than healthy controls at two days post-infection and 82% greater at five days post-infection. Lr2 had peroxidase activities 32% greater than healthy controls at two days post-infection and 36% greater at five days post-infection. Histological examinations showed that percent penetration, the number of hyphae per infection site, the number of haustoria per infection site, and the number of collapsed cells per infection site were related to infection type. At 24 hr post-infection 92% penetration was observed on the resistant line, but only 64% was observed on the susceptible line. Fungal development had essentially ceased in the resistant infection type at 24 hr post-infection; but in the susceptible infection type, fungal development increased greatly between 24 and 72 hr post-infection.

PLASMA CORTICOID LEVELS IN THE OVINE. R. Weigl, J. Tilton, W. Eide and M.L. Buchanan. Dept. of Animal Science, NDSU, Fargo, N.D.

Diurnal variation of plasma corticoid concentration were determined in 4 sheep. Samples were collected over a 24 hour period at 20 C and during periods of increasing temperatures (20 C to 37 C), with controlled lighting (12 hr). Corticoid levels were quantified by competitive protein binding and found to be 5.6 ng/ml of blood during light periods versus 4.4 ng/ml during darkness. The highest recorded values at 20 C were observed at 4 PM (17.9 ng/ml), falling rapidly to a low at 8 PM (1.9 ng/ml). Samples collected during periods of increasing temperature were found to have dramatic peaks in corticoid levels 3-4 hrs after exposure to increasing ambient temperature. Samples collected at 8 PM and subsequently at 8 AM were very low (2.6 ng/ml). Levels of corticoids assayed on the first day of exposure to 37 C were significantly ($P \leq .025$) higher than those noted on subsequent days of exposure. Adaptation was observed as evidenced by reduced corticoid levels in peripheral blood plasma following initial increases. The most repeatable measures of corticoid levels were noted in those samples collected at 8 AM.

TURNOVER RATE IN MIXED GRASS PRAIRIE. Warren C. Whitman. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak. 58102.

Primary production and turnover of above- and below-ground plant material was studied in a mixed grass prairie ecosystem during the period 1970-72. The study plot was located at the Dickinson Experiment Station in southwestern North Dakota and had been protected from grazing since 1961. Peak standing crop yields were 320, 303, and 343 g/m^2 for 1970, '71, and '72, respectively. The average old-crop standing dead ranged from 217 to 323 g/m^2, and average litter weight varied from 625 to 662 g/m^2. The mean total above-ground plant biomass remained near 1125 g/m^2 over the 3-year period. Below-ground production was estimated from the changes in below-ground plant material during the first and second season. Using the methods of Golley (1965) and Jenny et al. (1949) decay constants were estimated for above- and below-ground plant material. The decay constants for the above-ground material averaged 0.36 for the study period, while the decay constants for the below-ground material to a depth of 30 cm averaged 0.25, suggesting a somewhat slower turnover rate for the below-ground material. Turnover rates thus appear to be about 3 years for above-ground material and 4 years for below-ground material. Above-ground standing dead material showed a decay rate about 2½ times greater for the April-September period than for the October-March period.

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of the

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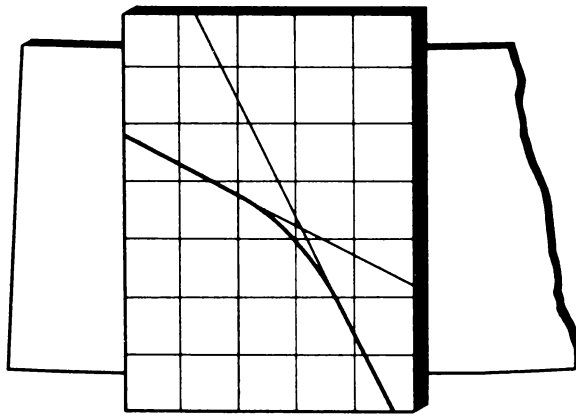

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PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE PAPERS

Volume 28, Part II September 1976

NORTH DAKOTA ACADEMY OF SCIENCE

(Official State Academy; founded December, 1908)

OFFICERS AND OTHER MEMBERS OF EXECUTIVE COMMITTEE, 1973-74

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INSTRUCTIONS TO AUTHORS FOR THE NORTH DAKOTA ACADEMY OF SCIENCE PROCEEDINGS

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.

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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.

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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.

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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.

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

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.

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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.

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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.

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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

JAMES R. REILLY

1918 - 1974

Dr. James R. Reilly was Editor of the North Dakota Academy of Science at the time of his death on June 28, 1974. Appointed Editor in 1972, he edited parts I and II of volume 26 and part I of volumes 27 and 28 of the Academy's Proceedings.

He was born at Tottenville, Staten Island, New York on November 2, 1918. Bachelor and Master of Science degrees were received from the University of Maine in 1941 and 1948; the Ph.D. was received from the University of Illinois in 1965. He married Catherine Merkler on May 22, 1943. His military service was with the U.S. Coast Guard in 1943-45 where he attained the rank of Lieutenant.

Dr. Reilly achieved national and international recognition as an authority on wildlife diseases. He acquired this knowledge through 12 years work as a game

pathologist with the New York State Conservation Department and 14 years research at the Universities of Illinois and North Dakota. During his later years Dr. Reilly was chiefly concerned with botulism but was also involved with a number of other bird and mammal diseases including leptospirosis. He published 30 articles, including the chapter on tularemia in Infectious Diseases of Wild Mammals published by the Iowa State University Press. Dr. Reilly, Professor of Biology at the University of North Dakota, taught graduate and undergraduate courses in the wildlife field and directed graduate students seeking the M.S. and Ph.D. degrees. He is also remembered with great affection by numerous other graduate students for the willingness and ability to help with thesis composition.

Dr. Reilly was an accomplished musician, playing the violin in the Grand Forks Symphony Orchestra and the baritone in the 9th District Legion Band, which was elected to represent the Legion in Hawaii in 1973. He was also a member of the Elks Band of Grand Forks and sang in the Holy Family Church Choir. The first Grand Forks Symphony concert of the 66th Season, November 16, 1975, was dedicated to him.

Dr. Reilly is sorely missed by his colleagues at the University of North Dakota. His crusty exterior hid a heart of purest gold, and he could refuse no one a requested favor. He is most affectionately remembered by a title bestowed upon him by a former departmental secretary—"a hard-boiled cream puff." He toiled long and diligently for the benefit of all concerned and his imprint will endure.

—Joe K. Neel

TYPE AND REFERENCE SECTIONS FOR A NEW MEMBER OF THE FOX HILLS FORMATION, UPPER CRETACEOUS (MAESTRICHTIAN) IN THE MISSOURI VALLEY REGION, NORTH AND SOUTH DAKOTA

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ABSTRACT

Field study in Emmons and Sioux Counties, North Dakota, and eastern Corson County, South Dakota has revealed a new stratigraphic unit of the Upper Cretaceous (Maestrichtian) Fox Hills Formation. Previously, workers had included this unit with the Colgate lithofacies (or member) which has its type area in Montana. Hayden clearly recognized its significance calling it "Bed Q." This is the resistant sandstone which capped Hayden's "Fox Ridge." It is probable that most of Hayden's exploration was along this ridge well into North Dakota. Recognition of the uniqueness of the unit, which we have called the Linton Member, has shed light on the meaning of much of Hayden's work.

The Linton Member consists of light olive gray to grayish brown, fine-grained, subangular, moderately to poorly sorted, indurated, siliceous sandstone containing volcanic shards. Cross strata and flat bedding are present locally, the unit being generally massive. Analyses indicate a range of lithologic constituents which place the rock between a feldspathic arenite and subgraywacke with about 28% matrix.

Vertical roots, *Equisetum*, and *Ophiomorpha* also characterize the unit. Wood fragments are abundant in the upper beds. Thickness ranges from 0.2 to 6.0 m. A type section is designated in N½ secs. 8 and 9, T. 132 N., R. 76 W., 1 mile E. of Linton, North Dakota. Reference sections are in NE¼NW¼ sec. 28, T. 130 N., R. 78 W., Emmons County, N. Dak. and 11 miles west of McLaughlin in northeastern Corson County, S. Dak. The Linton Member is interpreted as a channel sand, deposited in and along a major, wide, shallow, southward-flowing estuarine tidal river.

INTRODUCTION

Purpose.—Stratigraphic description of the Fox Hills Formation in the Missouri Valley Region of North and South Dakota is intricately entwined with the history of early explorations of the Territory. A great deal of controversy has revolved around the rocks which record the transition from marine to terrestrial depositional environments. Many of these issues are resolved for such units in Wyoming, Montana, and western North Dakota, yet stratigraphic sequences and facies relationships still are not understood in the Missouri Valley, the type area of the Fox Hills Formation.

Our work is an attempt to clarify and to begin to diagnose undescribed Fox Hills strata which occur over much of Emmons County, North Dakota and which extend eastward and southward into adjacent areas (Figure 1). The present paper defines a new lithologic unit at the top of the formation. It also attempts to place this unit in proper context with respect to historic study of the Fox Hills Formation since the work of Meek and Hayden in the mid-nineteenth century. Eventually all strata east of the Strassburg Channel (Fisher, 1952, p. 26) in Emmons County, and those of Logan and McIntosh counties as well, should be thoroughly

re-examined and redefined. We begin the process herein by designating "Bed Q" of Meek and Hayden (1857) as the Linton Member of the Fox Hills Formation.

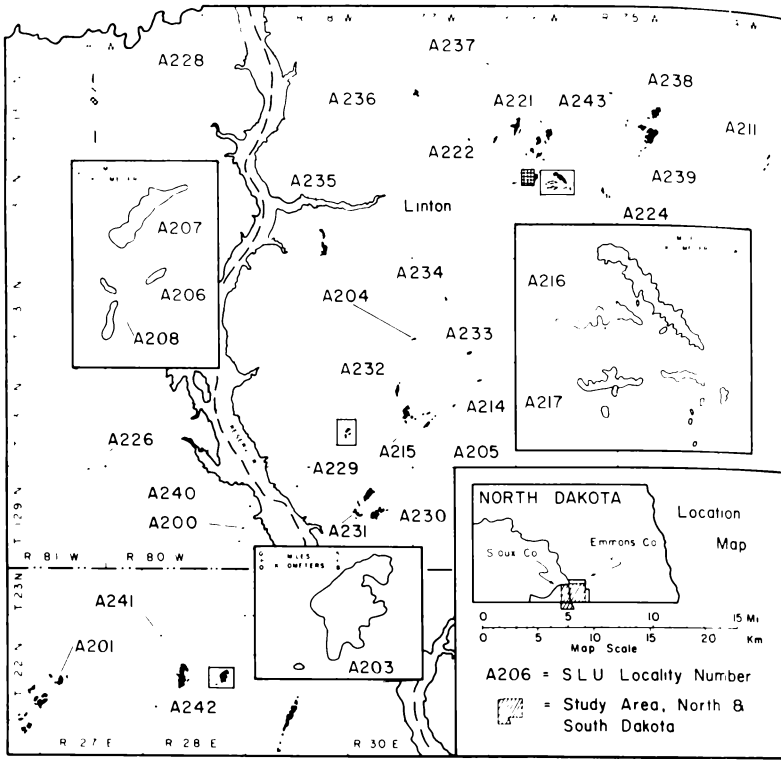

Figure 1. Map of major exposures of Linton Member with location of type (A216) and reference (A203, A206) sections.

Previous stratigraphic studies.—Formal recognition of the Upper Cretaceous (Maestrichtian) Fox Hills Formation was first made by Meek and Hayden in 1861 when the presently-used name was applied to rocks previously designated as "Unit No. 5" of the Cretaceous strata of the Missouri River Region (Meek and Hayden, 1856). Impressions of the formation and its confining beds, the Pierre below and the Hell Creek (Lance of some workers) above, upon which they based most of their discussions were developed largely from exploratory excursions conducted by Hayden along and adjacent to the Missouri River approximately between the present cities of Bismarck and Pierre. "Fox Ridge" on the divide between the Cheyenne and Moreau Rivers in South Dakota made a marked impression upon them, and the Fox Hills Formation was thought to have been named for rocks well exposed in that divide (Waage, 1968).

Attempts at more detailed description of Fox Hills and related Cretaceous rocks led to a multitude of stratigraphic misinterpretations and misunderstandings which rather confused the stratigraphic relationships for more than half a century. In large part, problems were due to long-distance physical correlations and to failure to recognize lateral lithofacies relationships which had resulted from rapid regression of the Maestrichtian sea and the terrestrial deposition which followed.

Several comprehensive historical treatments of the conceptual development of the formation are available in recent literature. That of Waage (1968), given in conjunction with a redescription of the stratigraphy of the type area in South Dakota, lends itself particularly well to explanation of the intricacies of geographical locations and probable routes of the early explorations of the region. Feldmann (1972) presented an equally useful account of previous studies in North Dakota. Some recent descriptions of Fox Hills rocks and their faunas were summarized by Erickson (1974).

Our discussion of previous work now focuses upon the uppermost sandstone unit of the formation in areas adjacent to the Missouri River. These rocks are lagoonal or estuarine deposits; their lower and upper contacts are often transitional, even gradational on occasion, between marine strata below and freshwater or terrestrial strata above. The brackish-water origin of invertebrates in these rocks was recognized by Meek and Hayden. Such fossils were common in "Bed Q," a gray, indurated-to-slightly-friable sandstone reaching a thickness of 30 feet (9.0 m) which they chose as the basal unit of the overlying "Great Lignite Group" (Meek and Hayden, 1857). In their opinion, this unit also represented the first "Tertiary" deposition in the region, a decision based largely upon Newberry's identification of floras in the Great Lignite Group as Miocene, and upon the absence of ammonites from Bed Q (Waage, 1968). Thus began a long stratigraphic controversy over the position of the Mesozoic-Cenozoic boundary in the northern Great Plains.

Calvert (1912) described the "Colgate sandstone member" of the Lance Formation (=Hell Creek Fm.) from sections at Colgate Station (N.P.R.R.) near the point where the Yellowstone River crosses the northwestern nose of the Cedar Creek anticline (center, south edge T. 15 N., R. 57 E.). Here, the member was about 175 feet (52.7 m) thick, a white (brown, lower) massive sandstone containing at least two leaf-bearing horizons. Knowlton (*in* Calvert, 1912) identified a collection of these leaves as being Tertiary in age, though Calvert (1912: 195) noted that elsewhere they were associated with dinosaur remains. From 1912 onward, the term "Colgate" has provoked a wide variety of interpretations and has received an overzealous use in the literature, providing a convenient departure for stratigraphic and philosophical disagreement.

Reasons for this are at least partially historic. Presence of a sandstone unit between the recognizably marine Fox Hills and the demonstrably terrestrial (fluvial, palludal) Hell Creek above places such a unit in a critical position. Paleobotanists, limited in their studies to terrestrial beds of the Fort Union and Hell Creek, established floral indices from these younger rocks, whereas invertebrate workers derived indices from molluscan remains in the older, marine rocks. The key sand-

stone unit, by virtue of its estuarine depositional environment, contained remains of both the land plants (defined as Tertiary) and the occasional marine mollusk of Cretaceous age. Was it of Mesozoic or Cenozoic age?

In 1910, Stanton effectively summarized the rock relationships, documenting this environmental transition. He interpreted the transition from marine to terrestrial depositional environments in the type area in South Dakota, in the Little Missouri River Valley of western North Dakota, and along Lance Creek in Wyoming. In 1911, Leonard presented a brief, incomplete, yet at that time, "modern" description of the stratigraphy in North Dakota. However, it was principally Stanton's presentation which brought the relationships to realistic perspective.

Thom and Dobbin (1924) recognized the similar stratigraphic position of the sandstone between the Fox Hills and Lance in Wyoming, the "Colgate" of Calvert, and the white sandstone occurring in the type area in South Dakota. This, and the subsequent work of Dobbin and Reeside (1929), solidified regional stratigraphic concepts of the upper Fox Hills-lower Hell Creek section formulating a basis from which, intuitively and logically, the pre-1968 concept of the section in the type area was to develop. Dobbin and Reeside (1929) provided the most definitive evaluation of the contact between the Fox Hills and Hell Creek Formations as applied to its transitional and gradational character. Theirs was the first major review of the "Colgate" sandstone that described it in relationship to rocks in the same stratigraphic position in Emmons County, North Dakota, and particularly in the Linton area.

Several recent works figure in our discussion. Todd (1910) presented some cogent glimpses of the lateral variability of rocks in the South Dakota section. These were overlooked during the late 1940s when mapping at geologic quadrangle scale in and around the type area in South Dakota began earnestly. During that work, Curtis (1952) introduced the name "Colgate Member" into the type area. That term came to be used extensively for any gray, cream, or white, indurated or non-indurated sandstone holding the "key" stratigraphic position at or near the Fox Hills-Hell Creek boundary in the region. Laird and Mitchell (1942) applied the name "Colgate" to such rocks in Morton County, North Dakota. Henceforth it has become common practice to label as "Colgate" all rocks fitting the general description given above, and occupying the "key" stratigraphic position.

Waage (1968) redefined the relationships of the type area in South Dakota in a well-conceived, useful manner. Recognizing the variable stratigraphic position of the Colgate Member in South Dakota, Waage defined the Iron Lightning Member containing Colgate and Bullhead lithofacies. This redefinition took into account the ephemeral nature of the Colgate sand facies which appears in both upper Fox Hills and lower Hell Creek sections. However, his conception of rocks in the "Colgate lithofacies" produced interpretve problems (Waage, 1968:129-151, "Butte Cap Problem"). In Emmons County, North Dakota, Fisher (1952) recognized the significance of rocks in this "key" position, but skirted any nomenclatorial question because his primary concern was structural in-

terpretation. Feldmann's (1972) stratigraphic summary likewise avoided the issue by calling all such rocks Colgate. Definition of the rock unit in this position has thus continued to be a problem in recent work. The relationship of the Linton Member to this issue and its historic significance to geologic study in the Missouri Valley Region will be discussed below.

Present field work.—Field work by Erickson in 1969 indicated that units of the Fox Hills Formation in Emmons County, North Dakota needed clarification relative to units in other areas of Fox Hills outcrop. Work in Emmons County further disclosed undescribed Fox Hills strata. Field work for this report was undertaken between late June and early August, 1973. All butte systems located in central and southwestern Emmons County were investigated, as were several buttes and outcrops in Sioux County, and related butte systems extending into northeast Corson County were examined. Fifty-four sections containing Linton strata, and 11 sections containing strata associated with the Linton Member were measured.

Where available, 7½-minute topographic maps were used to locate positions and elevations of the best exposures. However, only unedited advanced prints were available for the western one-half of Emmons County, and the lack of any topographic coverage for the eastern one-half of Emmons County necessitated other means of locating exposures. Aerial photographs were used for this purpose. Additional outcrops were located while travelling in the field.

Stratigraphic terminology in this report.—Erickson (1971) suggested the existence of an "unnamed" member at the top of the section, describing it as a blanket sandstone having an estuarine faunule. During that study, and in subsequent presentations, Erickson (1973, 1974) adopted Waage's (1968) revision of nomenclature where applicable, feeling that the Iron Lightning concept was useful in North Dakota.

We accept herein the member names Trail City, Timber Lake and Iron Lightning (with a Bullhead and Colgate lithofacies). We note that true Timber Lake sediments are scarce east of the Strassburg Channel of Fisher (1952) and suggest that Fox Hills units above the Trail City Member in that region are largely unnamed. Some Iron Lightning lithologies are present locally both east and west of the channel. The newly named Linton Member, by virtue of its butte-capping position, is at the top of the section.

DESCRIPTION OF THE LINTON MEMBER

Location and distribution.—Exposure of the Linton Member is limited in occurrence to buttes, as in Figures 3, 6, and 7, on which it forms a prominently displayed sandstone cap. Buttes are arranged in the area directly east, west, and north of Linton. In the southwestern quarter of Emmons County they form a northeast-trending, broken ridge which also appears west of the Oahe Reservoir in Sioux County where exposures of the Linton Member are found in T. 129 N., Rs. 79 and 81 W. Additional outcrops occur in the northeastern corner of Sioux County, just west of the town of Cannonball. In Corson County, South Dakota, buttes capped by the Linton Member continue the northeast trend established in

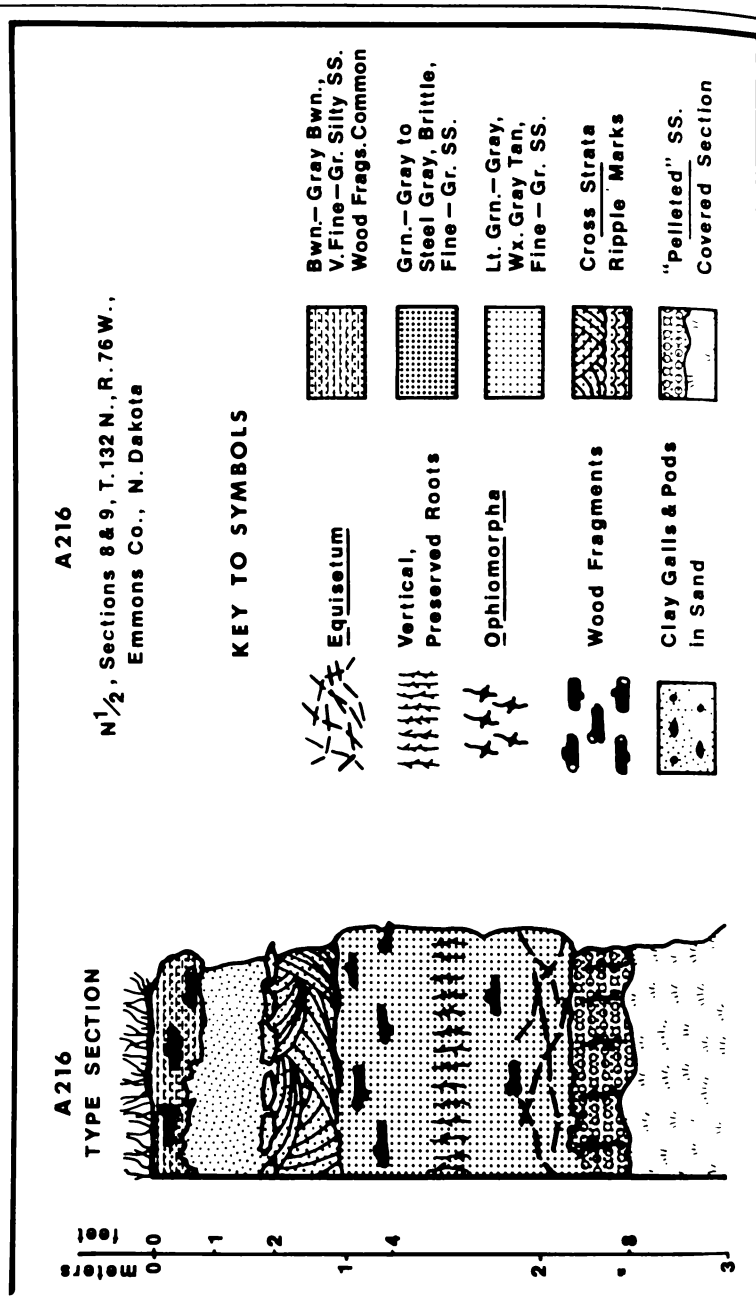

Figure 2. Schematic type section of *Linton Member*.

North Dakota. The unit was studied also on several buttes near McLaughlin, South Dakota.

Figure 1 shows known areas of exposure and is not a complete distribution map of Linton rocks. The unit is present well into the type area in South Dakota. It thins westward in Sioux County, although its exact lateral limits are not known; likewise the eastward extent is undescribed. Fisher traced it to the eastern edge of Emmons County where the stratigraphy became complicated by additional similiar units.

Thickness and stratigraphic position.—Exposures of Linton Member range in thickness from 0.2 m to 7 m, about 2 m being average. Although no locality offers a complete exposure due to prevalent slump blocks and hillslope talus around buttes, it is probable that 7 m approximates the maximum depositional thickness to be expected for the unit. Tops of those buttes which do not expose bare rock are covered by a soil composed of weathered glacial drift. Top and bottom contacts are not well understood because of poor exposures.

The basal contact on a butte on the south side of the Cannonball River in sec. 21, T. 134 N. R. 79 W. is gradational downward through thin, orange, lignitic sands into the top of a water-laid volcanic ash. The ash here is 3 or 4 m thick and rests on sediments of the Bullhead lithofacies. Eastward, at locality A206 in Emmons County, Bullhead sediment occurs 48.6 m below the Linton, and Timber Lake-like lithology occurs 62.5 m below its base. Animal burrows and slumps reveal transient glimpses of lower units. From these, it appears that the Bullhead lithofacies thins southeastward in Emmons County where its position is occupied by a complex, unindurated silty, very fine sandstone and sandy siltstone containing clay laminae, much lignitic matter and rare cleaner, flaggy, thin-bedded sandstone having a marine fauna.

Fisher (1952:39) described these flaggy "sea-green" sandstone ledges at several localities. In the type section (A216) the upper ledge occurs 29.5 m below the base of the Linton and 19.6 m above the volcanic ash which also thickens somewhat from Sioux into Emmons County. In the region around and southwest of the town of Linton the base of the member seems to have a gradational contact with the unindurated silty sands that lie below and which represent an unnamed Fox Hills facies.

Units overlying the Linton are also poorly known. However, on the largest, northernmost butte of a system in the SE¼SW¼ sec. 21, T. 130 N., R. 78 W., 1 m of chocolate-brown lignitic shale containing some jarosite, directly overlies the silty sandstone (variety 4, Table 1) of the Linton. An additional 12.3 m of very fine-grained silty sand capped by an indurated bed overlie this lignitic shale. The shale is taken as the basal unit of the Hell Creek Formation in our study.

A similar relationship was seen in a north-facing roadcut in the NW¼SW¼ sec. 30, T. 130 N., R. 78 W. There, the lignitic shale is overlain by 4.6 m of gray and orange sands containing clay laminae which in turn are covered by 4.3 m of dark gray bentonitic clay. This overlying sediment is suggestive of Hell Creek facies and supports our interpretation of the Linton Member as the top-most Fox Hills unit in this region. The basal Hell Creek lignitic shale is very difficult to

identify throughout most of Emmons County and care should be taken not to confuse it with lignitic units in or below the Linton thereby placing the contact too low in the section.

Textural and bedding characteristics.—Four lithologic varieties comprise the sediment of the Linton Member as diagrammed in Figures 2 and 5. Many of the characteristics of these are described in Table 1 and listed in their most common stratigraphic order of occurrence. Varieties 1, 2, and 3 are locally controlled manifestations of the same basic lithology. Control of variation is more likely due to post-depositional effects of diagenesis and differential lithification in response to local paleohydrology, than it is to major change in lithology. Slight lithologic differences, primarily in silt-clay content and degree of bioturbation, were responsible, however, for subtle permeability and porosity gradients within the paleohydrologic regime. Thus, depending upon these subtleties, variety 2 may lie beneath, above, or laterally adjacent to variety 3 making sequential interpretation of these lithologies difficult or impossible.

Table 1. Characteristics of lithologic variations in the Linton Member listed by most common stratigraphic position.

	Characteristics
Variety 4	Indurated, massive, loosely packed, poorly sorted, subangular, very fine-grained, silty sandstone. Thickness 0.3 m or less. Weathered color light gray (5Y7/1); fresh color dark brown (7.5YR4/2) to dark grayish brown (10YR4/2). Waterworn wood fragments abundant.
Variety 3	Indurated, brittle, massive, moderately packed, moderately sorted, angular to subround, fine-to very fine-grained sandstone. Fractures in curved, flaggy slabs. Weathered color light gray (2.5Y7/2) to light yellowish brown (2.5Y6/4); fresh color gray (5Y6/1) to light olive gray (5Y6/2). Contacts abrupt or gradational, thickness 0.5 m to 2 or 3 m. Large-scale cross-strata sometimes present. Contains clay galls, worn wood fragments, and occasional *Ophiomorpha*.
Variety 2	Indurated, sometimes brittle, massive, moderately packed, moderately sorted, moderately permeable, subangular to subround, fine-to very fine-grained, sandstone. Weathered color grayish brown (10YR5/2) to brown (10YR5/3); fresh color light olive gray (5Y6/2) to light gray (5Y7/1); often slightly mottled with iron stain. Occasionally with large scale, trough cross-strata. Contacts abrupt or gradational. Thickness 0.5 m to 2.5 m. Contains clay galls, worn wood fragments, preserved roots and casts, *Equisetum*.
Variety 1	Semi-indurated, "pelleted" (Fisher, 1952), non-bedded, friable, loosely packed, permeable, moderately sorted, subangular, fine-grained sandstone. Weathered color light brownish gray (2.5Y6/2) to light yellowish brown (2.5Y6/4); fresh color light gray (5Y6/1). Usually basal in position, not laterally persistent, 0.3 m or less in thickness, nonfossiliferous.

Usually, the "pelleted" sandstone (variety 1, Table 1) is basal. It is often gradational into a more indurated sandstone and its texture of small aggregates of sand seems to be partially a weathering phenomenon. In contrast variety 4 is constant in its physical appearance and always occupies a position at the top of the member. It is substantially different from the other lithologies in color and in higher silt and wood content.

Compositionally, the Linton Member ranges from a subgraywacke to a feldspathic arenite as determined from preliminary analysis of 38 thin sections. Cementation, where it has occurred, is with silica. All samples contain shards of volcanic glass in the silt fraction. Quartz and feldspar grains exhibit a high degree of angularity in contrast to occasional well-rounded glauconite grains. Although the sand-sized fraction is well sorted in the fine-to very fine-sand range a prominent silt-clay component leads to a slight bimodality of distribution denoted by our use of the terms "moderately" and "poorly" sorted in Table 1. This bimodality, which gives the sediment a moderately to poorly sorted overall distribution, may result from admixure of clay during deposition, a likely explanation for the origin of the glauconite grains as well.

The matrix of the Linton sandstones makes up nearly 30% of the rock and incorporates a volcanic shard fraction as noted above. This is distinctly unlike the Colgate lithofacies with which it has been most often confused. Dobbin and Reeside (1929) reported 66% matrix and specifically stated that volcanic material was absent from the Colgate. Our samples indicate that the Colgate may have as little as 48% matrix, still well in excess of the Linton. Likewise, the Timber Lake sandstones differ from the Linton by lack of volcanic shards, greater roundness, higher glauconite and heavy mineral content, and often by being better sorted. The Linton Member is compositionally and texturally distinct from other Fox Hills sandstone facies.

As noted earlier, nonuniform induration causes unusual weathering styles in Linton rocks and makes study of bedding features difficult. Most outcrops show no bedding. Occasionally clay pellets or galls are aligned and define bedding surfaces. More often bedding is in the form of two or three sets of large-scale trough(?) or planar cross-strata, each set being nearly 1 m thick (Figure 4). Cross-strata are common enough to give the impression that Linton deposition took place under the influence of moderately strong, persistent currents acting over a short period of time.

Ripple marks are rare in the Linton Member. Some butte caps in South Dakota, however, preserve both a thinly laminated sandstone, and unusual sets of lunate ripples indicative of uni-directional current flow. One bedding surface in Emmons County contains well preserved mud cracks. These are the only notable primary structures observed.

Fossil content.—Faunal remains are rare in the Linton Member, yet ichnofossils are rather common. Large specimens of *Ophiomorpha*, the burrow of a callianassid crustacean, can be found in most outcrops. Nowhere are these burrows clustered in thick networks as are characteristic of the upper Timber Lake sandstones, a significant difference between the two occurrences of this fossil. Trails and burrows of uncertain origin occur sporadically throughout Linton rocks and indicate the existence of an unpreserved infauna. In more southerly outcrops occasional external molds of *Tancredia americana* and, less commonly, *Cymbophora warrenana* and scaphitid ammonite fragments occur. Nowhere are these common. They do not occur in the unit in or near Linton.

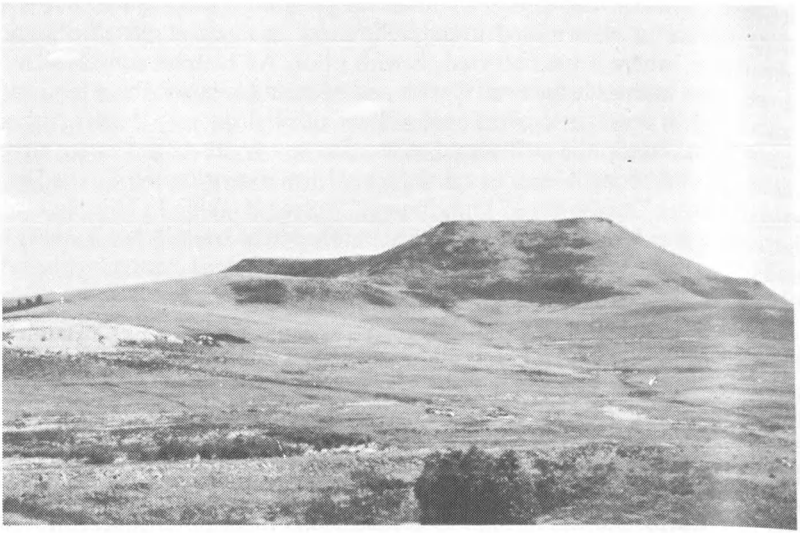

Figure 3. View of northwest tip of butte containing type section, A216. Note volcanic ash bed, left middle.

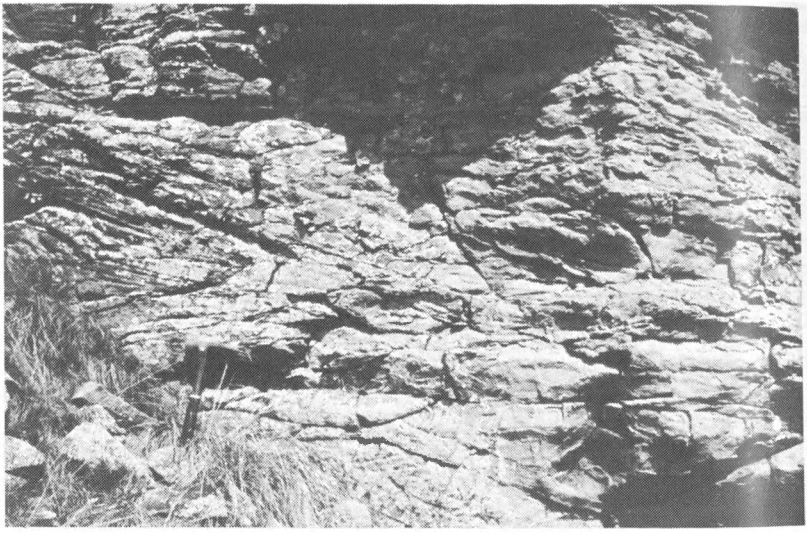

Figure 4. Remnant cross-stratification in Linton Member at A230. Hammer gives scale.

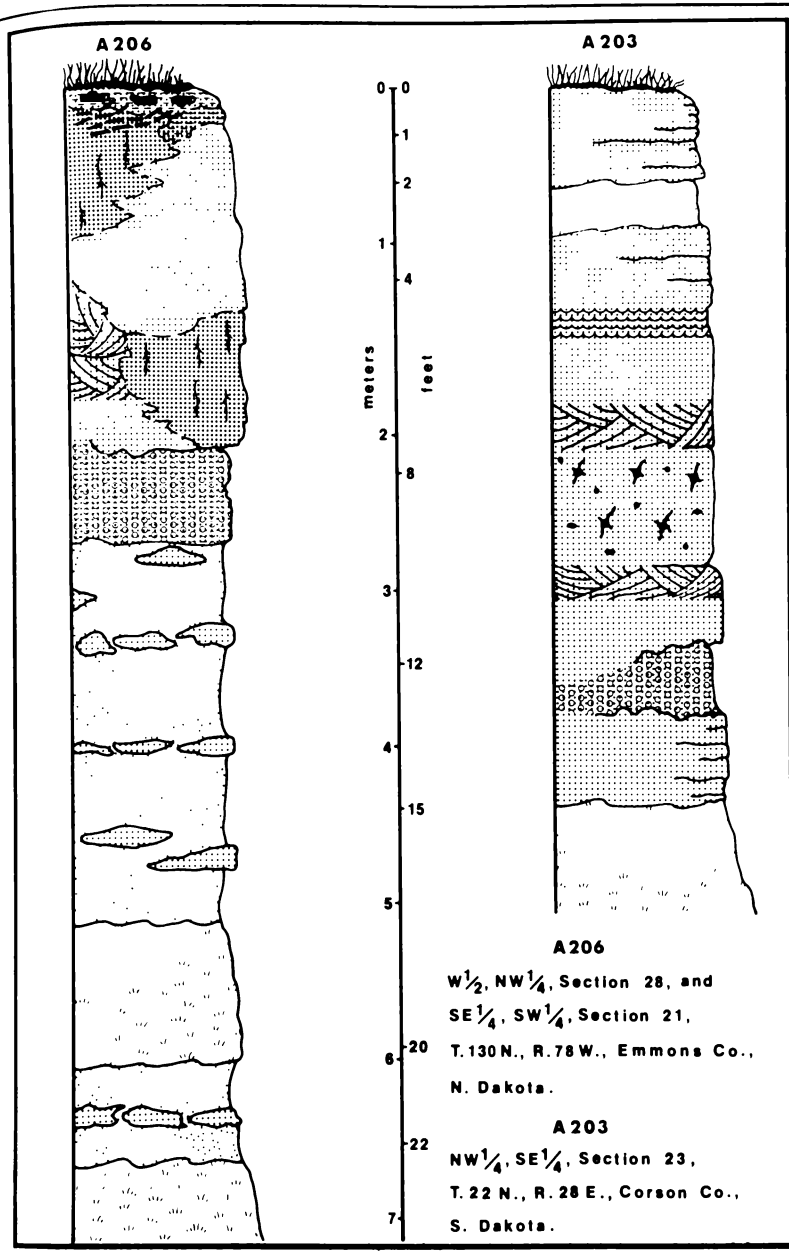

Figure 5. Schematic reference sections of Linton Member. Symbols as in Figure 2.

In Emmons County the member is characterized by the common occurrence of *Equisetum* found in both upright and flattened position. This rush was growing in and adjacent to the sand deposits which formed the member and can be relied upon for recognition of the member in the type area.

A zone of vertical root tubes and casts is also indicative of the unit (Figure 2). Most often these root systems are only a few milimeters in diameter, but they may be a foot or more in length. These should not be confused with the frequent occurrences of woody (now silicified) roots which may be as much as 2 m long and, in distribution, resemble a modern, sediment-engulfed mangrove root system. These types of root development are less common in South Dakota where they are often replaced in the section by wood "hash" of water-worn fragments up to many centimeters long.

Transported plant remains are not restricted to the South Dakota outcrops. Wood chips may be found throughout the outcrop belt. A variety of leaves have also been recovered. These appear in localized deposits generally including a few deciduous species and a variety of large palmetto. None have been transported far. Leaves are not useful for recognition of the member.

Plant remains other than leaves are surprisingly consistent in occurrence. We have little difficulty understanding why Meek and Hayden chose to consider Bed Q the basal unit of the "Great Lignite Group." The influence of terrestrial biota in it is very strong though the presence of *Ophiomorpha* confirms the estuarine character of its depositional environment.

TYPE AND REFERENCE SECTIONS

The type section for the Linton Member (Figure 2) is designated as a butte cap in the N½ secs. 8 and 9, T. 132 N., R. 76W., Emmons County, North Dakota. This is the longest (1.3 km at greatest extent), northwest-trending member of a butte system 1.2 km eastward from the eastern edge of Linton. Except at the butte's edge, soil covers the uppermost unit of the Linton Member, and aerial photographs show a cultivated strip which crosses the center of the butte top. At the northwest base of the butte (Figure 3) lies a well-known volcanic ash, mined at this locality for commercial purposes. The type section offers an exposure of the Linton Member's lithologic subunits, as well as limited exposure of some commonly associated sediment below the butte cap. Detailed descriptions of the type and reference sections are in Appendix A.

Two reference sections (Figure 5) are designated from outcrops containing representative lithologies, fossils, structures, outcrop and weathering characteristics of the Linton Member. Reference section A206 occupies the southernmost butte of a four-butte system in the NE¼NW¼ sec. 28, T. 130 N., R. 78 W., Emmons County, North Dakota. Reference section A203 was measured on a small butte directly southwest of a large ridge and butte complex in the NW¼SE¼ sec. 23, T. 22 N., R. 28 E., Corson County, South Dakota. These buttes are shown in Figures 6 and 7.

Figure 6. View, looking south, of butte containing reference section A206, Emmons Co. Buttes in distance mark southernmost exposure of the Linton Member in Emmons Co.

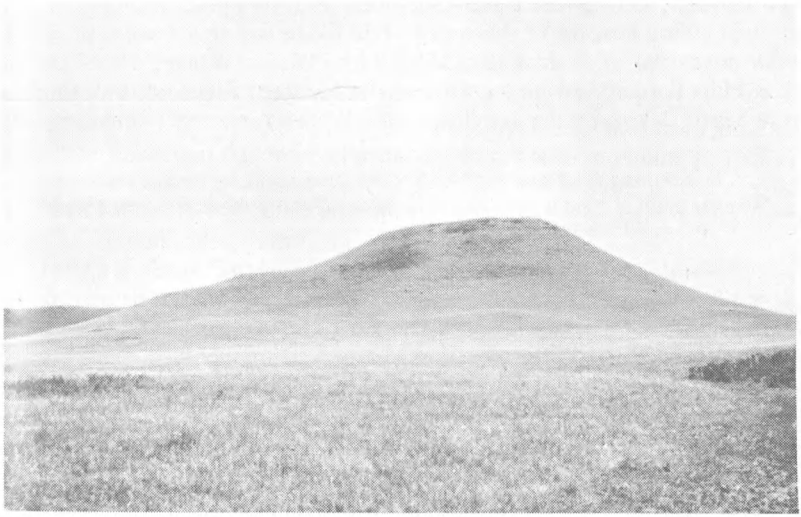

Figure 7. Reference section A203 caps this butte east of McLaughlin in Corson Co., S. Dak.; looking east.

HISTORICAL SIGNIFICANCE TO THE COLGATE PROBLEM

Our introductory remarks traced the literary history of the name "Colgate" from its Montana type area (Calvert, 1912) into the Missouri Valley (Curtis, 1952). No student of Fox Hills stratigraphy, with the exception of Hayden, had recognized the uniqueness of the Linton Member. Waage (1968) and Feldmann (1972) grouped these rocks under the term Colgate.

Hayden (1857) had an excellent concept of Missouri Valley stratigraphy *north* of the Grand River in South Dakota. He was intimately familiar with his Bed Q which held up "Fox Ridge." About Bed Q Waage (1968: 31) wrote:

> "One other subject relating to the upper contact of the Fox Hills Formation as interpreted by Meek and Hayden concerns the nature of "Bed Q", the limiting bed marking the base of their Lignite Group. . . . From their writings it is apparent that Meek and Hayden believed Bed Q to be a single widespread bed, but they were only slightly more fallible in picking out this unit at different localities than later geologists have been in trying to use the Colgate lithofacies as a mappable unit."

Meek and Hayden believed it to be widespread because, in fact, it is in the Missouri Valley, whereas the Colgate is not. The butte caps which remain are remnants of a more expansive unit than we presently see and easily lend themselves to Hayden's interpretation. The fluted Colgate lithofacies (Figure 8) occurs widely west of the Missouri Valley in many stratigraphic positions throughout the upper Fox Hills and lower Hell Creek as Waage demonstrated, and is a subdued, slope-forming lithology with no noteworthy butte-capping capability. Bed Q has little of this time-transgressive character.

To Hayden, Bed Q (the Linton Member) and the buttes it capped were the most outstanding features of the region. Fox Ridge *was* that system of buttes *as Hayden conceived of it.* Hayden (1857: 113-114; *in* Waage, 1968) described the Fox Hills Formation where it crossed the Missouri River north of the North Dakota-South Dakota border as follows, with Waage's accompanying remarks:

> "In describing his Formation No. 5 at the point where its outcrop crosses the Missouri north of what is now the South Dakota-North Dakota state line, Hayden (1857, p. 113-114) wrote:
>
> > 'Here it forms an extension of what is called Fox Ridge, a series of high hills having a northeast and southwest course, crossing the Missouri River into Minnesota at this point. Its northeastern limits I have not ascertained. In its southwestern extension it continues for a considerable distance nearly parallel with the Missouri, crosses the Moreau River about thirty miles above its mouth, then forms a high dividing ridge between the Moreau and Shyenne Rivers, at which locality it *first* took its name.' [italic's ours]
>
> This description is part of the text accompanying Hayden's first geologic map of the Upper Missouri country. On the map the words "Fox Ridge" extend northeastward across the Cheyenne-Moreau and Moreau-Grand divides."

Hayden obviously extended the use of the local name "Fox Ridge" to include the entire butte system held up by the Linton Member. The "high hills" to which he

referred are those buttes, rather than the Timber Lake tableland in the Cheyenne-Moreau Divide as Waage (1968) suggested. For this and other reasons, it is deemed most probable that the historic type area of the Fox Hills Formation as Meek and Hayden knew it began about at the mouth of the Grand River in South Dakota and extended northward into North Dakota perhaps to the mouth of Long Lake Creek in Emmons County. Failure to recognize the historical significance of the Linton Member is responsible for serveral misconceptions regarding Hayden's geologic interpretations.

In Emmons County, Feldmann (1972:31-32) included the Linton Member in his definition of Colgate as quoted below:

> "The uppermost member of the Fox Hills Formation in North Dakota is the Colgate Member. Although the term was originally coined for beds along the west flank of the Cedar Creek Anticline, it has been widely used in the Missouri Valley area since the work of Laird and Mitchell (1942). This unit presents perhaps the greatest variation in lithology of any of the members of the Fox Hills Formation in North Dakota. Near the type area, along the east flank of the Cedar Creek Anticline, in Bowman County, the unit is quite similar to that seen in the type area and consists of 36 to 40 feet of medium-grained, white, graywacke sandstone with thin scattered layers and partings of lignitic shale. The most characteristic feature of the unit in this area is the fluted surface observed on all weathered exposures. The unit can also be seen in similar aspect near Crowghost Cemetery in sec. 33, T. 134 N., R. 81 W., in Sioux County. As the unit is traced eastward and southeastward from this area, however, its character changes markedly and is normally characterized by less than ten feet of well indurated, white to cream colored, flaggy sandstone. This condition is observable on Redhorse Butte in Sioux County in sec. 21, T. 134 N., R. 79 W., along the Cannonball River flood plain in Sioux County, as well as on many butte tops in Emmons and Logan counties."

Furthermore, Feldmann did not adequately define Fox Hills facies east of the Strassburg Channel. He presented only one stratigraphic section from Emmons County (Plate 1, section 6), an erosionally truncated section ending in rocks of Timber Lake-like lithology. Fisher (1952) gave detailed sections of these rocks, but, as noted earlier, he made no attempt to formally define units within them. His unit "D" is equivalent to the Linton Member.

The term "Colgate" should be restricted to the white to light gray, very poorly sorted, generally unindurated, lenticular sand bodies with fluted weathering and few fossils. This rock may have 45-65% clay matrix and contains no volcanic shard component. It weathers to a very diagnostic fluted surface as seen in our Figure 8, as shown by Dobbin and Reeside (1929, Plate 4, c), and as noted by Feldmann (quoted above) for the Colgate in its type area.

Linton Member sandstones are light green to gray, poorly sorted, subangular in grain shape, moderately-to-well indurated, contain up to 40% matrix (usually about 28%), and have a substantial volcanic shard component. They never weather to a fluted surface and have a well defined *Ophiomorpha-Equisetum* fossil component. In contrast to a typical Colgate outcrop as seen in Figure 8, the Linton Member appears as in Figures 4 and 9.

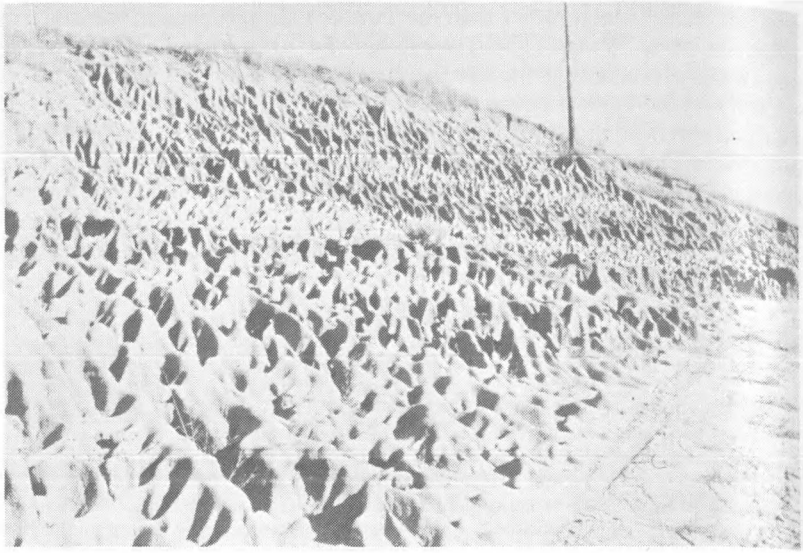

Figure 8. Characteristic weathered appearance of Colgate lithofacies to be compared with Figures 4 and 9 of Linton Member. Fence gives scale.

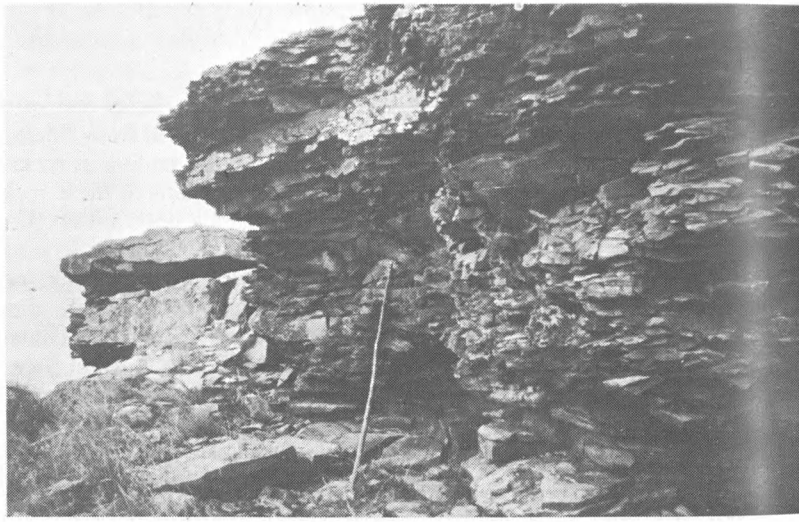

Figure 9. General outcrop appearance of Linton Member. Staff length is five feet.

The distinctions made above are sufficient to define sandstones here placed in the Linton. Such definition should aid in interpretation of the historic concept of the Fox Hills Formation and in future paleoenvironmental studies and geologic mapping of the formation.

PALEOENVIRONMENTAL INTERPRETATIONS

The *Equisetum* flora and mangrove-like root systems indicate that the sediment surface was at or slightly above the highwater line of the Maestrichtian sea. This is corroborated by the presence of large, sparsely distributed *Ophiomorpha*, a characteristic of *Callianassa* occurrences in estuarine environments today (Land, 1972). The waters were more brackish to the north where no marine bivalves occur, and more marine to the south where *Tancredia* and *Cymbophora* are found in the Linton.

Sandstone was deposited either as large shoals, somewhat analogous to point bars, in an estuarine, tidal river or deltaic distributary, or as a series of shoreline shoals along a narrow, north-trending, estuarine embayment. Source of sands was largely from reworking of previous Fox Hills deposits including the Timber Lake, Colgate, and the Emmons and Sioux County ash beds. The sea probably retreated south-southeastward from the local area at which time lignite-producing swamps flourished locally. This relationship became more complex eastward in McIntosh County.

Similar deposits in Wyoming have been described by Land (1972). He defined an estuarine channel sandstone with characteristics very similar to those of the Linton Member. Channels were marked by scour and lag deposits of gravel and oyster shells at their base. The base of the Linton Member is not sufficiently exposed in most areas to interpret analogous conditions. We do feel that they occur in some oyster hashes in Sioux County, but such relationships are not yet firm. The oysters may have been deposited in tidal channels tributary to the main estuarine river here described or in such channels meeting the estuarine shoreline along which sandy shoals of the Linton Member were forming.

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Douglas O'Brien has invested energy and ideas throughout the study and deserves particular thanks. The Haas family, formerly of Solen, North Dakota, has warmly hosted wet and weary field parties on a moment's notice for two summers. Dr. and Mrs. F. D. Holland, Jr. also graciously hosted the field party on several occasions and Bud Holland provided helpful criticism of the preliminary manuscript. Cynthia J. Munsell examined the botanical fossils from the Linton

Member, and Catherine Goodmen and Dale Chayes made preliminary petrographic analyses of its rocks. The manuscript was typed by Alice Quackenbush. We gratefully acknowledge the contribution each has made to this work.

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APPENDIX A

Type Section (A216)
Linton Member, Fox Hills Formation

Composite section beginning on NW tip of butte, extending to exposure on center of N side of butte, N½ secs. 8 and 9, T. 132 N., R. 76 W., Emmons Co., N. Dak.

Fox Hills Formation
Linton Member

Unit		Thickness (feet)
20.	Sandstone, silty, fine-grained, brown-gray brown, weathers gray brown; abundant plant fragments; 1.5 ft. maximum thickness.	0.8
19.	Sand, fine-grained, gray-tan; contains poorly indurated, fine-grained, sandstone pods above cross stratified beds.	2.2
18.	Sandstone, fine-grained, gray to gray-green, weathers gray-tan and brown-rust on fractures; massive; vertical preserved roots, some over 3 feet long; wood fragments, increasing towards top half of unit; *Equisetum* in lower half.	4.0
17.	Sandstone, fine-grained, gray, pelleted appearance; weathers gray-tan with some orange-rust color; weathers into BB-size pellets; vertical preserved roots; some thin clay laminae.	1.0
	Total thickness, Linton Member	8.0 (2.4 m)

Fox Hills, Undifferentiated

16.	Covered section.	90
15.	Sandstone, very fine-grained, sea green, weathers dark green-gray with some rusty laminae; thin bedded; fossils *Tancredia americana, Tellinimera scitula, Nucula, Cymbophora warrenana, Protocardia,* a scaphitid ammonite, *Ophiomorpha,* unidentified fish scales; fossils preserved in battered condition, spotty exposure.	7
14.	Covered section.	20
13.	Sandy silt, very fine-grained sand, tan; fossils *Euspira subcrassa, Goniocylichna bisculpturata?, Tancredia americana;* poor exposure.	1.5
12.	Sandy clay, very fine-grained sand, dark gray fissile.	1
11.	Sandy silt, very fine-grained sand, gray-tan, weathers tan with some rust color.	6
10.	Sandy silt, semi-indurated, very fine grained sand, gray-light tan with rusty sand laminae; bedded; gradational contact with unit below.	3
9.	Sandy silty shale, dark brown, weathers gray; lignitic; finely parted; few rusty sand laminae.	1.5
8.	Sandy silt, very fine-grained sand, gray-white, weathers gray-tan, bedded, some rusty laminae of sand; gradational contact with unit below.	2
7.	Sandy silty clay, tan-gray, irregular partings; rust colored sand filling burrow tubes; few laminae of lignitic material; may contain volcanic ash.	5
6.	Covered section.	3
5.	Silty shale, gray, weathers light gray-white; fecal pellets abundant; small wood fragments present; contains clay pods and volcanic ash.	1
4.	Covered section.	1
3.	Sandstone, green-gray, like unit 15 above; wood fragments, clay galls, *Ophiomorpha*; poor exposure.	20
2.	Covered section.	22
1.	Volcanic ash, white-light gray.	
	Total thickness, Fox Hills undifferentiated	188 (56.6 m)
	Total thickness measured	196 (59.0 m)

Reference Section (A206)
Linton Member, Fox Hills Formation

Section measured beginning on the southernmost butte of a four-butte system, extending downward to exposure in small valley directly SE of butte base, W½ NW¼ sec. 28, T. 130 N., R. 78 W., Emmons Co., N. Dak.

Fox Hills Formation
Linton Member

Unit		Thickness (feet)
16.	Silty sandstone, fine-grained, tan-gray, abundant angular wood fragments, some 2 inches × 3¼ inches; gradational contact with unit below.	0.6
15.	Sandstone, fine-grained, steel gray, weathers rust colored from iron stain; brittle, breaks in curved fracture; vertical preserved roots to 2 feet in length; *Equisetum* in horizontal position; thin laminae of wood fragments at top; grades laterally wtih unit below.	2.2
14.	Sandstone, fine-grained, gray-green to gray-tan, weathers in blotchy pattern; vertical preserved roots; shows textural variations, grades to more friable cross-bedded sandstone below, and grades laterally to fine-grained, steel gray sandstone, weathering rust colored; brittle with curved fracture; vertical preserved roots like in unit 16 above.	4
13.	Sandstone, fine-grained, gray to gray-tan; friable, weathers to small pellets; gradational contact with unit below.	2
	Total thickness measured, Linton Member	8.8 (2.7 m)
Fox Hills, undifferentiated		
12.	Sand, fine-grained, gray-tan; contains semi-indurated ledges of fine-grained sandstone.	8
11.	Covered section.	3
10.	Sand, fine-grained, orange-tan to gray with green tinge; some rusty ledges of fine-grained sand and clay.	2
9.	Covered section.	10
8.	Sand, fine-grained, orange-tan, green tinge, scattered small pieces of indurated fine-grained sand and clay.	1
7.	Sand, very fine-grained and silt, gray-tan.	1
6.	Covered section.	95
5.	Silty clay, brown-gray, poor bentonitic "popcorn" weathering.	1
4.	Covered section.	40
3.	Sandy, silty, clay, very fine-grained sand, gray-brown, bentonitic "popcorn" weathering.	2.5
2.	Covered section.	45
	Total thickness Fox Hills undifferentiated	208.5 (62.8 m)
Timber Lake Member (?)		
1.	Sand, fine-grained, orange with green cast; glauconitic; one rust-brown indurated ledge of calcareous cement containing *Ophiomorpha;* small concretions containing poorly preserved shells and small wood fragments; base covered.	7
	Total Fox Hills measured	215.5 (65.5 m)

Reference Section (A203)
Linton Member, Fox Hills Formation

Section taken from small butte, directly southwest of a large ridge and butte complex approximately 11 miles E. of McLaughlin, NW¼ SE ¼ sec. 23, T. 22 N., R. 28 E., Corson Co., S. Dak.

Fox Hills Formation
Linton Member

Unit		Thickness (feet)
5.	Sandstone, fine-grained, gray-tan, flaggy.	2
4.	Covered section.	1
3.	Sandstone, fine-grained, green-gray with "salt and pepper" appearance; weathers to pitted surface in places due to presence of clay pods and galls; *Ophiomorpha;* cross-strata, ripple marks; some massive blocks have slumped down butte side; gradational contact with unit below.	9
2.	Sandstone, fine-grained, green-gray, pelleted surface; unit not persistent.	1
1.	Sandstone, fine-grained, like unit 3 above; more flaggy; base covered.	2
	Total thickness measured, Linton Member	15 (4.5 m)

MINERALOGY OF THE CLAY-SIZE FRACTION OF THE PALEOCENE TONGUE RIVER AND SENTINEL BUTTE FORMATIONS NEAR MEDORA, NORTH DAKOTA

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ABSTRACT

X ray diffractograms of 87 samples of the Tongue River and Sentinel Butte Formations near Medora, North Dakota indicate that the principal minerals of the clay-size fraction of both formations are clay minerals of the montmorillonite, mica-illite, kaolinite and chlorite groups, and quartz, dolomite, calcite, K-feldspar and plagioclase. Montmorillonite is more abundant in the Sentinel Butte Formation than in the Tongue River. In both formations montmorillonite basal reflections are higher than either the 10 A mica-illite or the 7.1 A kaolinite and chlorite reflections. Mica illite, quartz and dolomite are generally more abundant in the Tongue River than in the Sentinel Butte. These results suggest that mineralogic criteria may be developed to distinguish these formations. Attempts to correlate clay mineralogy with depositional environments in the Tongue River Formation were not successful.

INTRODUCTION

The non-marine, Paleocene Tongue River and overlying Sentinel Butte Formations are well-exposed in the Little Missouri Badlands of western North Dakota. They consist mostly of silt and clay with minor amounts of sand, limestone, and lignite. The United States Geological Survey and others regard the Tongue River and Sentinel Butte as members of the Fort Union Formation whereas the North Dakota Geological Survey and we regard them as formations in the Fort Union Group.

Many geologic studies of the Paleocene strata of western North Dakota were published in the early 1900s in bulletins of the United States Geological Survey and the North Dakota Geological Survey (Hares, 1928; Herald, 1913; Leonard, 1908; Leonard and Smith, 1909; Leonard, Babcock, and Dove, 1925; Lloyd, 1914; Pishel, 1912). More recent work includes that of Benson (1952), Bergstrom (1956), Crawford (1967), Denson and Gill (1965), Hanson (1955), and Meldahl (1956). Royse (1967) has provided a good review of most of this work.

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There have been no published mineralogic analyses of the clay-size fraction of the Tongue River or Sentinel Butte Formations. Clark (1966) presented a few x-ray analyses of the "blue bed" of the Sentinel Butte Formations in the North Unit Theodore Roosevelt National Memorial Park. His results agree with those presented in this report. There have been no published detailed cross sections of the Paleocene strata across the Williston Basin. This is partly because of the lack of adequate criteria for distinguishing the units in the subsurface. This study was designed, in part, to help provide such criteria.

The objectives of this study were (1) to determine what relationships, if any, exist between depositional environment and clay mineralogy in the Tongue River Formation, and (2) to find what differences, if any, exist between the mineralogy of the clay-size fractions of the Tongue River and Sentinel Butte Formations.

METHODS

Field work.—The field work for this study was based on work by Jacob (1973) who constructed a detailed cross section of units of the Tongue River Formation near Medora, North Dakota and interpreted the depositional environments of the recognized units. More than 80 samples were collected from these units (Jacob, 1973, Figure 5) near Medora (NW¼ sec. 26, T. 140 N., R. 102 W.) by one of us (R.E.) during the summer of 1973. Seven samples of the Sentinel Butte Formation were also collected from outcrops along the south side of Interstate 94 about five miles west of Medora (E½sec. 13, T. 140 N., R. 103 W.)

Laboratory study.—The samples were analyzed for the mineralogy of the clay-size fraction using X-ray diffraction techniques. Samples were gently disaggregated with mortar and pestle and suspended in distilled water, using an electric blender. Ten ml of suspension of the less than 4-phi clay fraction was separated by pipette and allowed to evaporate on a glass slide. All slides were x-rayed using a Philips high angle diffractometer and copper K-alpha radiation from 4°2Θ to 32°2Θ at a scan speed of 2°2Θ per minute. Some slides were glycolated and then scanned again from 59°2Θ to 63°2Θ following standard procedures for identifying layer silicates (Warshaw and Roy, 1961; Carroll, 1970; Grim, 1968).

RESULTS

The minerals identified in the clay-size fraction of the Tongue River and Sentinel Butte Formations and the results of a comparison of X-ray diffraction intensities are summarized below and in Table 1.

The clay-size fraction of the Tongue River Formation contains: (1) relatively large amounts of clay minerals of the montmorillonite and mica-illite groups, lesser amounts of kaolinite, and a small amount of chlorite, as indicated by basal X-ray reflections; (2) quartz, dolomite and minor calcite, alkali feldspar and plagioclase; (3) Ca-montmorillonite as indicated by a broad basal peak at 14-15 Å; (4) abundant mica relative to illite as indicated by the sharp 10 Å peak; (5) possible abundant biotite in the mica fraction as indicated by the presence of a peak at 1.52-1.53 Å; (6) possible dehydrated halloysite rather than kaolinite as

Table 1. X-ray data for clay and non-clay minerals from stratigraphic units of the Tongue River and Sentinel Butte Formations. Clay mineral values are given as the percentage of samples having the highest intensity clay mineral peak at the position noted. Non-clay mineral amounts are estimated as abundant (ab), common (co), or scarce (sc) in the clay-size fraction of these samples.

Sample	A[a]	C	E	G	H	J	L	LS	CS	F	NC	TR1	TR2	SB
No. of Samples	5	5	12	10	15	16	11	3	10	32	15	87	76	7
Mineral														
Montmorillonite 14-15A	20	20	58	10	40	19	64	100	0	39	15	37	24	100
Mica-Illite 10A°	60	80	42	60	33	44	18	0	40	45	70	42	51	0
Kaolinite + Chlorite 7.1A°	20	0	0	30	27	37	18	0	60	16	15	21	25	0
Quartz 3.34A°	ab	ab	ab	ab	ab	co	sc	sc	co	ab	ab	co	co	sc
Dolomite 2.88A°	co	sc	ab	co	ab	co	sc	co	co	ab	co	co	co	sc

[a]A-L = units in Tongue River Formation (Jacob, 1973, Figure 5) collected near Medora, North Dakota.
LS = sand body in unit L.
CS = River—channel sand in unit C.
F = Flood basin units A, E and H.
NC = Natural levee and crevasse—splay units C and G.
TR1 = Average of all samples from the Tongue River Formation.
TR2 = TR1 minus samples of Medora Member, unit L.
SB = Sentinel Butte Formation samples collected near Medora, North Dakota.

indicated by peaks at 1.54 Å rather than at 1.48 Å; (7) similar kinds and amounts of clay and non-clay minerals in river channel, flood basin, natural levee, and crevasse-splay environments as shown by CS, F and NC in Table 1 with possible higher montmorillonite in the flood basin, mica illite in natural levee and crevasse splay, and a kaolinite-chlorite in channel sand enviroments; and (8) particularly abundant montmorillonite in the Medora Member (L, Table 1). The clay fraction of the Sentinel Butte Formation, in comparison with the Tongue River Formation, contains (1) more montmorillonite, and (2) less mica, quartz, and dolomite.

These results suggest that mineralogic creiteria may be developed to distinguish the Tongue River and Sentinel Butte Formations.

ACKNOWLEDGMENTS

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STRATIGRAPHY AND DEPOSITIONAL ENVIRONMENTS OF PALEOCENE DEPOSITS IN THE HUSKY LIGNITE MINE NEAR DICKINSON, NORTH DAKOTA

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ABSTRACT

Exposures in the Husky Lignite Mine near Dickinson, Stark County, North Dakota, are in the uppermost Tongue River Formation or lower Sentinel Butte Formation (Paleocene). Sand beds are about 3 m thick, have erosional bases and gradational tops, and become finer-grained and more silty and clayey from bottom to top. They contain large-scale curved sets of cross strata in the lower part and mixed large-scale sets of low-angle, straight, cross strata and small-scale sets of cross strata in the upper part. They are interpreted to have been deposited on point bars in high-sinuosity streams.

A FORTRAN program has been written to descirbe paleoflow characteristics of these streams based on Cotter's (1971) method of analysis. Results of this method indicate that the streams that deposited the sand beds were about 3 m (10 feet) deep, 18 m (60 feet) wide, had sinuosities of about 2, mean annual discharges of about 4.2 m^3/s (150 cfs), meander lengths of about 215 m (700 feet), drainage areas of about 520 km^2 (200 square miles), stream lengths of about 50 km (30 miles), and channel slopes of about 1 m (3 feet) per mile.

The beds surrounding the sand beds are interpreted as deposits of an alluvial plain. Brown to yellow brown clayey and sandy silt beds that contain climbing-ripple cross strata and limonitic nodules are interpreted as deposits of natural levees adjacent to the streams. Lignite beds and gray beds of clay and silty clay that have a high content of organic matter, distorted stratification, and iron-sulfide nodules are interpreted as deposits of flood basins marginal to the main streams. Beds of evenly laminated clay and silty clay are interpreted as deposits of lakes and ponds on the alluvial plain.

INTRODUCTION

Lignite-bearing Paleocene strata constitute the bedrock over 75% of the North Dakota part of the Williston Basin. The strata are almost completely unlithified and can easily be excavated with a pick. Many active open-pit lignite mines are present in this area and provide excellent exposures for sedimentologic study. This report is an example of how lateral and vertical successions of sedimentary sturctures, textures, and lithologies in these deposits can be used to decipher depositional environments. It also adds to the knowledge of paleogeographic conditions during part of the Paleocene Epoch in the Williston Basin and it provides more specific sedimentologic evidence for the fluvial origin of the deposits than the work of Royse (1970). The Husky Lignite Mine was studied and is located about 7 km southeast of Dickinson, Stark County, North Dakota (Figure 1).

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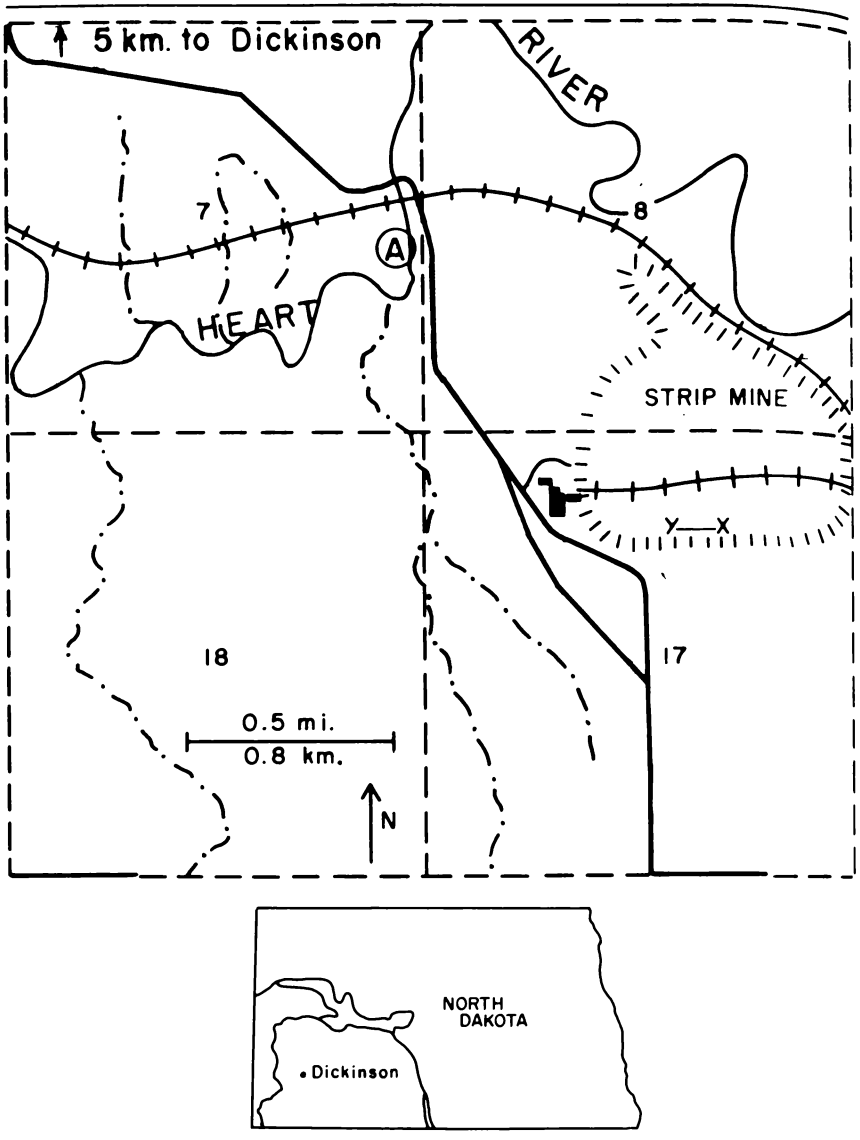

Figure 1. Location of Husky Lignite Mine near Dickinson, North Dakota. Line XY indicates location of Figure 2. Exposures of Tongue River Formation occur along the east bank of the Heart River at A. Numbers are sections in T. 139 N., R. 95 W., Stark County. The road is light-duty, unnumbered, and local.

Jacob's (1973b) classification of cross stratification is used throughout this report. The English system of measures is used because of the difficulty of converting Schumm's (1960) equations to the metric system. Grain-size analyses were performed by me with a settling-tube method described by Felix (1969) modified with a shutter-type release mechanism.

STRATIGRAPHIC SETTING

General.—In North Dakota the Paleocene Fort Union Group consists, in ascending order, of the Ludlow and Cannonball, Tongue River, and Sentinel Butte Formations. These units generally dip at very low angles toward the middle of the Williston Basin. Lignite in North Dakota is obtained entirely from the Ludlow, Tongue River, and Sentinel Butte Formations. These units represent the continental deposits that prograded into the sea as it withdrew from the Williston Basin during the early part of the Paleocene Epoch.

Husky Lignite Mine.—It is not certain whether the strata exposed in the Husky LIgnite Mine are in the Tongue River Formation or the Sentinel Butte Formation. A wide area, including the mine, has been mapped as the Sentinel Butte Formation (Carlson, 1969). However, outcrops of about 50 feet of Tongue River Formation occur at an elevation of 2350 feet along the banks of the Heart River about one-half mile west of the mine (location A, Figure 1). Outcrops of the Tongue River Formation at this high elevation indicate the presence of some previously unrecognized structure. The strata exposed in the mine itself, at an elevation of 2400 feet, have characteristics of both formations. The sand beds (Figure 2) are less friable, more laterally continuous, and darker gray and brown than the sand bodies of the Tongue River Formation, but the strata surrounding the sand beds are light yellow and gray as in the Tongue River Formation. This may indicate intertonguing of the two formations.

DEPOSITIONAL ENVIRONMENTS

Sand beds.—The depositional environments of the strata shown in Figure 2 must be interpreted in the light of fossil evidence, which indicates a terrestrial, fresh-water environment for both the Tongue River and Sentinel Butte Formations (Delimata, 1969; Yen, 1946, 1948). The sand beds of Figure 2 are interpreted as stream deposits. Modern streams can be classified either as meandering or braided, and meandering streams can be classified either as low-sinuosity ("straight" streams of most workers) or high-sinuosity streams. Moody-Stuart (1966) described deposits preserved in the geologic record that are characteristic of both stream types.

The two sand beds in Figure 2 are interpreted as high-sinuosity (point-bar) stream deposits. Fisk (1947) described some of the processes involved in the lateral accretion of point-bar deposits. Allen (1970a, 1970b) showed that point-bar deposits become finer grained upward, and that several vertical arrangements of sedimentary structures may be present. Small-scale sets of cross strata are in the upper rather than the lower parts of such sand beds.

Bernard et al. (1970) described modern examples of sand beds deposited in the

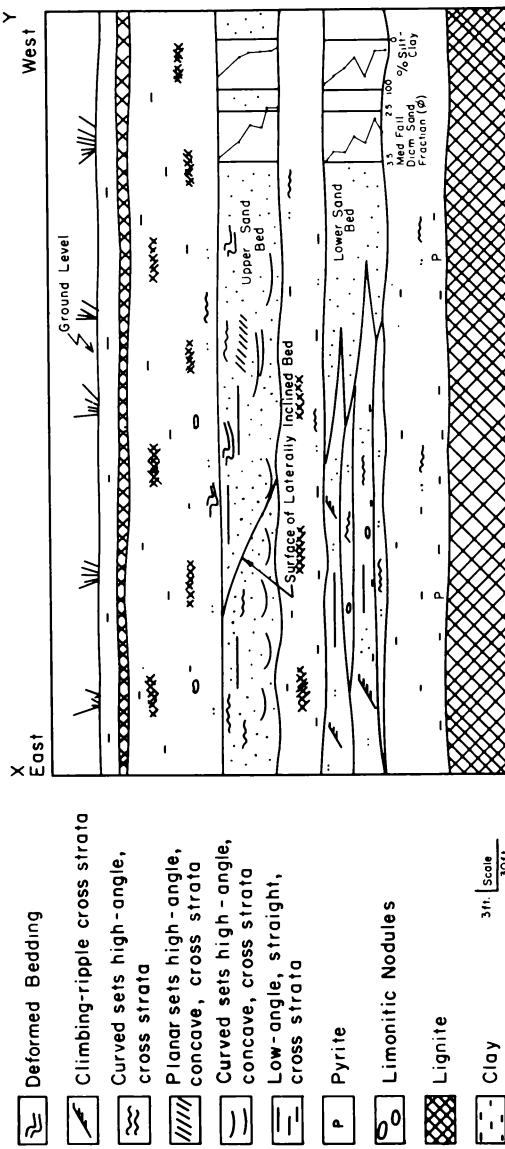

Figure 2. Diagram of part of south wall of Husky Lignite Mine near Dickinson, North Dakota. Line XY in Figure 1 shows the location.

Brazos River, a high-sinuosity stream. The beds average about 55 feet in thickness and become finer grained upward. They described "giant ripple cross bedding" (large-scale, curved sets of cross strata) in the lower and middle part of the beds, overlain by "horizontal bedding" (large-scale sets of low-angle, straight cross strata), which, in turn, is overlain by "small ripple cross-bedding" (small-scale sets of cross strata) at the tops of the beds. This sequence is very similar to that of the upper sand bed of Figure 2. The Brazos River deposits contain poorly bedded gravel at the base, but there is no gravel in the deposits studied here.

Laterally inclined beds with contacts that extend from the top of the upper sand bed to its base are present (Figure 3). Diagrams and photographs of laterally inclined beds such as these were shown by Allen (1965a), who called them ep-

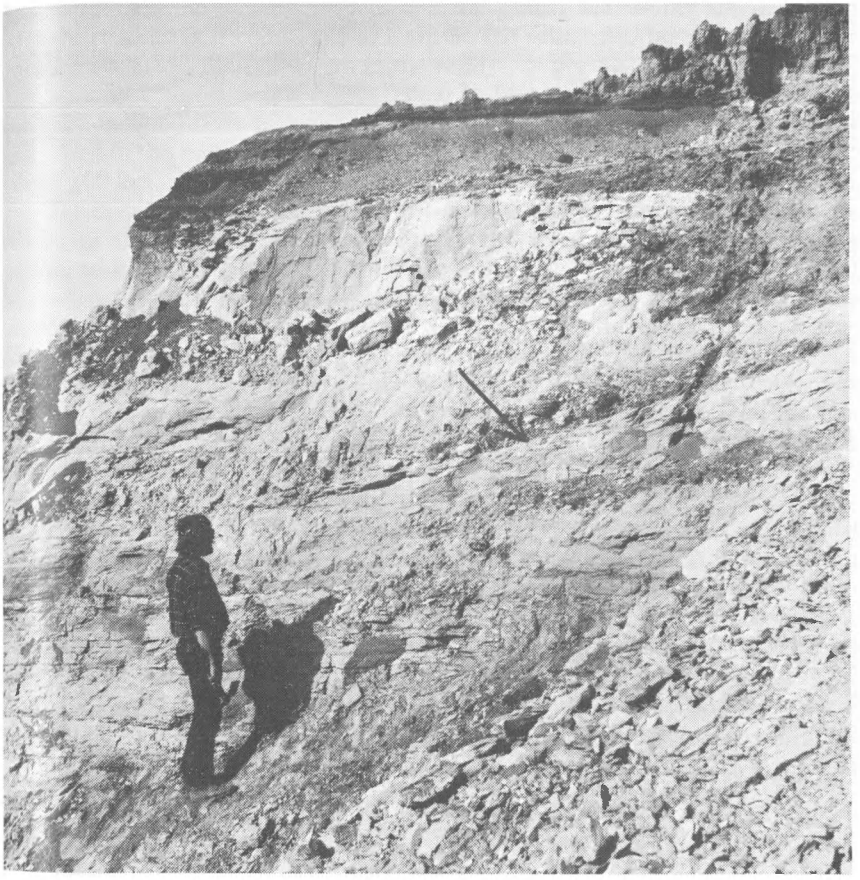

Figure 3. Surface of laterally inclined bed (arrow) in upper sand bed of Figure 2. The view is looking north on the mine-access road.

silon cross strata, although their characteristics do not match those of epsilon cross strata as originally defined by Allen (1963, Fig. 3D). Allen (1965a) attributed epsilon cross strata to lateral accretion on point bars in meandering streams.

Figure 4 shows how the sand beds of Figure 2 probably originated. R. J. LeBlanc, Sr. (written communication) attributed the surfaces of the laterally inclined beds to erosion toward the convex bank of the stream bend. These surfaces are not very abundant in the sand beds of Figure 2. This indicates that lateral accretion of the sand bed was not often interrupted by strong erosion. Maximum stream depth in the bends of the depositing streams was equal to the thickness of the sand bed (Figure 4).

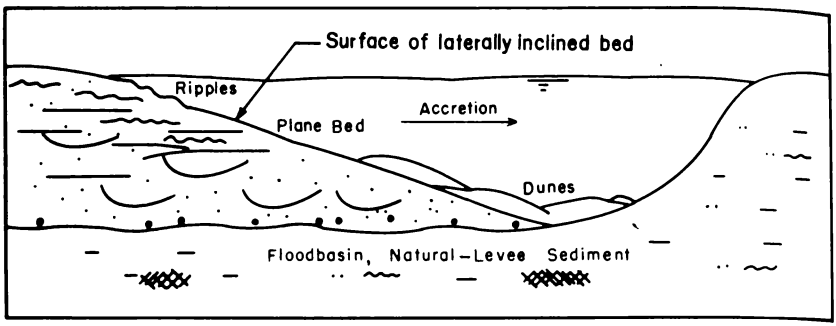

Figure 4. Model for origin of sand beds in Husky Lignite Mine. Lateral accretion on a point bar is taking place toward the concave side of a stream bend. The surface of the laterally inclined bed (Figure 2) forms when erosion of the point bar is followed by renewed lateral accretion. The thickness of the sand bed is about equal to the bankfull stream depth. Bedforms on the stream are labeled. The legend is given in Figure 2. Modified from Allen (1970a).

FLOW INTERPRETATIONS

Cotter's method.—The upper sand bed exposed in the mine wall (Figure 2) lends itself very well to the kind of analysis made by Cotter (1971) for the Upper Cretaceous Ferron Sandstone in Utah. A computer program in Fortran IV language was written by Robert P. Johnson (personal communication), and slightly modified by me, to perform operations similar to those outlined by Cotter.

The type of sediment in a stream channel can be characterized by the parameter M defined by Schumm (1960) as

$$M = \frac{SC \times W + SB \times 2D}{W + 2D}, \quad (1)$$

where SC is the average percentage of silt and clay (fraction finer than 0.074 mm) in the channel alluvium, SB is the percentage of silt and clay in the bank alluvium, W is the bankfull channel width, and D is the bankfull channel depth.

Schumm (1960, 1963) developed the following regression equations relating M to the width:depth ratio (F) and to the sinuosity (P), and relating P to F:

$$F = 225\ M^{-1.08}, \tag{2}$$

$$P = 0.94\ M^{0.25}, \tag{3}$$

$$P = 3.5\ F^{-0.2}. \tag{4}$$

In order to calculate M, values for the bankfull stream width and depth are necessary. As shown in Figures 2 and 3, the thickness of the sand bed (10 feet) is about equal to the bankfull stream depth in the bend. Schumm's equations were developed for straight reaches of streams. SC and SB probably do not change much between the straight reaches and the bends, but depths are less in the straight reaches as is commonly known. So the value used here for D is 9 feet, which is 10% less than the depth in the bend.

Bankfull channel width can be taken to be about 3/2 of the width of the laterally inclined beds as indicated by Cotter (1971). The width of these beds measured in the mine is about 65 feet. This indicates a bankfull channel width of about 100 feet.

The calculated width:depth ratio (F) is about 11. If equation 2 above is solved for F, the result is about 6. These values do not agree well, and suggest that the stream width was less than 100 feet, or, in other words, that the measured width of th laterally inclined beds is greater than the true width. It is possible that the mine wall is not perpendicular to the strike of the laterally inclined beds, and the measured value of the width of these beds is larger than the true value. The beds are not well enough developed to measure their orientation to check this possiblity. But if a stream width of 60 feet is used instead of 100 feet, the calculated width:depth ratio is 6.6, and this value agrees well with the value of about 6 obtained from equation 2. So a value of 60 feet is used for the stream width (W).

The sand exposed in the mine is very friable, and samples can be disaggregated and analyzed in the laboratory. The results give values of about 20% for SC (Figure 2) and SB is assumed to be 100%. Using these values, and those determined for W and D, M is calculated to be 39.

The sinuosity calculated by equation 3 is 2.3 and by equation 4 is 2.3. These results indicate a sinuosity of about 2.

Mean annual discharge (Qm) can be estimated using Schumm's (1968) equations, rearranged to give

$$Qm = \sqrt{\frac{WM^{0.39}}{37}}^{\frac{1}{0.38}}, \text{ and} \tag{5}$$

$$Qm = \frac{1}{0.29} \sqrt{\frac{D}{0.6M^{0.34}}}. \quad (6)$$

Equation 5 indicates a mean annual discharge of 150 cfs (cubic feet per second), and equation 6 indicates a mean annual discharge of 160 cfs. These results are extremely close to each other, and indicate a mean annual discharge of about 150 cfs.

Stream-meander length (L) can be estimated from the mean annual discharge using Schumm's (1968) equation

$$L = 1890 \frac{Qm^{0.34}}{M^{0.74}}. \quad (7)$$

Using values of 150 and 160 for the mean annual discharge, equation 7 gives values of meander length of 700 and 710 feet. This indicates a stream meander length of about 700 feet. This figure can be checked using Leopold, Wolman and Miller's (1964:297) empirical relation

$$L = 10.9\ W^{1.01}, \text{ or}$$

$$L \cong 11\ W. \quad (8)$$

Equation 8 indicates a stream meander length of about 660 feet, which agrees very well with the value of 700 feet.

Drainage area (A) can be estimated using Allen's (1970d:120) relation rewritten to give

$$A = 1.313\ Qm, \quad (9)$$

where Qm is mean annual discharge (cfs) and A is drainage area (square miles). Allen indicated that his formula applies only to drainage areas of less than 2.2×10^6 square miles (5.7×10^6 km^2). Using the values of mean annual discharge of 150 cfs and 160 cfs derived from equations 5 and 6, equation 9 gives results of 190 and 200 square miles. This indicates a drainage area of about 200 square miles.

Stream length (SL) can be estimated from the drainage area using Leopold, Wolman, and Miller's (1964) equation

$$SL = 1.4A^{0.6}.$$

Using the two values of the drainage area of 190 and 200 square miles obtained above, the stream length is calculated as 33 miles and 34 miles. This indicates a stream length of about 30 miles.

Channel slopes (CS) can be estimated using the equation of Schumm (1968):

$$CS = 60M^{-0.38}\ Qm^{-0.32}.$$

Using the value of mean annual discharge of about 150 cfs a channel slope of about 3 feet per mile is indicated.

As Cotter (1971) pointed out, Nordin, Simons, and Richardson (1965) indicated that dunes exist in the range between 1.5 Vc and 3.5 Vc where Vc is the critical erosion velocity for initiation of grain movements. For a grain size of about 2.7 phi (about 0.15 mm) (Figure 2), Figure 13 of Sundborg (1965) indicates a critical erosion velocity of about 1.3 (0.4 m) fps (feet per second) in water about 9 feet (3 m) deep, the depth of the depositing stream as shown above. So the mean velocity of the stream depositing the upper sand bed of Figure 2 may have been about 2.5 times this value, or roughly 3 (1 m) fps.

These flow characteristics indicate that the river that deposited the upper sand bed was not a major one on the alluvial or deltaic plain where the lignite formed. The characteristics are in the general range of some of the small rivers or bayous on the modern coastal plain of Texas or Louisiana.

Word of caution.—As Cotter (1971) pointed out, Schumm's (1960) equations were derived from data obtained in semiarid to subhumid regions. The absence of grass before the Miocene Epoch may have resulted in erosion and runoff conditions in pre-Miocene humid regions that were similar to those in modern semiarid to subhumid regions. The results of Cotter's (1971) method might be inaccurate to the extent that erosion and runoff conditions were different from those in modern semiarid to subhumid regions.

Climatic effects should be considered. Two high-sinuosity stream deposits of similar dimensions could result from deposition in two streams with different drainage areas, for example, if climatic conditions in the two cases were different. In a humid region—such as is indicated by the floral evidence in the deposits described here—the drainage area might be smaller than indicated by Schumm's equations.

As Cotter (1971) pointed out, Schumm's sediment load parameter (M) is based on measurements in straight reaches. A point-bar deposit in the geologic record formed in stream bends. Stream depths in the bends are greater than in straight reaches, and the silt-clay content in the bends may be different than in the straight reaches. Cotter's method will be inaccurate to the extent to which the silt-clay content and the estimated corrected depth are different from the actual values in the straight reaches of the depositing stream.

The ratio between the width of the active part of a point bar, not covered by vegetation (scroll bar of Allen, 1968:43), to the bankfull width of the stream may

be greater or less than the 2:3 value used by Cotter (1971). The accuracy of Cotter's (1971) method will be affected by the accuracy of this two-thirds relationship.

Another source of error is the natural scatter in the data used to establish the regression equations. The equations are only best-fit estimates, so that results obtained from the equations cannot be treated as facts.

Cotter's (1971) method of analysis can only give approximate results that must be considered in light of these possible inaccuracies. By considering the overall depositional setting of a stream deposit and by comparing results obtained using different equations, judgments can be made in individual cases as to whether the results of Cotter's method are reasonable. The flow environment of the stream deposit described here is in close accord with a depositional setting of a low-lying coastal plain as suggested by the deposits of the Fort Union Group.

STRATA SURROUNDING THE SAND BEDS

General.—The strata surrounding the sand beds probably were deposited on an alluvial plain as indicated by their internal characteristics and their association with the fluvial sand beds described above. The subenvironments of the alluvial plain, adjacent to the channel, are natural levee and flood basin (Allen, 1965a). Swamps, lakes, and ponds are located in the flood basin away from the main channel. Crevasse-splays form on the natural levees in places, and they may extend into the flood basins.

Lacustrine deposits.—The light to dark gray fine silt and clay that directly overlies the thick lignite bed at the base of Figure 2 shows very well developed planar laminae (Figure 5). These laminae indicate deposition in a lake that formed during foundering of the swamp in which the lignite formed. Coleman (1966) stated that these laminae are the most common type of sedimentary structure in the shallow-water lakes of the Atchafalaya basin in southern Louisiana. Wavy laminae and lenticular laminae, described by Coleman and Gagliano (1965) from the same lakes, are present along with the planar laminae.

Swamp deposits.—The lignite beds (Figure 2) are interpreted to have been formed in swamps in the poorly drained parts of floodbasins. Lignitized stumps of cypress trees and abundant fallen logs are visible on the upper surface of the thick lignite bed at the base of Figure 2 where the material over the lignite bed is removed during mining operations. These logs and stumps indicate swamps rather than marshes.

Beds of fine silt and clay, interpreted as deposits of swamps, contain abundant organic remains and are lignitic and pyritic in many places. They are light to dark gray, and, in a few places, they contain small-scale cross strata and various types of distorted or contorted laminae. These characteristics are very similar to those of the poorly-drained swamp environment described by Coleman (1966).

Deposits of well-drained swamps described by Coleman (1966) have less organic matter and pyrite and they have more nodules of limonite and calcite than poorly-drained swamp deposits. Beds interpreted as well-drained swamp deposits are less abundant in the mine than are poorly-drained swamp deposits. The

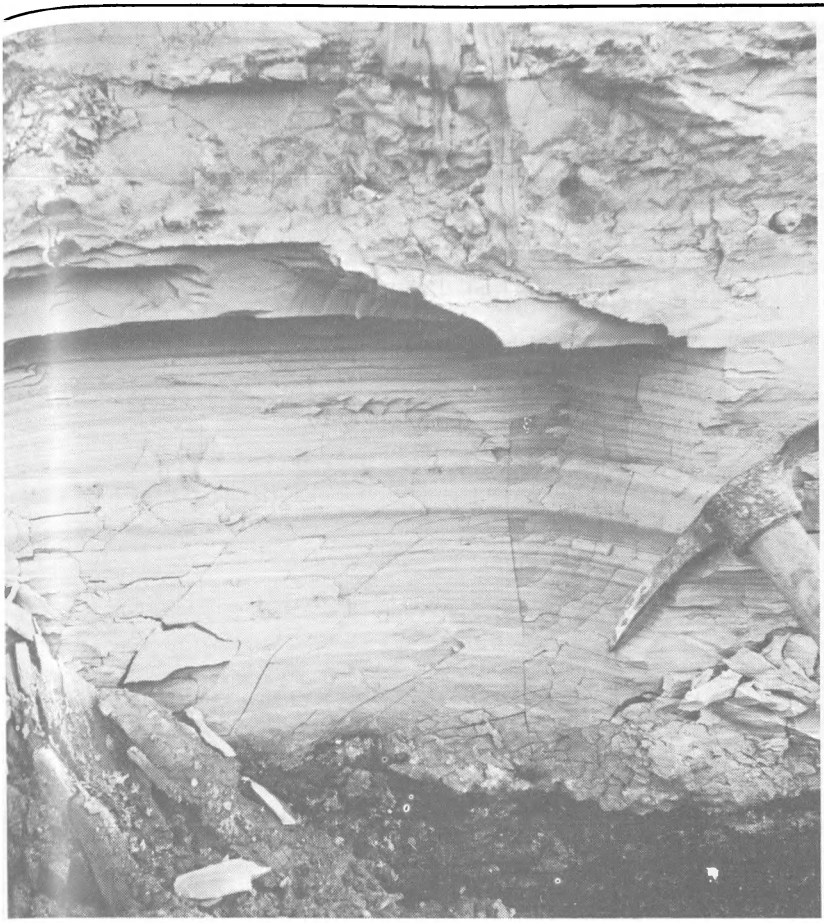

Figure 5. Planar laminae in bed of light to dark gray fine silt and clay that overlies thick lignite bed at base of Figure 2. This laminated bed is interpreted to be a lake deposit. The pick head is 0.3 m long.

deposits directly overlying the upper sand bed, which contain limonitic nodules, probably formed in a well-drained swamp.

Natural-levee deposits.—Natural levees are the sites of the highest rates of sedimentation on the alluvial plain (Allen, 1965a). Allen (1970c) showed that high sedimentation rates enable the formation of small-scale climbing-ripple cross strata. This sedimentary structure was reported only from the natural-levee environment on the Mississippi River deltaic plain by Coleman and Gagliano (1965, Table 1). It was also reported from natural-levee deposits by McKee (1966) and

Singh (1972), so it seems to be particularly diagnostic of the natural-levee environment.

Small-scale climbing-ripple cross strata (Figure 6) are very abundant in the yellow-brown to brown sandy silt deposits adjacent to the lower sand bed (Figure 2). Other types of small-scale cross-strata and horizontal, planar laminae that have been reported from natural-levee deposits (Allen, 1965b; Coleman and Gagliano, 1965; Singh, 1972) are also present. Limonitic nodules that have been reported from natural-levee deposits (Coleman and Gagliano, 1965; Donaldson et al., 1970) are present. These characteristics indicate that the deposits adjacent to the lower sand bed formed on a natural levee.

Figure 6. Small-scale, climbing-ripple cross strata in silt bed adjacent to lower sand bed (Figure 2). These cross strata resulted from high rates of sedimentation on a natural levee adjacent to the stream that deposited the lower sand bed. The pen is 0.15 m long.

Natural-levee deposits are not closely associated with the upper sand bed. This is probably due to the erosion of these deposits during lateral migration of the stream that deposited the sand bed. Natural levees are best developed on the concave sides of stream meanders and are removed during lateral migration.

SUMMARY

The strata exposed in the Husky Lignite Mine near Dickinson, North Dakota are in the uppermost Tongue River Formation or lower Sentinel Butte Formation. The sand beds exposed in the mine probably were deposited on point bars in high-sinuosity streams. The strata surrounding the sand beds probably were deposited on an alluvial plain. Yellow-brown to brown sandy silt beds that lack organic matter and contain small-scale, climbing-ripple cross strata and other types of small-scale cross strata, horizontal laminae, and iron-oxide-stained nodules were probably deposited on natural levees. Clay and silt beds that are light to dark gray, pyritic, and rich in organic matter were probably deposited in flood basins; those beds that are lignitic and contain small-scale cross strata and distorted bedding probably formed in marshy or swampy areas, and those beds that contain horizontal, planar laminae are interpreted as lake deposits. Lignite beds formed in swamps in poorly-drained parts of flood basins away from the main channels.

A computer program has been written that will calculate Schumm's (1960) parameter M, width:depth ratio, sinuosity, mean annual discharge, stream-meander length, drainage area, stream length, and channel slope for the stream depositing a tabular sand body that can be shown to be fluvial, and that contains what Allen (1965a) called epsilon cross strata. The results of the analysis outlined by Cotter (1971) indicate that the sand beds exposed in the mine were deposited in rather small streams having low flows and draining small areas. The streams had characteristics in the general range of some of the small rivers or bayous of the modern coastal plain of Texas or Louisiana.

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LATE CENOZOIC DRAINAGE EVOLUTION IN NORTHWESTERN McKENZIE COUNTY, NORTH DAKOTA

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ABSTRACT

Evidence for several stages in the evolution of the Yellowstone River drainage system is found within an area of 465 km² in northwestern McKenzie County, North Dakota. During the earliest stage, the Yellowstone deposited rusty-brown gravel containing chert and volcanic and plutonic rocks; the upper surface of this gravel is about 90 m above the present flood plain of the Yellowstone River. Subsequent downcutting was interrupted by a glacial advance that dammed the river, forming a lake in which laminated silt and clay were deposited. As the glacier retreated, a series of outlet channels drained the lake. Yellowstone drainage was re-established and gravel derived from local bedrock was deposited in the tributaries of the Yellowstone River. Drainage was again disrupted by two additional glacial advances. As the last glacier retreated, gravel containing chert and volcanic, plutonic, and carbonate rocks was deposited in a series of meltwater channels. The upper surface of this gravel is about 13 m above the present flood plain of Charbonneau Creek and is graded to about the level of the lowest terrace along the Yellowstone River. Gravel underlying this terrace contains chert and volcanic, plutonic, and carbonate rocks. Renewed downcutting established the present flood plains of the Yellowstone River, Charbonneau Creek, and the Missouri River.

INTRODUCTION

During the summer of 1973, I mapped the geology (Salomon, 1974) of six townships in northwestern McKenzie County, North Dakota (Ts. 151 and 152 N.; Rs. 102 W. to 104 W.) (Figure 1). Various stages in the evolution of the Yellowstone River drainage system are represented by sediment with or without related terraces. During pre-glacial time, the Yellowstone and Missouri Rivers flowed northward into Hudson Bay (Bluemle, 1972). During this time, the Missouri River formed a trench northwest of the study area (Figure 1), so pre-glacial sediments deposited by the Missouri River are not found in the study area. The Yellowstone River was blocked by at least three glacial advances. Evidence for only one lake formed by glacial damming was found in the study area. In this paper, I describe the sediments (Figure 2) deposited during the various stages, and discuss the stages of drainage evolution of the Yellowstone River in northwestern McKenzie County. I also relate my local conclusions to the regional conclusions of Howard (1960) and Alden (1932), who mapped large areas of eastern Montana and western North Dakota.

MATERIALS AND METHODS

Aerial photographs and 7½-minute topographic maps (1:24,000, contour interval 20 feet) were used for base maps, and the soil map of McKenzie County

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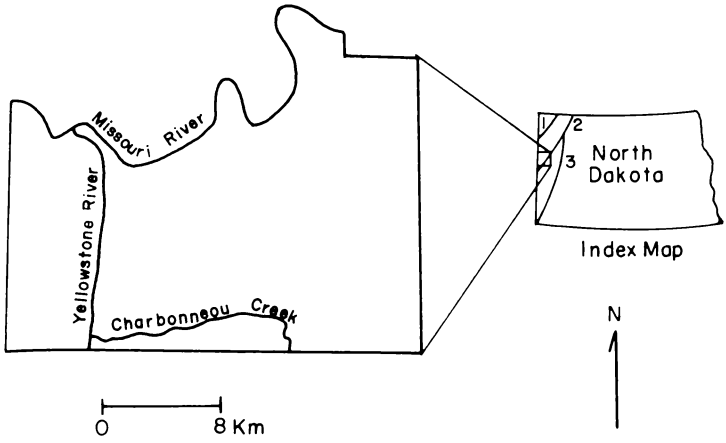

Figure 1. Location map of study area in northwestern McKenzie County, North Dakota. Location of preglacial Missouri (1), Yellowstone (2), and Little Missouri (3) Rivers from Bluemle (1972).

(Edwards and Ableiter, 1942) was used as a reference. A geologic map of the area was compiled at a scale of 1:63,360. All section-line roads were traveled and much of the area was traversed on foot. Numerous outcrops were examined and described, and samples of till and gravel were collected from many. Where outcrops were scarce, a hand auger was used to obtain samples.

The samples were analyzed in the laboratory during the fall and winter of 1973. The lithology of approximately 200 grains from the coarse-sand fraction (1 mm to 2 mm) of 75 samples of till was determined. The grains of these samples were separated into three categories: crystalline (igneous and metamorphic rock types), carbonate (limestone and dolomite), and shale. The lithology of the 8 - 19 mm fraction of gravel samples was determined. Pebbles were separated into eight categories: chert, quartzite, plutonic (granite and granitic rocks), volcanic (andesite, trachyte, rhyolite and basalt), limonitic, carbonate (limestone and dolomite), sedimentary (shale and siltstone), and "scoria" or clinker. At least 300 pebbles were counted in each of 17 samples. Three hundred is the minimum number needed to give the least variation between samples of the same gravel collected at a single outcrop. The 8 - 19 mm size fraction was chosen because fractions of smaller sizes were biased by the absence of less resistant sedimentary rocks and the fractions of larger sizes were biased by the small number of pebbles.

RESULTS AND DISCUSSION

In the study area, the earliest stage of Yellowstone drainage is represented by 12-17 m of rusty-brown gravel (Unit A, Figure 2) whose upper surface occurs about 90 m above the present flood plain of the Yellowstone River. This gravel

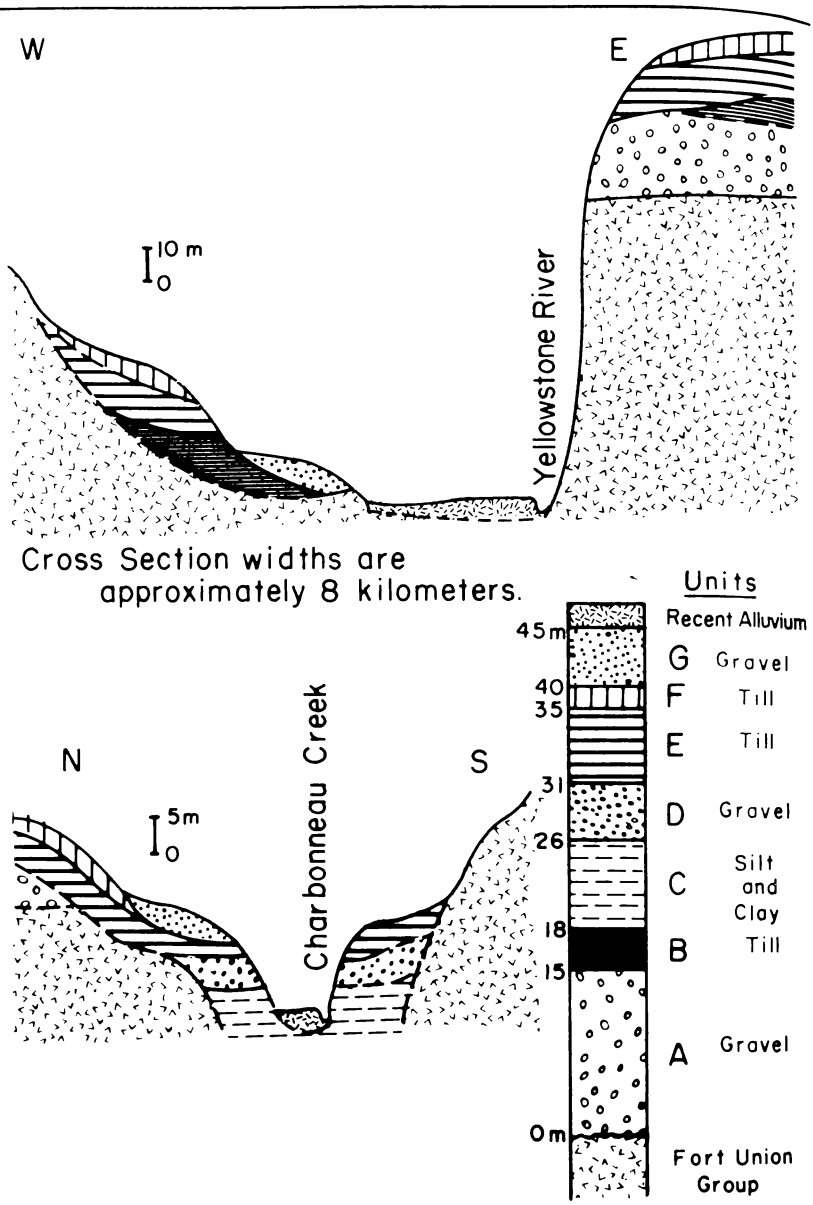

Figure 2. Schematic cross sections across the Yellowstone River valley and Charbonneau Creek valley in northwestern McKenzie County, North Dakota. The average thickness of stratigraphic units is indicated along the left side of the explanation.

consists predominantly of heavily iron-stained pebbles of chert, quartzite, and volcanic and plutonic rocks. It is lithologically similar to gravel deposited (Howard, 1960, p. 19) by the Yellowstone River. The lithology of the gravel of Unit A and its pebble imbrication indicate a northward direction of flow, and suggest that it was deposited by the Yellowstone River.

The following indirect evidence suggests that the age of Unit A is late Pliocene or early (pre-glacial) Pleistocene. First, no till has been found beneath Unit A. Second, the absence of carbonate pebbles in the gravel strongly suggests that no till was present in the area at the time of the deposition of Unit A. If the area had been glaciated, carbonate pebbles brought from the north or northeast would be present in the area and should occur in the gravel.

Alden (1932:44) suggested an early Pleistocene age for the surface underlain by Unit A. He traced this surface, his No. 2 bench (1932:51), along streams and rivers throughout eastern Montana and western North Dakota. Howard (1960:19) recognized the gravel of Unit A and traced a surface, underlain by Unit A in the study area, along the Yellowstone, Missouri, and Little Missouri Rivers, and along numerous streams in eastern Montana and western North Dakota.

The earliest known glacier in the study area deposited till (Unit B) containing abundant fragments of lignite, siltstone, limonitic concretions, and "scoria" from the Fort Union Group (Paleocene), pebbles from Unit A, and shale pebbles from the Pierre Formation (Cretaceous). Considerable downcutting by the Yellowstone River preceded this glacial advance. The exact elevation of the Yellowstone River at this time is not known, but it was considerably lower than the upper surface of Unit A. In some outcrops, till of Unit B occurs only 7 m above the present flood plain of the Yellowstone River. Gravel of Unit A underlies till of Unit B, 90 m above the present flood plain showing that glacial erosion was not responsible for the variation in elevation.

This glacier, of unknown age, dammed the Yellowstone River, forming a lake in which laminated silt and clay (Unit C) as thick as 8 m were deposited. The upper surface of Unit C along Charbonneau creek is at an elevation of about 10 m above the present flood plain of the Yellowstone, further evidence that downcutting by the Yellowstone preceded the glacier that dammed the lake. The relation of Unit C to sediment of glacial Lake Glendive, which existed in the Yellowstone valley in eastern Montana (Howard, 1960, p. 81-83), is not known.

As the glacier retreated, numerous pre-existing valleys were uncovered and acted as outlets for the lake. One of these outlets, in the southeast corner of the study area, is a broad channel with gently sloping sides, comparable in width to narrow parts of the Missouri River trench. This northeast-trending channel is truncated by the present trench of the Missouri River. The western end of this channel is occupied by Charbonneau Creek, a westward-flowing tributary of the Yellowstone River.

After the glacier retreated and the lake drained, the Yellowstone River drainage was re-established. Tributaries of the Yellowstone River deposited gravel (Unit D) consisting predominantly of locally-derived "scoria," sedimentary, and

limonitic rocks. Along Charbonneau Creek, Unit D overlies Unit C and beds of gravel of Unit D appear to dip westward. This suggests that Unit D was deposited after the draining of the lake to the northeast through the outlet channel discussed previously.

The re-established drainage of the Yellowstone River was interrupted by two additional glacial advances. The tills deposited by these advances (Units E and F) are very similar. In outcrops, it is difficult to differentiate the two units except where a boulder lag occurs between them (NE¼SE¼SE¼ sec. 29 and SW¼SW¼NE¼ sec. 30, T. 152 N., R. 104 W.). The coarse-sand lithology of the two units differs markedly, Unit E having about 60% crystalline and 35% carbonate fragments versus about 75% crystalline and 20% carbonate fragments in Unit F. Washboard moraines were formed during the deposition of Unit F; the orientation of these features suggests that the glacier advanced from the northeast. Striations on the boulder lag under till of Unit F also indicate a northeast-southwest direction of flow. No directional indicators associated with the deposition of Unit E were found, but it is reasonable to assume this glacier advanced from the north or northeast. The age of these advances is unknown. The glaciers undoubtedly dammed the Yellowstone River, but no lake sediment overlying Units E or F was found in the study area.

As the last glacier retreated, meltwater channels were developed on the upland area north of Charbonneau Creek. These channels were eroded into the till of Unit F and in places are very well preserved. The channels trend north-south and slope toward the south. The gravel occurring at the base of these channels contains predominantly chert and volcanic, plutonic, and carbonate rocks. The upper surface of this gravel is about 13 m above the present flood plain of Charbonneau Creek. The presence of the channels on the upland area suggests that the glacier filled the trenches of the Missouri and Yellowstone Rivers in the study area and prevented drainage through these trenches.

Drainage of the Yellowstone was re-established soon after the meltwater channels were developed, and the river deposited gravel consisting predominantly of chert and volcanic, plutonic, and carbonate rocks. The upper surface of this gravel forms a terrace about 13 m above the present flood plain of the Yellowstone River. This terrace appears to be graded to the level of the surface that occurs about 13 m above the present flood plain of Charbonneau Creek. The gravel (Unit G) underlying both surfaces is similar lithologically. Alden (1932:59) called this terrace the No. 3 bench and traced it throughout eastern Montana and western North Dakota. He indicated (pl. 1) that the gravel associated with this terrace was underlain by the till occurring in my study area. Howard (1960:21) called this terrace the Crane Creek terrace. In contrast to Alden, he indicated (pl. 1) that the gravel associated with this terrace was overlain by all the till occurring in my study area. Based on outcrops along the Yellowstone River and Charbonneau Creek, this gravel overlies the till in the area, as was suggested by Alden.

The present stage of drainage evolution in northwestern McKenzie County is the result of renewed downcutting establishing the present flood plains of the

Yellowstone River, Charbonneau Creek, and the Missouri River about 13 m below their lowest well-developed terrace.

Summary.—The Yellowstone River flowed northward over a surface about 90 m above its present flood plain during late Pliocene or early Pleistocene. Subsequent downcutting was interrupted by a glacial advance that dammed the river forming a lake, possibly glacial Lake Glendive of eastern Montana. The lake was drained by several outlet channels and the Yellowstone drainage was reestablished. Two additional advances disrupted the drainage. Meltwater channels were formed as the last glacier retreated. This meltwater deposited gravel whose upper surface is graded to the level of the lowest well-developed terrace along the Yellowstone River. This gravel does not underlie the till exposed at the surface in the study area, as indicated by Howard (1960). Instead the gravel overlies all the till in this area, as indicated by Alden (1932). Following the deposition of this gravel, renewed downcutting established the present flood plains of the Yellowstone River, Charbonneau Creek, and the Missouri River.

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PROJECT LIGNITE: CONVENIENCE FUELS FROM NORTHERN GREAT PLAINS PROVINCE LIGNITE

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ABSTRACT

The Office of Coal Research has established a project at the University of North Dakota having as its ultimate goal the determination of the feasibility of an integrated plant using solubilization, gasification, carbonization, and hydrogenation to produce a variety of upgraded fuel products from North Dakota lignite. Initially, emphasis was placed on the study of the solution-hydrogenation process to produce solvent-refined lignite (SRL). A small continuous process development unit was designed and is being constructed. Batch autoclave tests were conducted to obtain necessary design data. This paper reports initial results from the batch autoclave tests investigating various process variables and operating techniques.

INTRODUCTION

The Northern Great Plains Coal Province, which includes parts of the Dakotas, Montana, and Wyoming, contains extensive reserves of lignite and subbituminous coal. Though only lignite is found in North Dakota, its extensive reserves of an estimated 350 billion tons make the state the largest reservoir of coal in the United States. Lignite represents about 30% by weight of the total U.S. reserves, yet it has been utilized only to the very limited extent of approximately 0.5% of annual coal production. Utilization has been limited essentially to power generating stations and commercial heating.

The location of reserves in areas of low population density and at relatively great distances from large energy-consuming centers have been the factors that limited production. Lignite is chemically reactive, non-agglomerating, and can be strip-mined at high productivity to give a relatively low cost energy source. Sulfur and ash concentrations are lower than in most bituminous coals, though moisture content is much higher.

The combination of desirable physical and chemical properties together with low cost makes lignite a promising raw material for a number of energy-conversion processes. The present energy crisis and widespread concern for environmental protection have increased interest in conversion of lignite to more convenient and useful forms of energy. Economies could be effected by optimization of processing technology in an integrated coal refinery producing

several upgraded fuel products. The Office of Coal Research (OCR) has established a program of research and study at the University of North Dakota directed toward application of the refinery concept to lignite.

REASONS FOR PROJECT

The ultimate goal of the project is the conceptual design of an integrated lignite refinery complex to produce high quality gaseous, liquid, and solid fuels, and possibly a synthetic crude oil suitable as a feedstock for a petroleum refinery. The economic utilization of lignite in this way would aid materially in combating the national energy crisis as liquid fuels are not readily replaced in many applications.

The social, economic, and ecological benefits resulting from establishment of a chemical processing complex in North Dakota, a region of low population density where environmental control should be readily achievable, could be an important result of the project. A broader economic base and tax structure would be introduced into a primarily agricultural and ranching area and could dramatically alter the present trend of declining population. Other industries could be attracted to the area because of the availability of energy and of low-cost, basic organic chemicals derived from lignite.

A lignite refinery would produce a variety of products utilizing both novel techniques developed in the course of this project and the application, integration and modification of currently available technologies of coal and hydrocarbon processing. In such a refinery, liquefaction, hydrogenation, carbonization, and gasification processes could be combined to produce pipeline gas, synthetic crude oil, low sulfur-low ash boiler fuel, and industrial coke. At present, major activity nationally is directed towards gasification for production of methane. However, liquefaction is important and requires more development research.

SCOPE OF RESEARCH PROGRAM

The major object of the broad program of research and development is upgrading of Northern Great Plains Province coals to premium fuels and chemical products. To do this requires identifying specific steps in the overall process that have not been carried beyond the laboratory stage. These steps of the proposed commercial lignite refinery will be followed through four phases of development:

1. Bench scale research to determine the influence of process parameters.
2. Design and construction of a process development unit from bench scale results.
3. Operation of the process development unit to optimize the design parameters and provide data for scale-up calculations.
4. Development of a conceptual design for a pilot plant or demonstration plant.

Initially five process areas of the integrated lignite refinery seem to require development work prior to design of an integrated pilot plant. These areas are:

A. Production of solvent-refined lignite by solution-hydrogenation.
B. Hydrogenation of solvent-refined lignite to liquid products and recovery of various products of distillation.
C. Carbonization of lignite to give char, gases, and liquids.
D. Direct hydrogenation with lignite at pressures of 1000 to 1500 psi (68 to 102 atmospheres).
E. Drying of lignite as a process step prior to hydrogenation.

Economic evaluations for the projected commercial lignite refinery will be made at intervals during the course of the work.

EXPERIMENTAL WORK

The process selected initially for investigation is the solution-hydrogenation of lignite in a synthesis gas atmosphere (carbon monoxide plus hydrogen) using recycle solvents. To obtain the necessary data for design of the Process Development Unit (PDU), laboratory and bench scale studies are being made in which lignite is converted to liquid or low-melting solid products by hydrogenation using hydrogen, carabon monoxide or synthesis gas and a hydrogen-donor solvent.

EQUIPMENT AND PROCEDURES

The reactor for batch studies is a one-gallon stainless-steel stirred autoclave capable of processing coal slurries (coal plus a coal tar solvent) with added hydrogen and/or carbon monoxide at pressures to about 5000 psi (340 atmos.) and temperatures to about 900°F (473°C). The apparatus and operation have been already described (Skidmore et al., 1968).

The solvent was vacuum distilled from a coal tar fraction known as anthracene oil. The fraction boiling between 100° and 230°C at a pressure of 1 to 2 millimeters of mercury was recovered and identified as UND-MFRAO (University of North Dakota-Middle Fraction of Anthracene Oil). A useful parameter determined to characterize the solvent is the infrared ratio (IRR) and represents the ratio of absorbance at 3070 cm^{-1} to that at 2950 cm^{-1}. The absorbance at 3070 cm^{-1} is a measure of C-H stretching of hydrogen on double bond carbons, and that at 2950 cm^{-1} the C-H stretching of hydrogen on single bond carbons. Thus, the IRR ratio indicates the relative unsaturatedness or aromaticity of the solvent; the higher the value, the greater the proportion of aromatics present. With limitations, the lower the IRR ratio, the better the potential hydrogen donor characteristics of the solvent. The IRR is also determined on the solvent boiling range portion of the liquefied coal product.

Two different lignites were used in these studies. Lignite 71-1 was from the Baukol-Noonan mine at Larson, North Dakota, and was originally used in liquefication studies described by Wright and Severson (1972). Lignite 72-1 was obtained from the North American mine at Zap, North Dakota.

A relatively small quantity of lignite, usually enough for two autoclave runs, was obtained from the gross sample, and pulverized to pass 100 mesh. The usual

lignite charge to the autoclave was a little over 300 grams, corresponding to 200 grams of MAF coal. In some tests, water was added to replace moisture lost during pulverization to maintain a constant amount of water in the autoclave.

After completion of a run, the autoclave was cooled to about 400°F (205°C), and the product gases exhausted through a series of cold traps into a collection balloon. The gas was mixed in the balloon, sampled, analyzed, and metered. Hydrogen, methane, ethane, carbon monoxide, carbon dioxide, and nitrogen were determined by gas chromatographs whereas hydrogen sulfide was determined separately by using zinc ammonium sulfate absorption. Heavier hydrocarbons are usually not present in the gas stream. In addition to gas, water and light oil fractions were collected.

After discharge of gas, the autoclave was repressurized with nitrogen and the slurry, still at 400°F (205°C), was discharged through a dip-tube onto a heated Buchner funnel where the solvent and coal-derived liquids were separated from the mineral constituents and unconverted coal by filtration. The products collected were filtrate, filter cake, and autoclave residue.

The collected filtrate was distilled to recover a light oil fraction, a solvent fraction, and vacuum bottoms. The solvent-refined lignite (SRL) product is a major part of the vacuum bottoms. In most runs not quite as much solvent was recovered as had been charged. In continuous operation, there should be a slight net solvent production, but if not, recycle solvent would have to be prepared by widening the distillation cut to include some of the front end of the vacuum bottoms. The filter cake and other residues were extracted with pyridine to determine the amounts of adhering filtrate, and ash contents were determined on the filter cake, the residue, and the filtrate.

Complete material balances were made on each run, utilizing a IBM 370/165 system. The computer program calculates the amount of liquid products in the filter cake and residues, and distributes this quantity among the liquid fractions according to the distillation cuts of the filtrate. Also calculated are normalized gas compositions and consumption of hydrogen and/or carbon monoxide.

A summary of runs relevant to the discussions in this report is given in Table 1, with the exception of certain runs used to evaluate filtration temperatures in which product recoveries were low.

INFLUENCE OF TEST CONDITIONS: AUTOCLAVE EXPERIMENTS

Batch tests in the bench-scale autoclave were conducted primarily to define some operational parameters and to indicate the effect of process variables on yield of light liquids and SRL. A limitation of these small batch tests is the difficulty of determining accurately solvent recovery. For a commercial process, solvent make-up should be minimal or sufficient equivalent solvent should be generated from the lignite to maintain the process. Such data must be obtained from continuous operation on a somewhat larger scale, such as in the proposed process development unit (PDU). The influence of some of the variables and operating conditions for the batch experiments are discussed in the following paragraphs.

Filtration temperature.—A critical step in the SRL process is the filtration of reacted lignite-solvent mixture. Because the SRL is a high molecular weight material of high viscosity, filtration temperature must be relatively high. However, at overly high temperatures with the filtration equipment employed, solvent is flashed from the mixture, reducing filterability.

The effect of discharge temperature on the filtration equipment was studied in a series of tests where operating conditions were maintained constant except for filtration temperature. Anthracene oil (UND-MFRAO) was used as solvent. Below 400°F (260°C), filtration was difficult because of excessive solvent vaporization. The higher filtration temperatures also resulted in operational difficulties with the filter paper, which sometimes charred. At approximately 400°F (205°C), filtration characteristics seemed to be most favorable, and this temperature was selected as the standard filtration temperature for subsequent tests in which anthracene oil was used.

Influence of solution temperature and reaction time.—Solution temperature and the time required for solution are important design parameters, and several tests were run to determine the influence of these variables. The solvent-lignite ratio was maintained at 2 g of MFRAO per ram of moisture-ash-free lignite in a reaction atmosphere of 1:1 ratio of H_2 to CO. Initial pressure was a nominal 1000 psig. In all cases, yield parameters were corrected to a 100% material balance closure.

Reaction time should be minimized. However, in practice, runs of less than a half hour at reaction temperature presented operational difficulties in achievement of reproducible results. Results with the North American 72-1 lignite for otherwise identical conditions using 0.5 h and 2 h reaction times at nominal 400°C (750°F) are given in Table 1.

Table 1. Influence of reaction time on liquefaction (at 400°C).

Run number	422	418
Reaction time (h)	0.5	2.0
Yields, wt. %, MAF basis		
Net gas	35.2	49.4
Vacuum bottoms	83.2	61.5
Vac. bottoms + Lt. liq.	96.3	78.0
Net liquid	68.4	56.7
Total extracted	90.0	89.9
Solvent recovery		
Wt. % of solvent charged	86.1	89.4

Conversions are about the same at 0.5 h and at 2 h, but more gas and light liquids and less vacuum bottoms are produced at the longer time, apparently because of thermal degradation of the products. At the shorter time, vacuum bottoms yield was about 83%, and net liquid yield slightly over 68%. Since 0.5 h reaction time is about the practical minimum, that was taken as standard.

Runs were made with the Baukol-Noonan 71-1 lignite at 380°, 405°, and 430° in a H_2 and CO atmosphere with an initial pressure of 1000 psi to indicate

the optimum temperature for extraction. Run time at temperature was a half-hour, and test conditions were as with the reaction-time experiments. Data for tests at different temperatures are presented in Table 2. These data indicate yields to be generally higher at 405°C than either at 380° or 430°C. Thus, 400°C (752°F) appears to be near the optimum reaction temperature and was used as standard in subsequent tests.

Table 2. Influence of reaction temperature on liquefaction.

Run number	401	402	404
Run temperature, °C	380	405	430
Yields, wt. %, MAF basis			
Vacuum bottoms	57.4	62.2	56.6
Vac. bottoms + Lt. liq.	84.4	98.1	74.1
Net liquid	63.7	70.2	44.2
Total extracted	86.8	87.4	81.2
Solvent recovery			
Wt. % of solvent charged	89.6	86.1	85.0

It must be understood that the time-temperature relationships indicated as optimum can only be considered optimum for the particular system. Other conditions such as a different solvent, a different pressure, or a different type of lignite would perhaps give different results.

Influence of test atmosphere.—Initially in solution-hydrogenation work, it was thought that a high-hydrogen atmosphere was necessary. Later, work by other investigators indicated that a high degree of extraction could be obtained in a carbon monoxide atmosphere if the coal was of low rank with nearly full moisture content. Tests were made in a hydrogen atmosphere, in a carbon monoxide atmosphere, and in synthesis gas atmosphere (CO & H_2). If a carbon monoxide or CO & H_2 atmosphere would give satisfactory yields, processing costs would be reduced over those using pure hydrogen. In all tests, reaction temperature was a nominal 400°C (725°F), reaction time a half-hour, initial gas pressure 1000 psig (69 atmos.), and pulverized North American 72-1 lignite of approximately 30% moisture was charged. Solvent was the MFRAO (2:1 oil-maf lignite ratio), and the synthesis gas atmosphere was equal molar in H_2 and in CO. Results for tests in the three atmospheres using raw lignite are summarized in Table 3.

Table 3. Influence of hydrogenation atmosphere on liquefaction.

Run	410	408	412
Atmosphere	CO	CO & H_2	H_2
Yields, wt. %, MAF basis			
Light liquid	9.4	13.4	14.4
Vacuum bottoms	66.3	75.5	88.3
Vac. bottoms + Lt. liq.	75.6	89.0	102.8
Net liquid	42.6	75.5	57.7
Total extracted	82.8	87.2	82.2
Solvent recovery			
Wt. % of solvent charged	83.4	93.4	77.5

Highest gross yields were obtained in the hydrogen atmosphere, though considerable products must have been produced from the solvent, as judged by the low solvent recovery; and the lowest in the carbon monoxide atmosphere. Values for the CO & H_2 atmosphere were intermediate but here solvent recovery was good. Net liquid yields were highest with CO & H_2, as was total extraction, though in all cases extraction exceeded 80% of the maf lignite. It should be noted that optimum conditions were not determined for the various atmospheres. It is believed that with reduced solution temperatures relative yields would be favored in a carbon monoxide atmosphere. Yields were similar to those reported by Appell (1975) if differences in test conditions are considered.

A similar series of runs was made with an extracted lignite (described in the section. Influence of cations on extraction). The results of these tests are summarized in Table 4. In these tests, the highest gross yields were obtained in the CO atmosphere, and the least in the CO & H_2 atmosphere. However, because of higher conversion and higher solvent recovery with the CO & H_2, net liquid yields were essentially the same as with CO, and slightly higher than with H_2.

Table 4. Influence of hydrogenation atmosphere on liquefaction of extracted lignite.

Run	411	414	413
Atmosphere	CO	CO & H_2	H_2
Yields, wt. %, MAF basis			
Light liquid	11.3	12.4	14.4
Vacuum bottoms	91.4	69.6	78.6
Vac. bottoms + Lt. liq.	102.7	82.0	93.0
Net liquid	60.0	59.6	57.8
Total extracted	77.4	87.9	84.2
Solvent recovery			
Wt. % of solvent charged	78.7	88.7	82.4

Influence of cations on extraction.—Earlier work (Wright 1972) had indicated that the naturally occurring minerals in lignite had a catalytic effect on the reaction of hydrogen or carbon monoxide with the lignite. This work was done with a number of lignites of varying mineral content and composition, and with lignite to which sodium bicarbonate or potassium carbonate was added. The amount of hydrogen reacting and the conversion of the lignite could be related to the amount of sodium, potassium, and iron present in the lignite. The current work in which various cations are added back to extracted lignite is an extension of these previous catalytic studies.

In lignite, cations, particularly those of sodium and calcium, are associated with the coal substance rather than with the mineral ash. These cations are bound to the coal in such a fashion that they can be removed by an ion-exchange process. If lignite is washed with an acid solution, sodium and calcium concentrations are greatly reduced. Other cations of soluble salts can then be added directly to the coal substance by placing the acid-extracted coal in a solution of the desired cations. The concentrations of cation added can be controlled by varying solution concentration and time of contact.

A large sample of the North American 72-1 lignite was extracted in a glass

column with 1 normal HCl, and the ash content on a dry basis was reduced from 9.97 to 3.15 weight percent. Over 95% of the remaining ash was alumina, silica, and iron oxide. Various concentrations of cations such as sodium, potassium, iron, calcium, colbalt or nickel were added to the extracted lignite by reverse ion exchange. In cases where the cation formed a soluble acetate, this salt was used since the acetate does not add any "foreign" material to the lignite. In many cases individual cation concentration can be increased over the naturally occurring level without increasing other mineral components.

Analysis of the test series is incomplete, pending completion of ash analyses. However, some indication that cations do influence extraction can be noted from the data shown in Table 5. Test conditions were the same except that extracted and unextracted lignite were used. Solvent was MFRAO and the atmosphere, synthesis gas (CO & H_2). The Lignite contained about 30% moisture.

Table 5. Influence of ash minerals on liquefaction.

Run	408	414
Lignite	Unextracted	Extracted
Yields, wt. %, MAF basis		
Net gas	27.2	27.1
Vacuum bottoms	75.5	69.6
Vac. bottoms + Lt. liq.	89.0	82.0
Net liquid	75.5	59.6
Total extracted	87.2	87.9
Solvent recovery		
Wt. % of solvent charged	93.4	88.7

The various liquid yield parameters were reduced when extracted lignite was processed though the total extracted was about the same. Perhaps addition of sodium or calcium to levels higher than those naturally occurring would increase yields, although with the raw unextracted lignite the yields are already quite high. However, it may be possible to achieve high yields under milder conditions if the cation concentration is increased.

Influence of solvent quality.—For the solution-hydrogenation experiments, the distilled solvent was prepared in large batches, and each batch was used for a number of tests. Two batches of solvents were used, one for Runs 401 through 415 and the other for Runs 416 through 422. For reasons as yet unexplained, perhaps because of differences in the distillations, or perhaps because of stratification and non-uniform sampling in the 55-gallon drum of anthracene oil, the two solvents were different as regards the IRR. The first sample had a value of 1.29, and the second 1.46. Runs to establish base case conditions were made with each of the solvents. The results of these runs compare the two solvent batches in Table 6.

The solvent with the higher IRR gave higher yields of gas and vacuum bottoms, and about the same of light liquid, but solvent recovery was less. As a result of the higher gas yields, the net liquid yield was lower although total extracted was higher. If, as was theorized, the solvent with the higher IRR had lower hydrogen donor properties, this seemed to result in increased fragmentation of both coal and

Table 6. Influence of solvent quality on liquefaction.

Run	408	422
Solvent IRR[a]	1.29	1.46
Yields, wt. %, MAF basis		
Net gas	27.2	35.2
Light liquid	13.5	13.1
Vacuum bottoms	75.5	83.2
Vac. bottoms + Lt. liq.	89.0	96.3
Net liquid	75.5	68.4
Total extracted	87.2	90.0
Solvent recovery		
Wt. % of solvent charged	93.4	86.1

[a]IRR: Infra-red ratio, ratio of absorbance at 3070 cm^{-1} to absorbance at 2950 cm^{-1}. Higher values indicate higher aromaticity.

solvent molecules. The available hydrogen may stabilize larger fragments and give increased liquid yields. Additional work with a wider variety of solvents is planned.

CONCLUSIONS

The scope and the purpose of Project Lignite are presented to indicate the relationship with the lignite research program being conducted at the University. Preliminary results from a number of solvation-hydrogenation bench-scale experiments with North Dakota lignite and the operational procedures employed are described. General trends indicated from various tests were:

1. For each solvent, an optimum temperature exists for filtration of lignite-solvent slurry. Approximately 400°F (205°C) was found most suitable for anthracene solvent.

2. Solution temperature and reaction time are interacting variables which are also important design parameters. One-half hour at 405°C seems most suitable in terms of product yields for tests using anthracene oil and North Dakota lignite in an atmosphere of carbon monoxide and hydrogen.

3. High conversions are possible in hydrogen, hydrogen plus carbon monoxide, or carbon monoxide atmospheres. Yields of solvent-refined coal and light liquids and solvent recovery are most favored with full moisture lignite and carbon monoxide atmosphere.

4. Cations have a catalytic effect on solution-hydrogenation of lignite. Tests with added cations indicated that those naturally occurring in North Dakota lignite may be sufficient for good solution characteristics.

5. The hydrogen donor capability of the solvent, as indicated by the IR ratio (ratio of aromatic to alipatic hydrogens), affects the efficency of solution hydrogenation.

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STUDENT PROJECT IN RENAL SURGERY: PHASE II. EXPERIENCE WITH AUTOGRAFTS

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ABSTRACT

The overall goal of the project is the study of the effect of artificial perfusion solutions on dog kidney and the development of a solution suitable for preservation.

Autografts have been attempted in two ways: bilateral nephrectomy with unilateral autograft and unilateral nephrectomy with unilateral autograft. One unilaterally nephrectomized animal survived until removal of the contralateral kidney. Refinements in surgical, anesthetic, and laboratory techniques have been suggested.

Perfusion techniques are under development. A balanced salt solution has been selected for testing, a perfusion chamber has been constructed and histological preparations from a perfused kidney have been examined.

INTRODUCTION

The ultimate purpose of the student project is the development of a balanced salt solution suitable for perfusing organs for preservation and transplantation. At present, many preservation techniques involve the use of serum. A balanced salt solution would be preferable to serum perfusate for the following reasons: it eliminates blood-borne pathological conditions since it can be autoclaved and serum cannot; it eliminates the problem of immunological reaction between serum protein in the perfusate and kidney protein from the donor since there is no protein in the artificial perfusate; and artificial perfusate would be a universal solution that is easily transported and preserved, less expensive, and more readily available than serum as a perfusate.

Tests can be devised to monitor the functioning of a preserved kidney *in situ*, but the final functional test will involve successfully grafting the organ after preservation.

MATERIALS AND METHODS

General.—The 10 dogs used were mongrels of both sexes, of unknown ages, and weighed 25-50 pounds. They were in good health, except for one animal who, after autograft, was found to have roundworms. All surgeries were done using sodium pentobarbital anesthesia in the dosage of 30 mg/kg of body weight. The dogs were respirated through an endotracheal tube. Trans-thoracic needle electrodes inserted subcutaneously were used in conjunction with an impedance pneumograph and a preamplifier to monitor respiration and electrocardiogram. A cathode ray oscilloscope was used for visual display and an audio amplifier was used to produce an audio signal with each heart beat. Temperature was monitored by means of a telethermometer connected to a rectal probe.

Surgical Technique.—Two surgical procedures were used: bilateral nephrectomy with unilateral autograft and unilateral nephrectomy with unilateral autograft.

Bilateral nephrectomy with unilateral autograft (''bilaterals'') was performed in the manner described by Stewart (1971). The blood vessels used for anastomosis were dissected free of surrounding tissue in the iliac region leaving an intraperitoneal pocket with which to cover the kidney following autograft. The left kidney was used for the autograft because it had longer vessels and was more accessible than the right kidney. Following nephrectomy of the left kidney, in which the renal capsule was left as intact as possible, the kidney was immediately flushed with heparinized (one unit per ml) isotonic (0.85% sodium chloride) saline and placed in an ice slush of the same solution. A roller pump was used to flush the isotonic saline through the kidney to remove blood and avoid the problem of clot formation. The kidney was then implanted in the iliac region.

The autograft was performed using an end-to-side anastomosis of the renal vein to the internal iliac vein (the common iliac vein was used if the internal iliac vein was too small). The renal artery was transected proximal to its bifurcation, which was present in all dogs, and anastomosed end-to-end to the internal iliac artery, the distal end of which was previously ligated and cut. Both anastomoses were done with BV-1 needles preset with 6-0 black silk. The ureter was reimplanted using a submucosal tunnel technique described by Stewart (1971). Nephrectomy of the contralateral kidney in these cases was done at the time of autograft. Contralateral kidneys were used for perfusion studies immediately after removal.

Unilateral nephrectomy with unilateral autograft (''unilaterals'') was done exactly the same as the ''bilaterals'' except that nephrectomy of the contralateral kidney was delayed for several weeks to give the autografted kidney time to heal (Belzer et al., 1967).

Laboratory tests.—Laboratory tests included red blood cell (RBC) and white blood cell (WBC) counts, differential white cell counts, blood urea nitrogen (BUN), hematocrit (Hct.), and, in some cases, hemoglobin (Hb.).

BUN was determined spectrophotometrically by Nesslerization; RBC and WBC counts were done on the hemacytometer and/or Coulter Counter; Hct. was done by the macro method.

Pathology examinations were made on perfused and post-mortem kidneys. Tissue sections 8 microns thick were prepared by standard histological techniques (Willey, 1971), using Bouin's fixative, hematoxylin and eosin stain, alcohol series and paraffin infiltration on the Autotechnicon automatic dehydrator and infiltrator. Post-mortem examinations were carried out using a standard Y-incision and selected organs and surgical techniques were photographed with Kodak Ektachrome film.

Perfusion technique.—The perfusion technique was modified from Belzer (1967). The perfusion system was modified from one developed at the University of Minnesota (Moberg et al., 1971). Kidneys were perfused for 24-48 h using a modified salt solution resembling Tyrode electrolyte solution (Table 1). A phosphate buffer system was used instead of a carbonate buffer system since the pH of a phosphate buffer system is not dependent upon the Pco_2 of the solution. Air was bubbled through the solution.

Table 1. Composition of phosphate-buffered Tyrode solution.

Compound	Concentration (μmoles/l)
NaCl	136.89
KCl	2.68
Na_2HPO_4	8.10
KH_2HPO_4	1.47
$CaCl_2 \cdot 2H_2O$	0.68
$MgCl_2 \cdot 6H_2O$	0.49
$C_6H_{12}O_6$ (glucose)	5.55

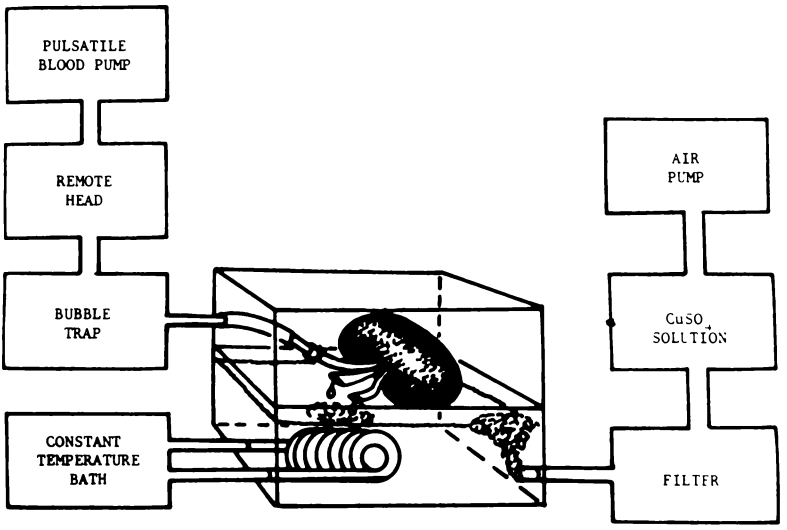

Figure 1. Components of the perfusion system. See the text for explanation.

The arrangement of the various components of the perfusion system is shown in Figure 1. Air was pumped through a flask of copper sulfate (0.1%) to retard mold growth, then through a glass wool filter that removed particulate matter. The air was then bubbled into a plastic perfusion chamber. A glass coil in the chamber was connected to a constant temperature bath to maintain the temperature of the perfusate at about 6°C. A pulsatile blood pump was used to pump the perfusate through a remote head and into a bubble trap before the perfusate entered the chamber.

Since every kidney perfused had a bifurcated artery, only one branch was cannulated and perfused. To determine which areas of the kidney received the perfusate, either a visual examination was made of the coloration of the tissue or a vital dye (Evans blue, 25 mg%) was added to the perfusion system immediately before removal. With the addition of the vital dye, perfused areas could be identified by noting the distribution of Evans blue in the cortex. Reimplantation of perfused kidneys was not attempted in this study.

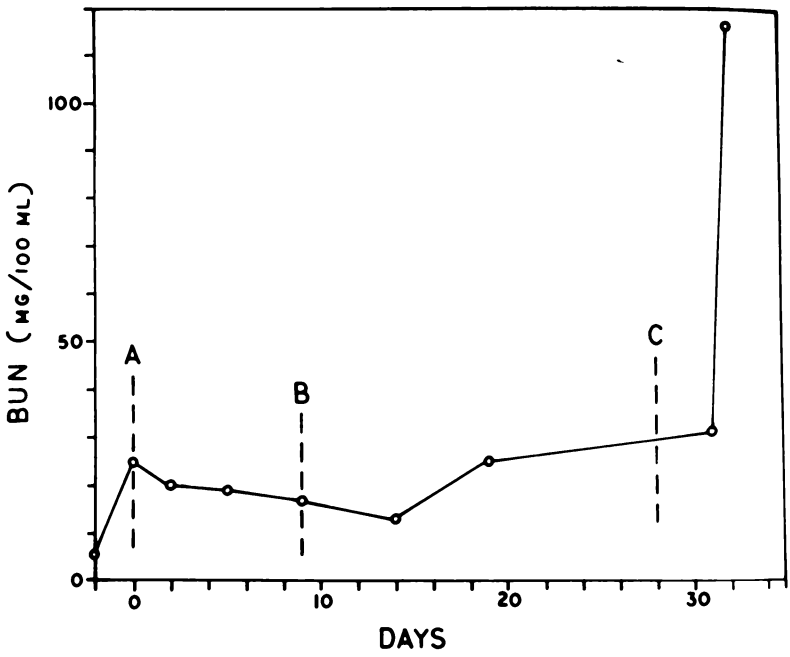

Figure 2. Graph of BUN vs. time for a "unilateral" dog. "A" indicates time of autograft. "B" indicates emergency surgery for repair of sutures. "C" indicates removal of contralateral kidney. Note the sharp rise in BUN following contralateral nephrectomy.

RESULTS

Surgical technique.—Eight of the 10 dogs were "bilaterals" and two were "unilaterals." One of the "bilaterals" was terminated post-surgically because of a parasitic infection with roundworms. Of the remaining seven, two survived for more than one day. The other five dogs either died during surgery or never regained full alertness.

The two "unilaterals" survived surgery, but one dog died early on the second post-operative day due to overdose of anesthetic during emergency surgery for hemorrhage. Post-mortem examination showed that the renal artery had become twisted, and cut off the blood supply to the autografted kidney. The remaining "unilateral" dog survived a total of 33 days after autograft, but only 4 days following removal of the contralateral kidney, at which time the dog died of uremic poisoning before peritoneal dialysis could be initiated. Figure 2 shows the BUN measurements of this animal. The BUN rose sharply following nephrectomy of the contralateral kidney at "C," indicating that the grafted kidney was not functioning properly. "A" indicates time of the autograft with the con-

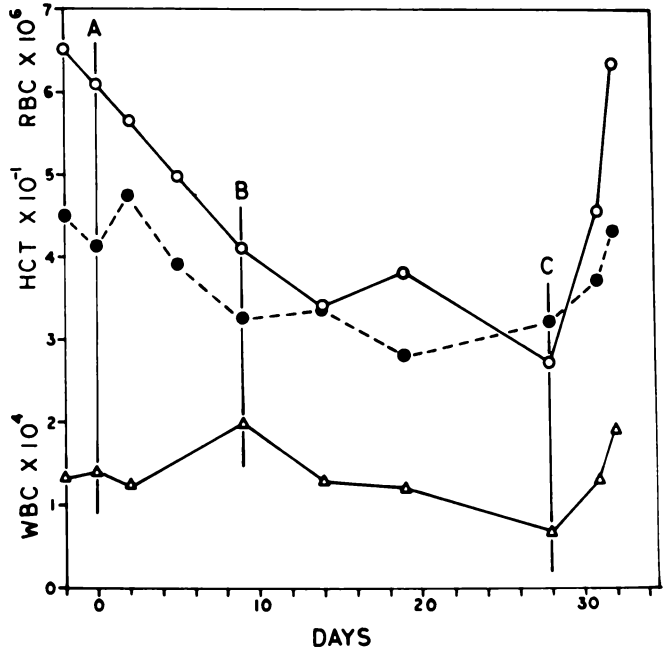

Figure 3. Graph of RBC and WBC counts, and Hct. vs. time in the same dog as in Figure 2. "A" indicates time of autograft. "B" indicates emergency surgery for suture repair. "C" indicates contralateral kidney. Vitamin B_{12} was given at "C" and for the next three days.

tralateral kidney left undisturbed. "B" indicates time of emergency surgery for replacement of sutures removed by the animal. "C" indicates removal of the contralateral kidney.

Other lab tests done on this dog included RBC and WBC counts and hematocrit (Hct.) (Figure 3). The letters "A", "B", and "C", indicate the time of autograft, emergency surgery, and contralateral nephrectomy. The sharp rise in RBC count is probably due to initiation of vitamin B_{12} therapy on days 29 through 32 for anemia and malnutrition, since vitamin B_{12} stimulates erythropeoiesis (Beck, 1971).

Intussusception, a telescoping of one section of intestine into another, was discovered during post-mortem examination of two of the "bilateral" dogs. This is consistent with the results of Dempster et al. (1955), who encountered a high incidence of intussusception following abdominal surgery involving the kidney being placed in the iliac region.

The two "unilateral" dogs were awake and alert following surgery, as opposed to the "bilaterals," who never regained full alertness.

Perfusion technique.—Three canine kidneys were perfused for 48 h but only gross or histological examinations were done on these kidneys. As would be expected, perfusing only one branch of the bifurcated artery resulted in cell damage to the non-perfused portions. Figure 4 is a photograph of a section from a non-perfused portion of a canine kidney. Advanced cell deterioration and vacuolization is apparent. Figure 5 is a photograph of a section from a perfused section of the same kidney as shown in Figure 4. There is very slight cell deterioration but the perfused tissue is otherwise normal.

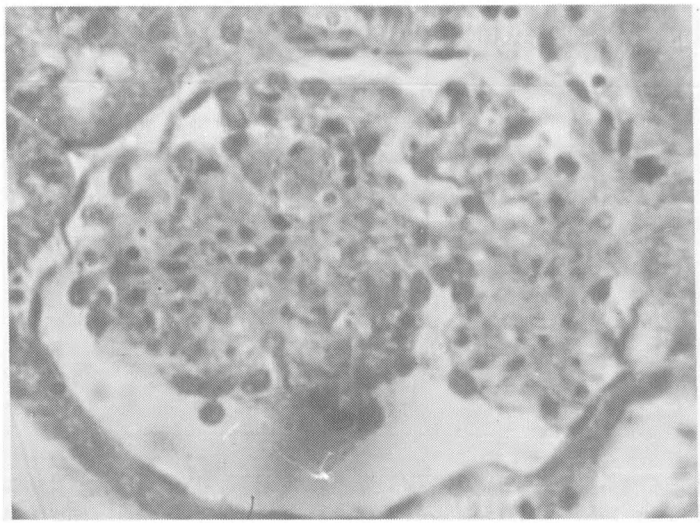

Figure 4. Non-perfused portion of a canine kidney. Advanced cell deterioration and vacuolization is apparent. Magnification 450X.

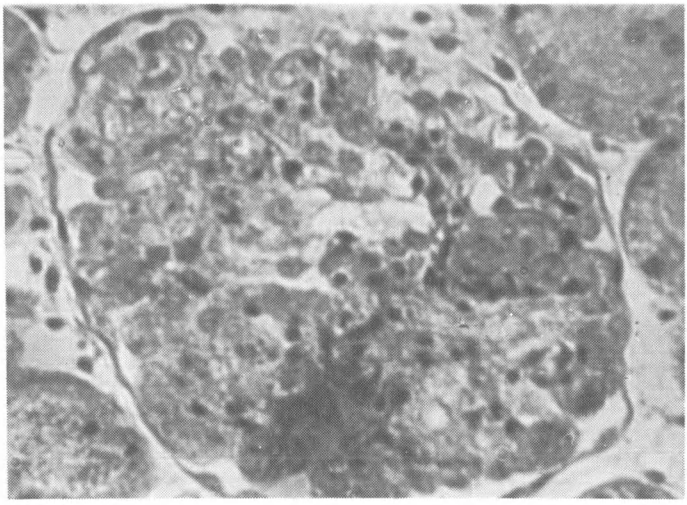

Figure 5. Perfused section of the same canine kidney as in Figure 4. There is only slight cell deterioration. The tissue appears essentially normal. Magnification 450X.

DISCUSSION

Surgical techniques.—Due to the failure of "bilateral" dogs to regain full alertness with sodium pentobarbital anesthesia and the relative alertness of "unilateral" dogs following surgery with the same anesthesia, barbiturate poisoning must have been a major contributing factor in the death of "bilateral" animals. Sodium pentobarbital is eliminated by the kidneys and is contraindicated in a major operation (Adriani, 1947). Sodium pentobarbital is also irretrievable once administered, individual tolerances making it difficult to determine the correct dosage. Other disadvantages include decreased urine formation and hypothermia. This anesthetic is also not excreted rapidly or properly if any renal necrosis is present (Baker, 1972). In an autograft, necrosis is inevitable.

For the above reasons, we propose changing the method of anesthesia. Methoxyflurane is an anesthetic gas which produces excellent muscle relaxation without depressing respirations (Adams, 1958). Since gaseous anesthesia is eliminated via a system that is not traumatized by abdominal surgery, i.e. the lungs, this type of anesthesia is indicated for future surgeries. If sodium pentobarbital is to be used, a "unilateral" procedure is indicated.

Perfusion technique.—Histologically, those portions of kidneys perfused with phosphate-buffered Tyrode solution appear to be well-preserved relative to non-perfused portions. The non-perfused portions (Figure 4), which stained poorly, had general advanced cellular deterioration, as evidenced by lack of distinct boundaries, glomeruli plugged with cellular debris, both in cross-section and

longitudinally, and vacuolization, indicating poor cellular integrity. The perfused sections (Figure 5), on the other hand, had clear cellular borders, intact glomeruli, and stained visibly better than the non-perfused sections, indicating that at the time of histological preparation, these sections were better preserved. However, further research is needed before any concrete conclusions regarding the viability of kidneys perfused with phosphate-buffered Tyrode solution can be drawn. Physiological measurements, such as glomerular filtration rate, Po_2, and Pco_2, will be initiated in future experiments.

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THE EFFECTS OF A LOW LEVEL OF DIETARY CADMIUM IN RATS

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ABSTRACT

The effects of 5 μg Cd/ml of drinking water on some biochemical and physiological parameters in male and female rats were studied. After 205 days, cadmium had no significant effect on weight gain, feed and water intakes or feed efficiency. Total cadmium intake in the cadmium-treated males and females was 29.0 and 32.1 mg over the 205-day, experimental period. In vivo retention of ^{24}Na was greater in the cadmium-treated males than in the control males at 161 days. In vivo retention of ^{42}K was significantly greater in the cadmium-treated males than in the control males at 189 days but the opposite was true of the cadmium-treated females in comparison with female controls. No significant differences were found between treatments in systolic blood pressure or serum cholesterol. There were highly significant differences ($p < 0.01$) in intakes of NaCl for control and cadmium-fed males at 269 days. The urinary excretion of ^{35}S was not significantly different for treated and control rats.

INTRODUCTION

In several animal experiments, the toxicity of orally administered cadmium has been reported (Bunn and Matrone, 1966; Cousins et al., 1973; Doyle et al., 1972, 1974). High dietary cadmium concentrations have been found to cause anemia, growth depression, adverse reproductive performance and chromosomal hypodiploidy. Low levels of dietary cadmium have been shown to cause hypertension in rats (Schroeder, 1964b), and to significantly ($p < 0.05$) reduce storage of Cu in liver, spleen and testicles of sheep (Mills and Dalgarno, 1972; Doyle, 1973). Specific microorganisms show an elevated growth response to low levels of Cd (Doyle, 1973).

The objectives of the experiment were to determine the effects of 5 μg Cd/ml in drinking water on various biochemical and physiological parameters of male and female rats over an extended period of time. The possible role of inorganic food factors in the production of hypertension was also investigated.

MATERIALS AND METHODS

Twenty male and twenty female, Long Evans rats, approximately 21 days old were allotted at random to one of two treatments. Treatment 1 was administered 5 μg Cd/ml and 4.5 μg Cr/ml drinking water whereas treatment 2 was the control and received 0 Cd and 4.5 μg Cr/ml in drinking water. The basal diet (Table

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1) was fed *ad libitum* and was chosen so as to maximize the effects of the cadmium (relatively high sodium with other essential elements not given in excess). Feed and water intakes were measured daily, and body weight was measured at frequent intervals. ^{24}Na retention was determined by injecting each rat with 2.8 μCi ^{24}Na intraperitoneally and then counting the rats in a Packard Armac Liquid Scintillation Detector, Model 446, at intervals over a period of 73 h for the males and 71 h for the females. The same procedure was followed for ^{42}K with the exception that 2.5 μCi ^{42}K was injected per rat and counting was over a period of 78 h.

Systolic blood pressure was measured by anesthetizing each rat with 0.35 mg/Kg pentobarbitol, intraperitoneally. Ten minutes after the injection each rat was kept in a 38°C environment for 10-20 min. Measurements were performed on a Narco Biosystems Desk Model Physiograph-Electrosphygmomanometer-Pulse Transducer system by the method of Friedman and Freed (1949). Systolic blood pressure was taken as the lowest of three consecutive measurements.

Serum cholesterol was determined by the fluorometric method of Carpenter et al. (1957).

The retention of methionine was determined by injecting rats with 5 μCi/100 g body weight of L-methionine ^{35}S and counting the radioactivity in the urine every 24 h for 6 days by using the Nuclear Chicago Liquid Scintillation System Mark II and Tye and Engel (1965) Liquid Scintillation Counting solution - DTN.

To test whether the cadmium-fed animals had a preference for sodium chloride, five cadmium-fed and five control rats of each sex were housed in ACME metabolism cages, one to a cage, for a period of 14 days. Rats in the cadmium-fed group given a choice of solutions containing 5 μg Cd/ml, 4.5 μg Cr/ml plus 0.2 M NaCl or 5 μg Cd/ml plus 4.5 μg Cr/ml. The control rats were given a choice of solutions containing 4.5 μg Cr/ml plus 0.2 M NaCl or 4.5 μg Cr/ml. Intakes of the solutions were measured daily. Bottles for each cage were rotated daily to eliminate position preference, and bottle nipples and stoppers were changed daily to eliminate nipple preference.

Standard errors of the means were calculated for all data. Student's t test was used as a measure of significance. Homogeneity of regression was tested as described by Daniel and Wood (1971).

RESULTS AND DISCUSSION

The average composition of the basal diet is shown in Table 1. There were no significant differences in body weight gain or food consumption for rats on the various diets (Table 2). This result is in agreement with data reported for rats (Decker et al., 1958), for dogs (Anwar et al., 1961) and for lambs (Doyle et al., 1972, 1974). Furthermore, cadmium had no significant effect on feed efficiency (Table 2) or water intake (Table 3). However, it reduced the water intake of the cadmium-treated males relative to the water intake of the control males and had a significant effect on the cadmium intake of all of the cadmium-fed animals (Table 3). Decker (1958) and Schroeder (1964a) reported the same results for Sprague-Dawley and Long Evans rats.

Table 1. Average composition of basal diet.

Diet composition	%	Vitamin mix	%	Salt mix	%	Minerals	μg/g of diet
Glucose (anhydrous)	58.67	Folic acid	0.004	Na_2SeO_3	0.0003	Se	0.05
Sprayed egg white	19.99	Biotin	0.008	KI	0.0007	I	0.17
Corn oil	10.00	Menadione	0.010	$Cu(AC)_2$	0.053	Cu	5.60
Solka floc	3.00	Pyridoxine	0.010	$Zn(AC)_2$	0.131	Zn	13.00
Salt mix	3.29	Vitamin B_{12}	0.012	$CaCO_3$	0.177	Fe	35.00
Vitamin mix	5.05	Riboflavin	0.016	$FeCl_2$	0.374	Mn	45.00
		Thiamin	0.020	Mn $(AC)_2$	0.602	Mg	400.00
		Niacin	0.079	$MgCO_3$	4.162	K	2000.00
		Ca panthothenate	0.079	KCl	11.441	Na	3935.00
		Vitamin A palmitate	0.079			Ca	5200.00
		Vitamin D_3	2.346	NaCl	30.000	P	4000.00
		α-tocopherol	0.791				
		Choline chloride	1.971	$CaHPO_4$ (dibasic)	52.951		
		Inositol	1.971				
		Glucose (anhydrous)	94.606				

Table 2. Body weight gain, feed intake and feed efficiency of rats fed 5 μg/ml cadmium over 205-day period.[1,2]

Treatment	Sex	No. animals	Body weight gain Total g	Per day g	Feed intake g/rat/day	Feed efficiency g feed/g gain
5 ppm Cd	M	9	300[2] ±13[a]	1.47 ±0.06[a]	13.81 ±0.87[a]	9.46 ±0.14[a]
Control	M	9	293 ±24[a]	1.43 ±0.12[a]	12.88 ±0.75[a]	9.17 ±0.45[a]
5 ppm Cd	F	10	189 ±8[a]	0.92 ±0.04[a]	9.39 ±0.40[a]	10.07 ±0.09[a]
Control	F	9	185 ±9[a]	0.90 ±0.05[a]	9.37 ±0.37[a]	10.36 ±0.38[a]

[1]Mean±SEM.
[2]Data with different superscripts are significantly different ($p<0.05$).

Table 3. Water and cadmium intake of rats fed 5μg/ml cadmium over 205-day period.[1,2]

Treatment	Sex	No. animals	Water intake ml/rat/day	Cadmium intake total/rat in mg
5 ppm Cd	M	9	28.28±2.07[a]	28.98±2.12[a]
Control	M	9	36.28±5.07[a]	0.05±0.004[b]
5 ppm Cd	F	10	31.33±3.83[a]	32.11±3.92[a]
Control	F	9	33.37±2.52[a]	0.05±0.004[b]

[1]Mean±SEM.
[2]Data with different superscripts are significantly different ($p<0.05$).

Table 4. Systolic blood pressure and serum cholesterol levels of rats fed 5 μg/ml Cd in drinking water.

Treatment	Sex	No. animals	Day	Blood pressure[1,2] in mm Hg	Cholesterol[1,2] mg%
5 ppm Cd	M	10	94[3]	125±4[a]	
		10	155		57±7[a]
		10	171[4]	123±7[a]	
		5	212		70±5[a]
Control	M	10	94[3]	128±6[a]	
		9	155		50±5[a]
		9	171[4]	118±8[a]	
		5	212		89±6[a]
5 ppm Cd	F	10	94[3]	117±3[a]	
		10	155		51±2[a]
		10	171[4]	116±4[a]	
		5	212		74±4[a]
Control	F	10	94[3]	119±4[a]	
		9	155		55±3[a]
		9	171[4]	107±6[a]	
		5	212		75±8[a]

[1]Mean±SEM.
[2]Data with different superscripts are significantly different
[3]Non-anesthetized.
[4]Anesthetized.

Systolic blood pressure was not affected by cadmium intake up to 94 days but was greater but not significantly so for both the treated and control groups of both sexes after 171 days (Table 4). However, Perry and Erlanger (1974a) found that

cadmium levels as low as 2.5 μg Cd/ml in drinking water caused a significant ($p < 0.05$) elevation in systolic blood pressure after 6 months; Lener and Bibr (1970), however, failed to produce hypertension in Wistar rats given 5 μg/ml Cd in their drinking water for 16 months. Schroeder and Vinton (1962) reported that 5 μg Cd/ml fed to rats in their drinking water caused severe hypertension after 200-240 days. Differences in mineral content of the diets may account for these conflicting results. The most obvious difference between our diet and the diets of Perry and Erlanger (1974a) and Schroeder and Vinton (1962) is the level of zinc. Table 1 shows the zinc concentration to be 13 μg/g of diet whereas the concentration in the water was less than 0.01 μg/ml. Perry and Schroeder added 50

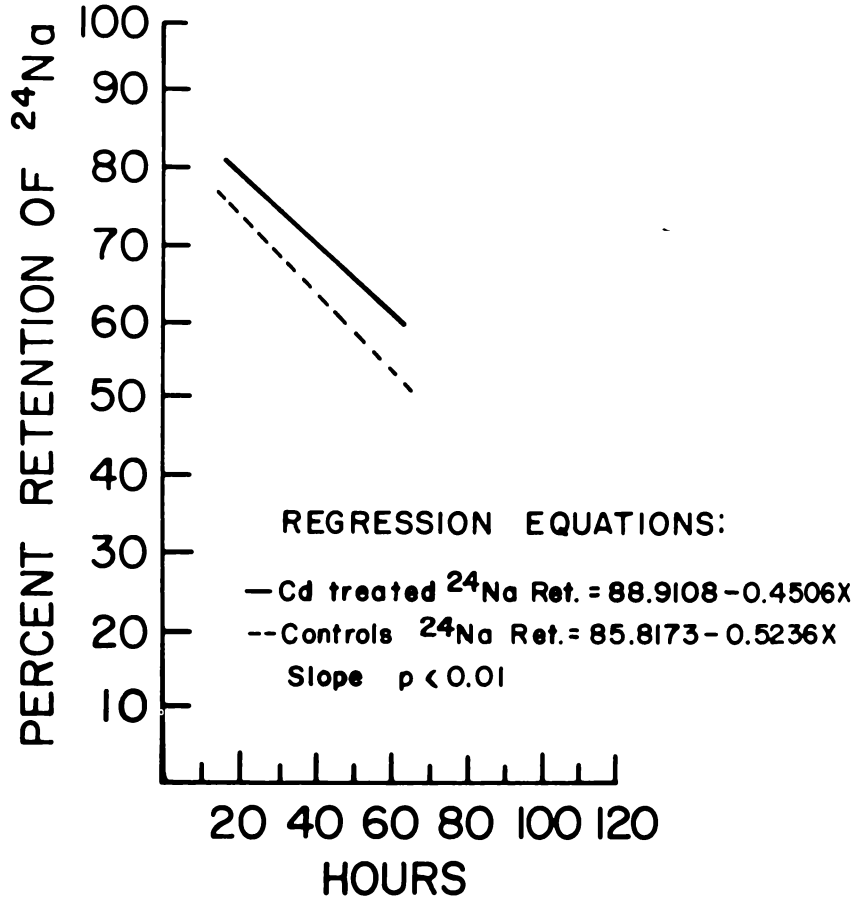

Figure 1. The relation between percent ^{24}Na retention and time in cadmium-treated and control male rats.

μg Zn/ml of drinking water whereas their diet contained 22 μg Zn/g. Hence, the zinc intake of our rats was, at most, only one-fifth the intake of Perry's and Schroeder's rats. As zinc is an antagonist of cadmium and vice versa, it would seem that our rats should become hypertensive sooner than Perry's or Schroeder's. The selenium added to our diet was only 0.05 μg/g and hence was not an anti-Cd hypertensive factor (Perry and Erlanger, 1974b). The apparent anti-hypertensive effect of our diet may have been due to the relatively high calcium level (0.52) as Schroeder et al. (1967) have shown that the incidence of Cd-hypertension in female rats fed 0.41% of dietary calcium was partly alleviated by 200 μg/ml Ca in drinking water. Furthermore, Piscator (1973) reported that the absorption of ingested cadmium is dependent on calcium intake. Hence, it seems possible that the relatively high level of calcium in our diet may have reduced the absorption of cadmium and thus increased the time period required to produce hypertension. The relationship between dietary calcium and sodium must also be emphasized. Douglas and Langford (1969) found that more rats became hypertensive when the calcium:sodium ratio in the diet was reduced. As the ratios reported in this experiment and by Schroeder were 1.33 and 1.05, the higher level of Ca in our diet may have acted in a protective manner against the dietary sodium.

The percent retention of ^{24}Na was greater in the cadmium-treated males and females than in the controls after a period of 161 days (Table 5). As analysis of individual time periods did not reveal significant differences in retention, the slope of the disappearances between 17 and 66 h was calculated utilizing all of the available data (Figure 1). The differences were significant ($p < 0.01$). As these animals were not hypertensive, these results suggest that increased sodium retention precedes the occurrence of cadmium-induced hypertension. Sodium retention has been reported to occur in some humans with hypertension (Dahl et al., 1962). The increased retention of ^{24}Na due to cadmium is believed to be a result of increased renin and aldosterone secretions by the kidney and adrenal cortex (Perry and Erlanger, 1973). The percent retention of ^{42}K was significantly ($p < 0.05$) greater in the cadmium-treated males than the control males whereas the reverse was true in the treated and control females (Table 6). The greater retention of ^{42}K as evidenced by the female controls was the result expected as sodium retention is associated with increased loss of potassium. However, the high retention of ^{42}K in the cadmium-treated males was unexpected. Vander (1962) reported decreased urinary sodium and potassium excretion in dogs injected with cadmium chloride and concluded that cadmium does not increase sodium reabsorption by enhancing sodium - potassium exchange mechanisms, as this would have resulted in an increased potassium excretion. A similar conclusion may well apply to the potassium data reported for male rats in this experiment. The biological half life (T 1/2) and fractional rate of disappearance (K) of the injected radiopotassium (plots appeared linear after 30 h) for the different treatments are shown in Table 7. They show large differences between treatments for both values.

The sodium chloride intake of the cadmium-treated males was significantly greater ($p < 0.01$) than that of the control males (Table 8). More than 45% of the

Table 5. Percent retention of ^{24}Na in rats fed dietary cadmium for 161 days.

Treatment	Sex	No. animals	^{24}Na retention[1,2]% 17h	22h	41h	45h	49h	65h	71h	73h
5 ppm Cd	M	6	81.2[d] ± 3.3		70.6[d] ± 4.7		66.7[d] ± 4.4	59.7[d] ± 4.1		59.3[d] ± 3.9
Control	M	6	76.9[a] ± 3.2		64.0[a] ± 3.3		60.7[a] ± 3.3	51.6[a] ± 3.2		51.1[a] ± 3.1
5 ppm Cd	F	6		68.2[a] ± 3.0		56.7[a] ± 2.9			54.6[a] ± 2.6	
Control	F	6		63.6[a] ± 0.9		51.3[a] ± 2.1			51.1[a] ± 2.2	

[1]Mean ± SEM.

[2]Data with different superscripts are significantly different ($p < 0.05$).

Table 6. Percent retention of ^{42}K in rats fed dietary cadmium for 189 days.

Treatment	Sex	No. animals	^{42}K retention[1,2]% 6h	18h	30h	42h	54h	66h	78h
5 ppm Cd	M	8	93.5[a] ± 2.1	88.2[a] ± 2.5	85.9[a] ± 2.3	83.7[a] ± 2.3	81.2[a] ± 2.3	80.5[a] ± 2.5	78.5[a] ± 2.6
Control	M	8	79.2[b] ± 6.3	73.9[b] ± 6.0	71.4[b] ± 5.7	69.4[b] ± 5.7	66.9[b] ± 5.5	66.3[b] ± 5.5	63.7[b] ± 5.2
5 ppm Cd	F	8	77.0[a] ± 6.4	73.8[a] ± 6.6	71.9[a] ± 6.4	68.8[a] ± 6.1	67.5[a] ± 6.1	65.2[a] ± 5.6	66.6[a] ± 6.0
Control	F	8	94.9[b] + 3.9	94.3[b] ± 5.2	91.4[b] ± 5.2	87.8[b] ± 5.0	85.0[b] ± 4.9	82.4[b] ± 5.0	84.5[b] ± 4.4

[1]Mean ± SEM.

[2]Data with different superscripts are significantly different ($p < 0.05$).

Table 7. Apparent body retention of ^{42}K in rats fed dietary cadmium for 189 days.

Treatment	Sex	No. animals	Body retention ^{42}K T ½[3] Days [1,2]	Body retention ^{42}K K value % body loss/day
5 ppm Cd	M	8	$13.69^{a} \pm 1.55$	5.06
Control	M	8	$7.76^{b} \pm 1.51$	8.93
5 ppm Cd	F	8	$6.63^{a} \pm 1.31$	10.90
Control	F	8	$12.45^{b} \pm 2.00$	5.57

[1]Mean ± SEM.

[2]Data with different superscripts are significantly different. ($p < 0.05$)

[3]Biological half life.

Table 8. Mean intakes[1] of NaCl solution and water by cadmium-fed and control male and female rats given choice of both solutions for 14-days.

	Sex	Control		Cadmium-fed	
		Water[2]	NaCl solution[3]	Water[2]	NaCl solution[3]
		in ml/100 g body wt.		in ml/100 g body wt.	
Mean	M	6.92 ± 0.15^{a}	2.91 ± 0.31^{a}	5.30 ± 0.24^{a}	4.40 ± 0.42^{b}
	F	7.21 ± 0.36^{a}	4.53 ± 0.30^{a}	7.66 ± 0.74^{a}	5.46 ± 0.60^{a}
% of total water intake	F	70.5	29.5	54.6	45.4
	F	61.4	38.6	58.4	41.6

[1]Mean ± SEM.

[2]Data with different superscripts for water are significantly different ($p < 0.01$)

[3]Data with different superscripts for NaCl solution are significantly different ($p < 0.01$).

Table 9. Cumulative ^{35}S urinary excretion of rats fed 5 μglml of cadium in drinking water for 244 days.

Treatment	sex	No. animals	Percent excretion in urine[1,2]						
			12h	24h	48h	70h	96h	120h	144h
5 ppm Cd	M	5	7.39 ± 0.45[a]	8.90 ± 0.31[a]	12.04 ± 0.50[a]	13.42 ± 0.47[a]	14.67 ± 0.48[a]	15.66 ± 0.52[a]	16.34 ± 0.50[a]
Control	M	5	5.93 ± 0.90[a]	7.39 ± 1.13[a]	10.93 ± 1.11[a]	12.61 ± 1.07[a]	13.81 ± 1.08[a]	14.71 ± 1.14[a]	15.43 ± 1.13[a]
5 ppm Cd	F	5	5.05 ± 0.76[a]	8.68 ± 0.96[a]	11.30 ± 1.00[a]	13.08 ± 1.03[a]	14.16 ± 1.14[a]	15.12 ± 1.24[a]	15.94 ± 1.24[a]
Control	F	5	4.40 ± 1.15[a]	7.94 ± 1.53[a]	10.24 ± 1.60[a]	12.01 ± 1.57[a]	13.25 ± 1.63[a]	14.24 ± 1.65[a]	14.97 ± 1.60[a]

[1]Mean ± SEM

[2]Data with different superscripts are significantly different ($P < 0.05$).

total fluid intake was saline water for the cadmium-treated rats whereas only 29% was saline water for the controls. This disagrees with the results reported by Schroeder (1964a) who found that thee was no significant difference in the intakes of NaCl between cadmium-fed and control rats. However, Schroeder's data do show that the cadmium-treated animals consumed more NaCl/100 g body weight by choice than did the control animals. The data reported here appear consistent with the sodium retaining action of acutely injected cadmium in dogs, as reported by Vander (1962). The sodium chloride intake of the cadmium-treated females was not significantly different from that of the female controls (Table 8).

There were no significant differences in serum cholesterol of treated and control animals (Table 4). However, the levels were much greater in the controls than in the cadmium-treated males. Schroeder and Balassa (1965) reported similar results for male and female rats.

The urinary excretion of ^{35}S was not significantly different for treated and control rats (Table 9) indicating that the cadmium-treated rats retained methionine as well as did the controls.

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ZINC DEFICIENCY IN THE PRENATAL RAT

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ABSTRACT

Previously it was observed that offspring of rats fed a zinc-deficient diet during the last trimester of pregnancy showed impaired behavior at 6 weeks of age even though they had been fed adequate zinc from the time of delivery (Halas and Sandstead, 1974). This paper describes biochemical findings on fetuses taken by caesarean section on day 21 of pregnancy from dams fed the zinc-deficient diet from day 14 of gestation. The findings are compared with those on fetuses from pair-fed (undernourished) and ad libitum-fed control dams which had been given adequate zinc. Fetuses from zinc deficient and pair-fed dams weighed less than fetuses from ad libitum dams. Brain weight of fetuses from the zinc-deficient dams was lower than that of fetuses from either the pair-fed or ad libitum-fed dams. Liver weight of fetuses from both the zinc-deficient and pair-fed dams, and placental weight from the zinc-deficient dams were lower than for animals of the ad libitum control group. Concentrations of DNA, RNA, and protein was higher in the brains of the zinc-deficient fetuses, but the total amounts of DNA, RNA, and protein were similar in all groups. Total liver DNA, RNA, and protein was reduced in fetuses of both the zinc-deficient and pair-fed dams; the total amounts in the placentae were not affected.

INTRODUCTION

Zinc is an essential nutrient for plants, animals, and man, its main uses in the body being related to enzyme functions and to the biosynthesis of nucleic acids and protein. Deprivation of zinc in experimental animals therefore affects growth, and in particular is manifested in those tissues in which there is active cell proliferation and enlargement. Hurley et al. (1971) showed that severe zinc deficiency in the rat for the whole or for part of pregnancy resulted in malformed fetuses. Every organ system was affected, including the brain. More recent studies of the rat have shown that prenatal zinc deficiency has adverse effects on behavior. The male offspring of dams fed a zinc-deficient diet from the 14th through the 19th day of pregnancy were rehabilitated with adequate zinc until testing at 6 weeks of age (Halas and Sandstead, 1974). The present study was therefore designed to examine the biochemical effects of maternal deprivation of zinc during the last trimester of pregnancy on the fetal brain; fetal liver and the placenta were also examined.

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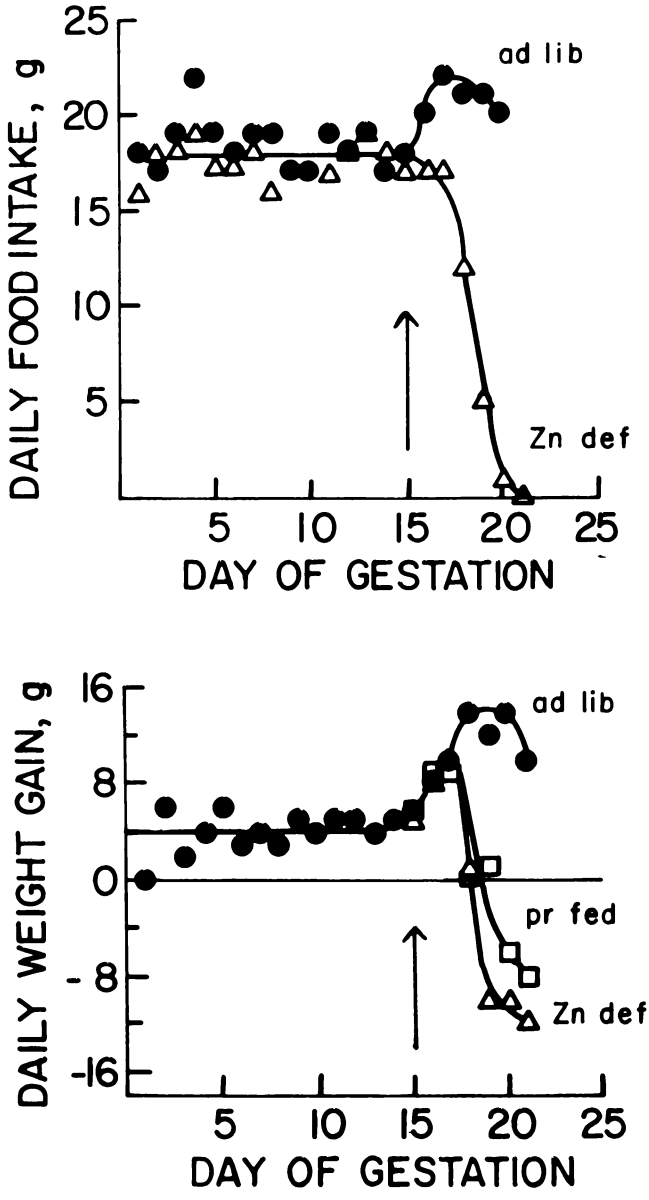

Figure 1. Daily food intake and daily weight gain during gestation for the zinc deficient, pair-fed, and ad libitum-control rat dams.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats (225-250 g) were bred and pregnancy was confirmed with vaginal smears. From the first day of pregnancy, the dams were fed ad libitum a biotin-enriched 20% sprayed egg white diet (Luecke et al., 1968) containing all known essential nutrients except zinc, which was present at less than 1 ppm by analysis. Drinking water containing 25 ppm zinc was freely available. At the beginning of day 14 of gestation the dams to be made zinc deficient were continued ad libitum on the purified diet, and changed to double-distilled, deionized water. A second group of pregnant dams was individually pair fed to the zinc-deficient dams for the whole of gestation, and a third zinc-adequate control group was fed ad libitum throughout gestation. On the morning of day 21, the fetuses were delivered alive by caesarean section and weighed; the placentae were placed into ice-cold buffered sucrose. The 8 fetuses around the median weight for the litter were decapitated, and their brains minus the olfactory bulb and brainstem were removed by blunt dissection and placed in ice-cold buffer. The fetal livers were also placed in the buffer, and each tissue was individually weighed. The pooled tissues were then homogenized and triplicate aliquots were taken for chemical analysis. DNA analyses were based on Burton's modification of the diphenylamine procedure (Burton, 1956); RNA was measured using the procedure of Fleck and Munro (1962), and protein was estimated using Hartree's modification (1972) of the Lowry procedure (Lowry et al., 1951).

RESULTS

Daily food intakes and daily weight gains are represented in Figure 1. Four days after zinc deprivation was started on day 14, the food intake declined in the

Table 1. Weight of fetuses, fetal brains, fetal livers, and placentae for the three experimental groups (means ± SE).

Group	Fetus g	Fetal brain g	Fetal liver g	Placenta g
Zinc deficient (9)[1]	3.98[2] ±0.11	0.15[2] ±0.01	0.23[3] ±0.01	0.46[3] ±0.02
Pair fed (8)	4.34[3] ±0.13	0.18 ±0.006	0.25[3] ±0.01	0.52 ±0.02
Ad libitum (8)	5.15 ±0.15	0.18 ±0.008	0.36 ±0.02	0.55 ±0.02

[1]Numbers in parentheses are the number of litters represented.
[2]Significantly different from the pair fed control, $P<0.05$.
[3]Significantly different from the ad libitum control, $P<0.01$.

zinc-deficient group, reaching zero by day 21. In contrast, the ad libitum-fed zinc-adequate dams increased their food intake for the last few days of gestation, when they also showed an increased rate of weight gain. Both the zinc-deficient and pair-fed dams stopped gaining weight by day 18, and thereafter both groups lost weight. The zinc-deficient group lost weight faster than the pair-fed group which had been given the same amount of food but had been given adequate zinc in their drinking water.

The fetuses from the zinc-deficient dams were smaller than the fetuses from the pair-fed dams, which, in turn, were smaller than the fetuses from the ad libitum-control dams (Table 1). Brain weight (0.15 g) of fetuses from the zinc-deficient dams was less than that of fetuses from the pair-fed and ad libitum-control dams (0.18 g), whereas liver weight of fetuses from both the zinc-deficient and pair-fed dams was less than that of fetuses from the ad libitum-control dams (Table 1). The placentae from the zinc-deficient dams were aiso smaller than those from the control dams.

Brain composition was affected by the lack of zinc. The concentrations of DNA, RNA, and protein were highest in the brains of fetuses from the zinc-deficient dams, intermediate in the brains of fetuses from the pair-fed dams, and lowest in the brains of fetuses from the ad libitum-control dams (Table 2). However, the total content of DNA, RNA, and protein in the brain did not differ significantly among the three groups. In the liver the concentrations of DNA, RNA, and protein were similar for the three groups (Table 3), but the fetuses from the zinc-deficient and pair-fed dams had less total DNA, RNA, and protein in their livers, reflecting the lighter weight of the livers from the zinc-deficient and pair-fed groups in comparison with the ad libitum-control group. Placental composition of DNA and RNA was not affected by either zinc deficiency or undernutrition (Table 4), but protein concentration was higher in the placentae from the zinc-deficient dams than from the pair-fed controls.

Table 2. Brain composition of fetuses from dams of the three experimental groups (means ± SE).

Group	DNA		RNA		Protein	
	mg/g	total mg	mg/g	total mg	mg/g	total mg
Zinc deficient (6)[1]	4.06[2] ±0.70	0.57 ±0.06	4.94[2] ±0.66	0.68 ±0.03	130[2] ± 15	19.9 ± 1.2
Pair fed (6)	3.49[2] ±0.20	0.61 ±0.02	4.13[2] ±0.20	0.71 ±0.02	121 ± 13	21.2 ± 2.1
Ad libitum (4)	2.94 ±0.16	0.58 ±0.04	3.49 ±0.15	0.69 ±0.03	92 ± 5	18.1 ± 1.1

[1]Numbers in parentheses are the number of litters represented.
[2]Significantly different from the ad libitum control, $P<0.05$.

Table 3. Liver composition of fetuses from dams of the three experimental groups (means ± SE).

Group	DNA		RNA		Protein	
	mg/g	total mg	mg/g	total mg	mg/g	total mg
Zinc deficient (6)[1]	3.11 ±0.31	0.68[3] ±0.10	11.08 ± 1.21	2.30[2] ±0.10	178 ± 19	37[2] ± 2
Pair fed (6)	3.36 ±0.23	0.83 ±0.04	8.79 ±1.25	2.20[2] ±0.31	156 ± 10	39[2] ± 3
Ad libitum (4)	2.87 ±0.16	1.16 ±0.17	9.54 ±0.52	3.82 ±0.43	144 ± 13	56 ± 2

[1]Numbers in parentheses are the number of litters represented.
[2]Significantly different from the ad libitum control, $P < 0.01$.
[3]Significantly different from the ad libitum control, $P < 0.025$.

Table 4. Placental composition for dams of the three experimental groups (means ± SE).

Group	DNA		RNA		Protein	
	mg/g	total mg	mg/g	total mg	mg/g	total mg
Zinc deficient (6)[1]	1.24 ±0.09	0.62 ±0.04	3.70 ±0.38	1.73 ±0.13	226[2] ± 19	108 ± 8
Pair fed (6)	1.22 ±0.08	0.65 ±0.02	4.10 ±1.19	2.11 ±0.53	179 ± 6	97 ± 5
Ad libitum (4)	1.15 ±0.14	0.61 ±0.08	2.99 ±0.27	1.57 ±0.16	175 ± 14	95 ± 16

[1]Numbers in parentheses are the number of litters represented.
[2]Significantly different from pair-fed control, $P < 0.025$.

DISCUSSION

The speed with which the zinc deficiency developed was striking. The latter third of pregnancy places great nutritional demands on the maternal rat, since at the beginning of the third trimester (day 14) the fetus weighs only 1/6 of its birth weight (Winick and Noble, 1965). The zinc content per fetus has been given as 12 μg on day 15, 32 μg on day 18, and 102 μg on day 22 (Feaster et al., 1955).

This marked acceleration in the accumulation of zinc corresponded with the time when the zinc-deficient dams began eating less and losing weight. Zinc in maternal tissues such as bone and liver has been shown to be inaccessible to developing embryos even in the face of teratogenic zinc deficiency (Hurley and Swenerton, 1971).

The differences observed in brain composition suggested that some component other than the ones measured was affected. A concomitant change in lipid content of the brains might have occurred. Lipid concentration in the brain of zinc-deficient suckling rats has been shown to be reduced in comparison with that in brains of pair-fed controls (Sandstead et al., 1972).

Zinc deficiency and undernutrition had a similar effect on fetal liver. A lower liver weight and lower total DNA, RNA, and protein have also been described during maternal protein deficiency in full term fetuses (Zeman and Stanbrough, 1969).

In the rat the placenta becomes functional during the second trimester (Nicholas, 1971), and it seemed that neither the zinc deficiency nor the undernutrition affected its composition as much as they affected the brain or liver. The reduction in weight and increase in protein concentration in the placentae from the zinc-deficient group might have reflected a change in lipid content.

Each of the tissues studied responded differently to the stresses of zinc deficiency or restricted feeding. Of particular interest in this study were the effects on the brain. The perinatal period is a critical time for growth of the brain in the rat. Therefore, one might expect to find residual behavioral sequelae of the zinc deficiency in animals raised to adulthood after nutritional rehabilitation. Indeed, such has been found to be the case in studies which are currently underway in our laboratory (Halas and Sandstead, 1974).

ACKNOWLEDGMENTS

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THE EFFECT OF AN ORAL HYPOGLYCEMIC AGENT ON EMBRYONIC MORTALITY IN RATS

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ABSTRACT

Virgin female rats were used in six trials to determine the effect of the hypoglycemic agent Dymelor (Eli Lilly) on embryonic mortality. Rats were fed the agent for various lengths of time post breeding at two different dose levels. Blood glucose levels were determined and the glycogen content of various tissues analyzed. Also, the ovulation rate, number of viable embryos, and detectable resorptions were noted. In six trials the percentage embryonic mortality for the control (C) and treatment (T) groups was: C = 19.9, 28.0, 26.4, 34.8, 24.5, and 28.5; and T = 28.0, 20.1, 16.1, 17.1, 16.4, and 14.8 ($P \leq .01$) In trial IV the muscle glycogen content was higher in the control group ($P \leq .01$) whereas the fetal glycogen content was higher in the treatment groups in trials III and IV ($P \leq .01$) and VI ($P \leq .05$). The number of detectable resorptions were higher in controls than in treatment animals in trials IV-VI ($P \leq .01$). The data suggest a significant benefit of a hypoglycemic agent, possibly through making available the maternal stores of glycogen to the developing embryo. It appears that no further benefits would be derived from any higher drug level or extended length of feeding beyond 5 mg per day per animal for six days.

INTRODUCTION

Embryonic mortality is a severe problem facing our livestock producers today. In swine, fertilization rate of released ova approaches 95-100% (Spies, 1959:163; Nalbandov, 1968:202). However, the likelihood of all fertilized ova resulting in live pigs at parturition is small. Embryonic death losses in swine reach 25-50%, with the most critical period of embryonic mortality apparently in the first 25 days after conception (Pomeroy, 1960:57; Perry, 1954:303; Lerner et al., 1957). One of the main factors affecting embryonic mortality is nutrition; however, the actual relationship has not yet been elucidated (Robertson et al., 1951:841). Therefore, the purpose of this study was to gain further knowledge in this area by determining the effect of the oral hypoglycemic drug Dymelor on embryonic mortality.

MATERIALS AND METHODS

Experimental procedure.—One hundred sixty-three virgin female rats, weighing approximately 200-210 g, were used in this study. These animals were divided into six trials using rats of the Sprague-Dawley strain (Trials I through IV) and the Holtzman strain (Trials V and VI). Each trial consisted of a control group and a series of treatments as follows:

Trial I. Treatment group fed 5 mg Dymelor/animal/day for 6 days post breeding;

Trial II. Treatment group fed 10 mg Dymelor/animal/day for 12 days post-breeding;

Trial III. Treatment group fed 5 mg Dymelor/animal/day for 6 days post-breeding;

Trial IV. Treatment group fed 5 mg Dymelor/animal/day for 12 days post-breeding;

Trial V. Treatment group fed 10 mg Dymelor/animal/day 6 days post-breeding; and

Trial VI. Treatment group fed 5 mg Dymelor/animal/day for 6 days post-breeding.

After detection of a vaginal plug, bred females were randomly assigned to a control or treatment group. An initial fasted blood sample was taken on day 1 of pregnancy via heart puncture, after which the females were started on trial. At slaughter a final blood sample was taken, the uteri were excised, and three reproductive characteristics noted: total number of viable embryos, total number of ovulations, and the total number of detectable resorptions. Also, 100 mg samples of maternal liver and muscle tissue were taken along with 100 mg of fetal tissue. Samples were placed in individual centrifuge tubes containing 1 ml of 30% potassium hydroxide and refrigerated (16 C) until analyzed for glycogen content.

Method of analysis—Whole blood glucose concentrations were determined using Glucostat (Worthington Biochemical Corp.), a prepared reagent for the quantitative colorimetric determination of glucose developed by Teller (1956:31c). Following the method of Seifter (1950:191) the tubes containing previously collected tissue samples were analyzed for glycogen content using anthrone reagent.

Oral hypoglycemic agent.—Dymelor is an acetohexamide produced by Eli Lilly. This drug is a sulfonylurea characterized by the presence of an acetyl group in the para-position of the phenyl ring and the incorporation of a cyclohexyl group in the urea moiety. Chemically, it is N-P-acetylphenylsulfonyl-N-cyclohexylurea (Figure 1).

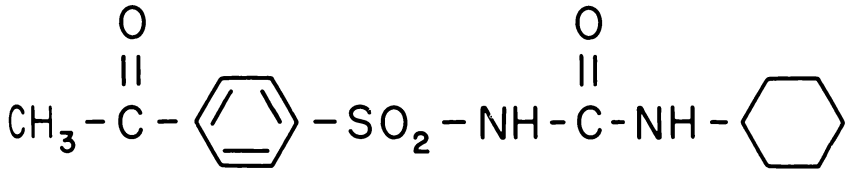

Figure 1. The drug Dymelor.

Statistical analysis.—Statistical analysis of the data was made using a generalized least squares analysis of variance program (Hammond, 1971). The program calculates analysis of variance (AOV) by the least squares method for missing data. Tables 2 and 3 contain means and standard errors of the mean. The values presented in Table 1 are means and their standard deviations.

RESULTS

Blood glucose levels.—All whole blood glucose levels were well within the normal accepted range of the rat, which is 75 - 150 mg/100 ml blood. Values of the combined trial means shown are all non-significant, which could be expected due to the type of drug and the time of blood sample collection (Table 1).

Tissue glycogen levels.—In only one trial (Trial IV) was the muscle control group significantly higher than the treatment group ($P \leq .01$) whereas the fetal glycogen content was higher in the treatment groups in trials III and IV ($P \leq .01$) and VI ($P \leq .05$). These levels may mean more nutrients are being made available to the developing embryo. The general trend and significant difference in trial III ($P \leq .05$), concerning liver levels, may indicate an increased activity in glycogenolysis (Table 2).

Reproductive performance.—Differences in ovulation rates were non-significant, which would be expected since all animals ovulated prior to treatment implementation. The number of resorptions was significantly less in treatment animals ($P \leq .01$). Also, the number of viable embryos was significantly higher ($P \leq .01$) and the percentage embryonic mortality was significantly lower ($P \leq .05$) in the treatment animals (Table 3). These results indicate some decrease in early embryonic losses can be achieved by reducing the level of plasma sugar present in the maternal blood system during early gravid stages. The oral hypoglycemic compound may also have acted to expedite the availability of glycogen stores to the fetus as indicated by a trend of elevated glycogen levels in treated females compared with control females.

Table 1. Blood glucose levels (combined trials).

	N	Initial[1]	Final[1]
Control	82	109.6±7.3	107.4± 8.9
Treatment	81	114.1±8.4	110.6±13.6

[1]mg/100 ml

Table 2. Tissue glycogen levels.

	Muscle[1]		Fetal[1]		Liver[1]	
	Control	Treatment	Control	Treatment	Control	Treatment
Trial I	21.8± 7.3	19.8±10.7	3.3± 1.3	3.8±2.1	2.2± 2.2	2.5± 2.0
Trial II	8.9±12.5	9.1±10.7	6.8±7.6	9.5±6.4	9.7±11.1	10.4± 9.2
Trial III	4.8± 2.5	6.5± 3.2	2.9±1.5	12.6±7.8[2]	3.7± 1.7	14.0±11.4[3]
Trial IV	4.5± 2.2	2.4± 1.5[2]	7.6±5.3	8.6±2.3	4.3± 3.0	5.0± 4.4
Trial V	2.8± 1.6	2.5± 1.5	441±2.8	9.5±2.7[2]	3.7± 3.2	4.2± 3.1
Trial VI	3.1± 1.3	4.7± 2.7	4.8±1.9	7.8±1.6[3]	2.2± 1.5	4.9± 4.1

[1]ug/100mg
[2]$P \leq .01$
[3]$P \leq .05$

Table 3. Reproductive performance (combined trials).

	N	Ovulation rate	Resorption rate	Number of embryos	Embryonic mortality (%
Control	82	14.1±0.5	1.9±0.5	10.3±0.8	26.9
Treatment	81	14.2±0.2	1.3±0.4[1]	11.6±0.9[1]	8.8[1]

[1]P≤.01

Table 4. Percent embryonic mortality (%)

	Trial I	Trial II	Trial III	Trial IV	Trial V	Trial VI
Control	19.9	28.0	26.4	34.8	24.5	28.2
N	15	15	15	15	15	7
Treatment	28.0	20.1	16.1	17.1[1]	16.4	14.8[1]
N	15	13	15	15	15	8

[1]P≤.01

Embryonic mortality.—With the exception of trial I the percentage embryonic mortality was reduced in all treatment groups, being highly significant (P≤.01) in trials IV and VI (Table 4). Even though the treatment embryonic mortality was significantly lower than the controls, actual effects of the drugs may be greater when applied to the normal population because the control embryonic mortality (26.9%) was in the low range in relation to that which occurs normally (25-50%).

DISCUSSION

The results of this series of experiments indicate that maternal nutrition in the very early stages of gravidity plays a very important role in the amount of embryonic wastage that occurs in litter-bearing mammals. Blood glucose levels were not significantly altered, which is to be expected because of time of sampling. Tissue glycogen sampling indicated an increase in fetal glycogen levels due to treatment. Resorption rate was significantly reduced when the groups were combined. These results were further substantiated by the increase in number of viable embryos. When the data for all trials were combined, percentage embryonic mortality was observed to be significantly reduced in the treated group. These results followed the same pattern as work reported by Battaglia and Meachem (1969:5) who used chlorpropamide. The cellular mechanisms by which these hypoglycemic compounds produce their effects are not known. It is tentatively concluded that these compounds improve prenatal survival by causing the transfer of nutrients needed by the embryo from the maternal system to the embryonic systems.

SUMMARY

The addition of an oral hypoglycemic agent (Dymelor) to the diet of pregnant rats apparently reduced embryonic mortality. Possibly this reduction was due to the ability of the drug to make available maternal stores of nutrients to the developing embryo indicated by increased glycogen levels in the fetal and liver tissues of the treatment animals. It would appear that no further benefits would be derived from any higher drug level or extended length of feeding than 5 mg Dymelor/day/animal for six days.

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PRIMARY PRODUCTION IN CULTURALLY-ENRICHED LAKE SALLIE, MINNESOTA FOLLOWING WEED HARVEST

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ABSTRACT

Phytoplankton photosynthesis and respiration in Lake Sallie, Minnesota increased from 1972 to 1973, both being greatest in August. Maximum gross primary production was 780 mg/m³/h in 1973. Considerable daily and seasonal variation in production was found at all depths. Photosynthesis was often inhibited by high light intensities. Maximum production moved to or near the surface and greatest efficiency occurred (maximum, 34.98 mg C/m³/h/ly/h) when bloom conditions developed and incident radiation declined. Possible effects of aquatic macrophyte removal on phytoplankton production are discussed, as are relations of production maximum to phosphorus concentrations.

INTRODUCTION

Eutrophication.—Man's addition of nutrients to a body of water is termed cultural eutrophication, which often results in increased primary production and noxious blue-green algal blooms. The effects of sewage nutrients on lake enrichcation after nutrient income reduction have been described (Hasler, 1947, 1969).

Study area.—Detroit Lakes, Minnesota, has for more than 60 years contributed sewage nutrients to Lake Sallie (Figure 1) (Larson, 1961). Biofiltration, aeration, retention in a stabilization pond, and flow through a peat bed follow primary treatment of wastes. These methods have proved insufficient and an average of approximately 1000 kg of phosphorus enters the lake each month (Smith, 1972).

Lake Sallie forms part of the Pelican River Watershed in Becker County, Minnesota (95° 54′23″-95° 54′00″W, 45° 54′00″-47° 26′00″N); the Pelican River is contributory to the Ottertail River. The river's watershed area lies between eastern boreal forest and western plains, and is in gravel outwash plains, bounded by sandy moraine to the east and clayey moraine to the west (Allisn, 1932). Lakes in the watershed are kettle holes, formed when large ice blocks were left behind as glaciers retreated, melted and created shallow depressions (Zumberge, 1952).

MATERIALS AND METHODS

Sampling.—A station located east of the hilus in kidney-shaped Lake Sallie was the site of production measurements and was at the lake's maximum depth of 16 m. Production measurements were made weekly during the open water season in 1972 and 1973; light penetration studies were made in 1972.

Physical features.—Light penetration was determined by use of a submarine relative irradiance meter with green, blue, and red Schoot glass filters to separate

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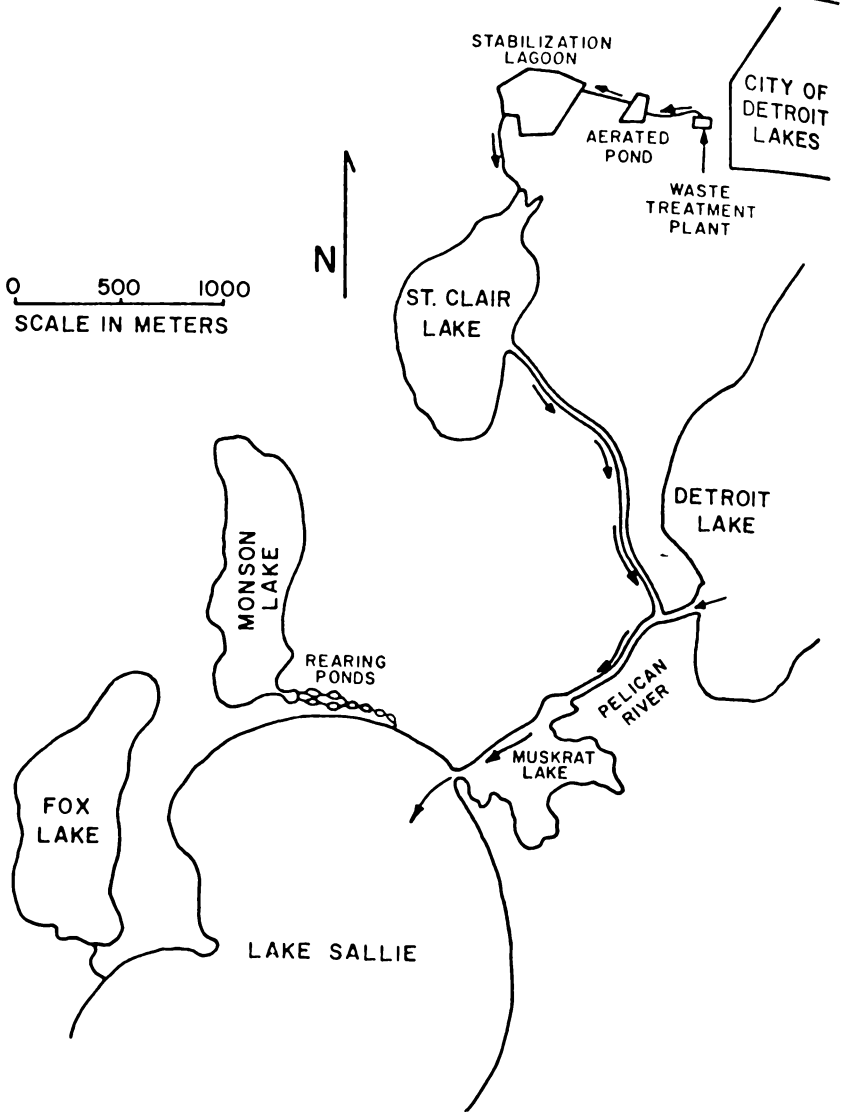

Figure 1. Flow route of wastewater effluent from Detroit Lakes to Lake Sallie.

spectral bands. Incident radiation was monitored by pyrheliometer in order to calculate the efficiency of light energy utilization.

Chemical features.—Analyses were according to Standard Methods

(American Public Health Association, 1965, 1971), except nitrate nitrogen, which was by the cadmium-mercury reduction column method (Strickland and Parsons, 1972), and total phosphorus, which followed persulfate digestion (Krawczyk, 1969).

Biological features.—The light and dark bottle oxygen method (Gaarder and Gran, 1927) was used for measurement of primary production. Samples were taken from 0, 0.5, 1.0, 2.0, and 5.0-m depths with an opaque, polyvinyl chloride Kemmerer sampler. Duplicate light and dark bottles were returned to the sampled depth and another bottle was filled to determine initial oxygen concentration. Two-hour incubation periods were adopted as standard. Calculation of gross and net production and respiraton, and conversion to mg C fixed/m^3/hr was by the following equations, using a photosynthetic quotient of 1.25 (Rhyther, 1956):

Gross primary production = 0_2 increase + respiration loss

Net production = 0_2 increase in light bottle

Respiration = 0_2 loss in dark bottle

$$\text{mg C m}^3\text{/hr} = \frac{f(\Delta 0_2)}{N \times 1.25} \times 0.375$$

$f = Na_2S_2O_3$ correction factor

N = number of incubation hours

$\Delta 0_2$ = change in oxygen concentration

$$0.375 = \frac{\text{atomic weight of C}}{\text{molecular weight of } 0_2}$$

RESULTS AND DISCUSSION

Light penetration.—Light entering water is reduced by absorption and scattering due to particulate matter and absorption by dissolved materials and phytoplankton (Hutchinson, 1957). Light penetration decreased as algae became more concentrated in 1972. Algal accumulations at the surface increased absorption and scattering. Pearsall and Ulloyott (1934), and recently Smith (1972) for Lake Sallie, found minimal penetration during periods of blue-green algal abundance. Except for variations occurring in the upper 1 m, due to absorption or the "immersion effect" (Westlake, 1965), transmittance decline was logarithmic with depth (Figure 2).

Transmittance of red light was greater than green during June, but less in July and August, when its penetrance was reduced by heavy blooms of blue-green algae. Vollenweider (1961) found that eutrophic lakes in general absorb strongly in the blue region and transmit longer wavelengths. Since chlorophyll "a" is most efficient in red and blue wavelengths (Round, 1965), a decline in transmission of those rays would be significant with respect to the Chlorophyta and less so to the Cyanophyta, whose phycobilins are capable of transferring energy to the chlorophyll molecule (Emerson and Rabinowitch, 1960).

Primary production.—In order to compare phytoplankton production following weed harvest to prior conditions, the procedures of Smith (1972) were

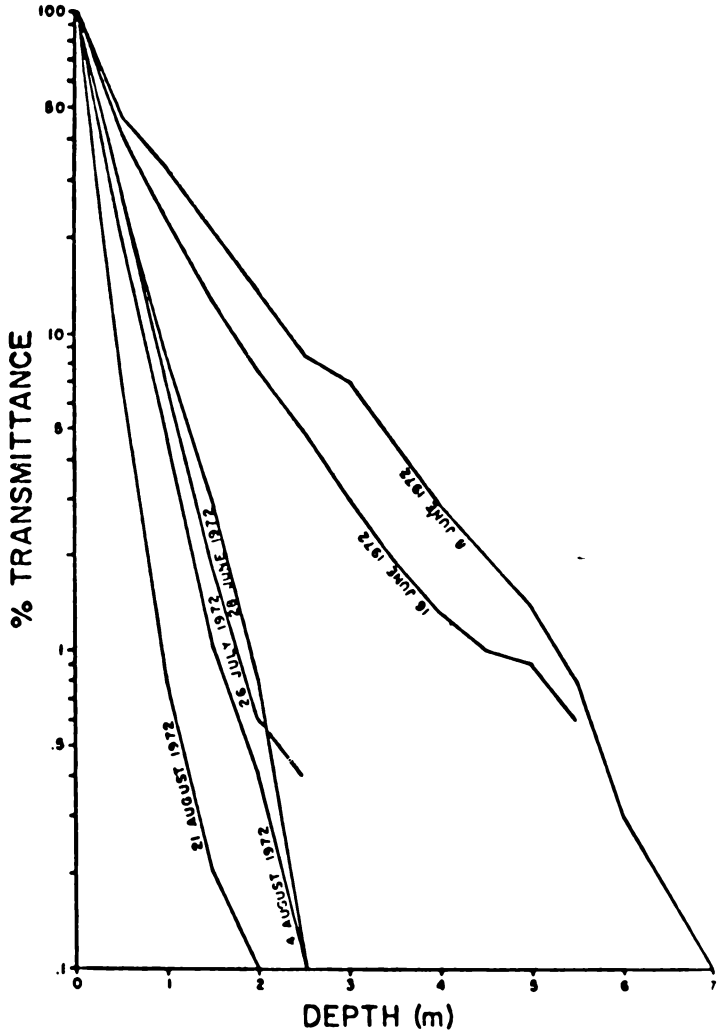

Figure 2. Transmittance of total light in Lake Sallie on selected dates in 1972.

continued. Before weed removal (1969) dense growths of rooted aquatic plants were found to a depth of 3 m and *Potamogeton praelongus* Wulf existed down to 6 m. At this time, vascular plants covered 34% of the total bottom area (Peterson, 1971). Severe reduction of weed populations followed harvesting and net productivity and respiration increased (Smith, 1972), seeming to confirm the antagonism between algae and aquatic plants reported by Hasler and Jones (1949)

and Fitzgerald (1969). It is supposed that the relationship is one of direct competition or the result of an antibiotic effect, the latter presumably from among the large amounts of organic compounds secreted by aquatic angiosperms (Wetzel and Manny, 1972).

Considerable variation in algal production on a daily and seasonal basis resulted from differing spatial distribution and species of algae present, cloud cover, and angles of light incidence. Depression of production from 1100 - 1300 h was common on clear days in both 1972 and 1973 but not on cloudy days when increases often occurred. Steeman-Nielsen (1962) suggested that high light intensities might inactivate the photosynthetic mechanism by photo-oxidation and cause production declines. The depression of photosynthesis is most often limited to only the upper 1 m, because as light is attenuated with depth, it becomes undersaturated and will not result in photoinhibition.

During early stages of thermal stratification, production was low and most intense at some distance below the lake surface. Maximum production occurred nearer the lake surface with the development of surface blooms, which reduced light penetration to lower levels. This resulted in a seasonal vertical movement of the zone of maximum production.

From 1972 to 1973 gross production increased, as did respiration, both being greatest in August (Figure 3). The greatest primary production increase was found at 0.5 m (Figure 4), with little change occurring at lower depths. Smith (1972) observed net production increases and respiration decreases from 1969 - 1971, unaccompanied by zooplankton decline. Net production continued to increase in 1972 and 1973, but respiration did as well, reversing the trend observed by Smith. This indicates a decreasing efficiency of phytoplankton in macronutrient utilization (Figures 5 and 6).

Photosynthetic efficiency increased following June lows in 1972 (Table 1). Efficiency was high in June 1973, and more consistent, albeit lower, in July and August. Declining solar radiation occurs in August, but production efficiency increased during that month in 1972 and 1967, and in 1969 - 1971 (Smith, 1972). Peak abundance of surface blue-green algae at this time probably explains the apparent paradox.

Daily production maxima showed high positive correlation with total phosphorus concentrations ($r = +0.942$ for both years combined; for 1972, $r = 0.506$ and in 1973, $r = 0.947$). Megard (1972) found a linear relationship between phosphorus and production maxima in summer, but not in spring and fall. A regression analysis of the data results in the equation $y = 40.065 + 0.746X$. Phosphorus in Lake Sallie is sufficient to maintain production at high levels, while light and nitrogen may at times be low. Ammonia may be depleted and nitrate may reach lows of 2 $\mu g/l$ in surface waters during summer. At this time, rainfall contributions of nitrate and ammonia, along with stratification blowouts (which occur two to three times each summer) and resultant ammonia circulation, may be significant to continued production.

In 1972, 59,486.81 kg wet mass of weeds were harvested, which contained 14.99 kg of P and 11.75 N (Miekicki, 1973). Reduction of aquatic plants

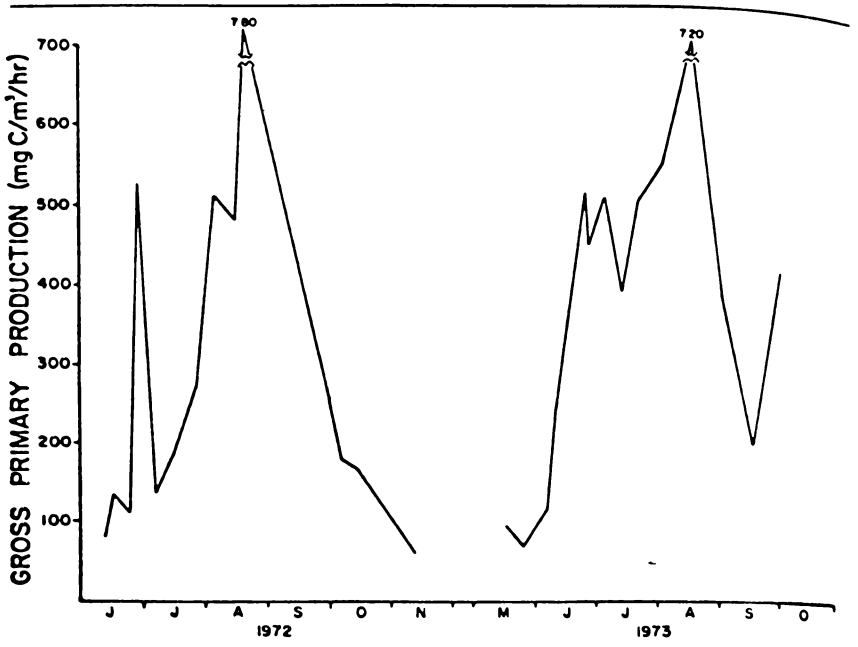

Figure 3. Maximum surface water gross primary production in 1972 and 1973.

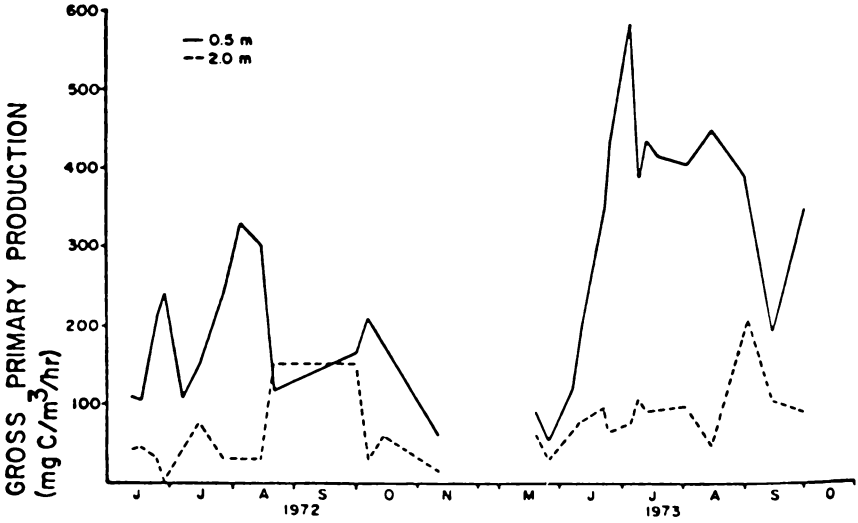

Figure 4. Maximum gross primary production at 0.5 and 2.0 m in 1972 and 1973.

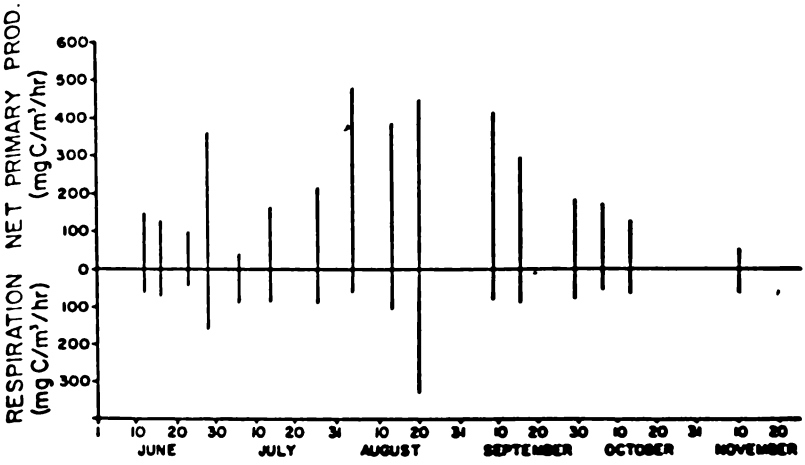

Figure 5. Maximum net primary production and respiration rates of phytoplankton in surface waters, 1972.

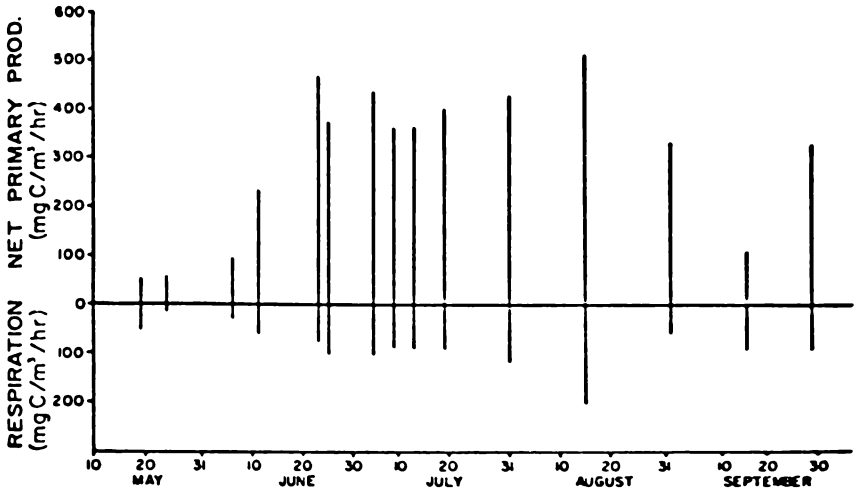

Figure 6. Maximum net primary production and respiration rates of phytoplankton in surface waters, 1973.

Table 1. Efficiency of gross primary production in Lake Sallie surface waters (mg C/m³/hr/ly/hr).

Date	0900-1100 h	1100-1300 h	1300-1500 h
12 June 1972	2.45	0.45	0.96
16 June	6.38	2.87	2.39
23 June	2.23	2.01	1.76
28 June	9.78	____	____
14 July	5.08	3.48	8.87
26 July	18.44	16.30	12.10
4 August	13.25	7.65	7.69
14 August	14.35	10.76	11.69
21 August	14.81	34.98	28.22
30 September	____	6.62	4.23
7 October	5.92	4.35	____
14 October	____	4.29	3.21
11 November	24.00	14.63	____
19 May 1973	2.55	1.79	____
24 May	6.83	2.38	____
6 June	5.11	1.78	2.66
11 June	9.16	9.75	10.76
23 June	7.29	10.02	12.71
25 June	14.26	11.69	5.43
4 July	____	7.79	12.56
8 July	10.25	10.09	7.79
12 July	____	7.65	6.87
15 August	____	____	15.42
1 September	____	24.07	____
15 September	14.00	12.41	____
29 September	20.57	11.67	10.69

served to decrease the direct completion for available nutrients of the plants with phytoplankton and also decreased any antibiotic effects they may have (Fitzgerald, 1969). Attempts to harvest in late August 1973 met with minimal sucess, since there had been little regrowth of weeds following three years of harvest. The unchecked growth of remaining plants may have been sufficient to explain, in part, the decline in net phytoplankton primary production in 1973. But their greatest influence may have been in serving as a substrate for the luxuriants growths of the green alga, *Rhizoclonium* sp., which in turn, competed for nutrients. It is expected that future increases in rooted aquatic biomass will lead to a diminution of blue and green algal blooms.

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USE OF FLUORESCENT ANTIBODY FOR THE RAPID DETECTION OF *SALMONELLA* IN WATER

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ABSTRACT

The fluorescent antibody (FA) technique was used as a means of detecting *Salmonella* in water. Water samples were passed through a membrane filter (pore size 0.45 μm) to concentrate the organisms. The entire filter and accompanying organisms were immersed in 15 ml of Selenite Cystine Broth (SCB) (Baltimore Biological Laboratory), and incubated at 43 C. After 12 h incubation, the membrane filter was removed and the SCB was passed through another membrane filter and washed with 50 ml sterile phosphate buffer (pH 7.2). The filter with enriched bacteria was re-enriched in SCB and incubated at 43 C for 8 h. Smears and touch slides were then made from the broth and filter and the direct FA procedure was applied with Bacto-FA *Salmonella* Poly (Difco, Detroit, Michigan). Slides were examined by use of an AO Fluorolume microscope. The presence of any rod shaped, fluorescing bacteria was designated as a positive result. Biochemical and serological confirmation followed. The FA technique is as sensitive as the cultural technique in detecting *Salmonella* in freshwater and is much faster.

INTRODUCTION

Up to the present, the detection of *Salmonella* from freshwater has been dependent on time-consuming biochemical analysis followed by serological typing. The value of this conventional method may be questioned on the basis of both time lost and sensitivity. Although there is a definite need for a rapid and reliable diagnostic method for *Salmonella* in water, there is no report on the use of fluorescent antibody (FA) as a method to help alleviate this particular problem. The application of the FA method has been emphasized by Abshire and Guthrie (1973:201) and Lieber and Martin (1965:99) in the rapid identification of other microorganisms in water. However, the rapid determination of *Salmonella* by use of FA is put to extensive use only by the food industry (Insalata, Schulte, Berman, 1967:1145), whereas the use of the FA reaction for screening water samples has yet to reach its full potential.

This study was designed to compare the FA technique for the detection of *Salmonella* in water with the conventional cultural method and to evaluate the

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potential of the FA method as a means to rapidly monitor freshwater sources of interest to public health authorities.

MATERIALS AND METHODS

Samples.—Water samples were collected at 10 sites from a North Dakota river draining a large agricultural area. Cultures of *Salmonella typhimurium* and *Escherichia coli* were obtained from Carolina Biological Company, Burlington, North Carolina, and grown in Trypticase Soy Broth (Baltimore Biological Laboratory) at 37 C for 24 h. After incubation, 0.5 ml of both cultures were inoculated in 100 ml sterile phosphate buffer for concomitant evaluation. Stained Bacto-FA slides (Difco, Detroit, Michigan) (Thomason et al., 1971:876) were used as controls for proper microscopic setup and alignment and as controls for the technique and reagents. Fluorescent antibody used throughout this work was Bacto-FA Salmonella Poly (Difco).

Membrane filter: selective enrichment.—Samples (100 ml) of water were passed through a membrane filter (Gelman, Curtin Scientific Co., Minneapolis, Minnesota) with a pore size of 0.45 μm to concentrate the organisms. The entire membrane filter was immersed in 15 ml of Selenite Cystine Broth (SCB) (BBL) and incubated at 43 C for 12 h without agitation.

Membrane filter: selective re-enrichment.—After incubation, the original membrane filter was discarded and the SCB containing the selected organisms was passed through another membrane filter. After the filter holder was washed with 50 ml of phosphate buffer (pH 7.2), this membrane filter was subsequently placed in another 15 ml of SCB and incubated at 43 C for 8 h.

Cultures.—Selective re-enrichments of SCB were streaked on XLD Agar (Difco) (Taylor and Schelhart, 1973:941) and Hektoen Agar (HE) (Difco) (King and Metzger, 1968:580). The XLD and HE plates were incubated at 37 C for 24 h. Typical colonies were picked to Kliglers Iron Agar (Difco) and Lysine Iron Agar (Difco), and those cultures exhibiting typical *Salmonella* reactions were confirmed serologically. Both cultural and FA procedures are listed in Figure 1.

FA staining procedure.—A loopful of re-enriched SCB was placed on flourescent antibody slides (Clay Adams, Parsippany, New Jersey), and air dried. The remaining SCB was passed through a membrane filter and touch slides were made of the filter surface and also air dried. The samples were then fixed by immersing the slides in Kirkpatrick Fixative (Difco) for 3 min and then immersed in ethanol for 1 min. The slides were air dried and stained with one loopful of Bacto-FA Salmonella Poly antisera. To prevent drying, the slides were placed in a humidified petri plate prepared by placing a filter paper moistened with distilled water in the plate cover. After incubating for 30 min at 37 C, excess serum was washed off by placing the slides in a Coplin staining jar containing phosphate buffer (pH 7.8) for 10 min. Upon removal from the buffer, the slides were rinsed in distilled water to remove residual salt. Slides were mounted under a coverslip and buffered Bacto-FA Mounting Fluid (Difco) and examined under darkfield and ultraviolet illumination. A positive slide was recorded when one or more strongly fluorescing bacilli were observed.

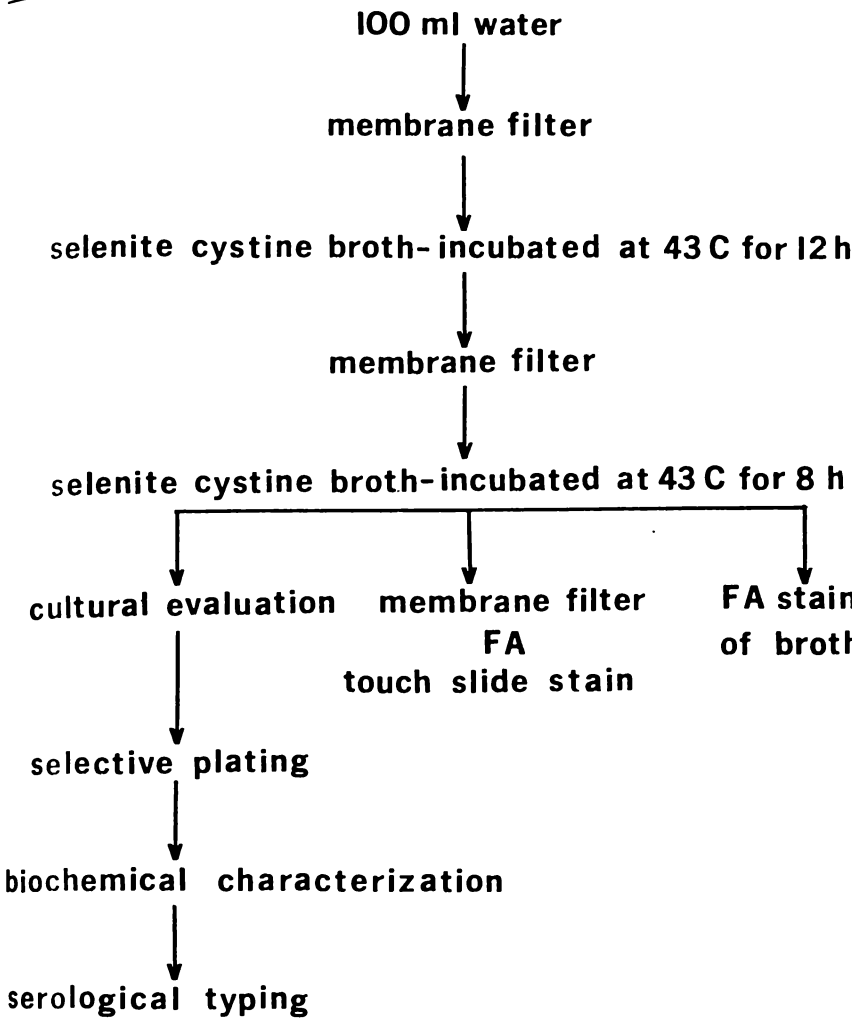

Figure 1. Flow chart of cultural and FA procedures for detecting *Salmonella* in water.

RESULTS AND DISCUSSION

Of the two *Salmonella* detection procedures for 10 river sampling sites, the cultural procedure detected four *Salmonella*-positive sites and five *Salmonella*-positive sites were detected by the FA procedure. This agrees with results

published by Goepfert and Insalata (1969:465), Harrington et al. (1970:1898), and Thomason (1971:1064), all of whom found the FA procedure to be at least as sensitive as cultural methods. These investigators also reported that one of the problems of the FA is the tendency to indicate the presence of *Salmonella* in samples that are culturally negative. In our study, the remaining sites proved to be both culturally and FA negative.

Upon examination of the known cultures using the two methods, only *Salmonella* was recovered, since the toxicity of the SCB completely inhibited *Escherichia coli.* This inhibition takes place mainly due to the presence of lactose and selenite in the SCB. The lactose is utilized by *E. coli* that produces acid and lowers the pH, which, in turn, activates the selenite (that inhibits the growth of *E. coli*) and disposes of the organism. SCB was employed since Haglund et al. (1964:447) reported the quelching of fluorescence in the FA reaction by tetrathionate.

Small variations were observed between the FA positive slides and the commercially prepared slides that were used as controls. This was probably caused by the effect of the SCB enrichment on *Salmonella*. Occasionally, yeast-like cells would pick up the conjugate and exhibit a characteristic apple-green stain, which, according to Fantasia (1969:708), was due to similar antigens in both yeast and *Salmonella*, specifically the somatic group H antigens.

Even though the cultural method as an indication for the presence or absence of an organism in a given sample is widely accepted, it should be recognized that this method is not the most efficient nor the most sensitive. This study has shown the FA method to be at least as sensitive as the cultural method in detecting *Salmonella* in freshwater; therefore, recovery of FA positive samples when cultural ones are negative does not mean that the FA procedure gives false positives but rather, the FA procedure should be accepted as correct.

The FA method for *Salmonella* monitoring shows potential. Diagnosing a body of water for hazardous pathogens need no longer take up to 7 days, but could be done in 48 h. The rapidity of this method would be of great significance for the public health profession, since questionable water could either be cleared or evaluated further before a dangerous situation arose.

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