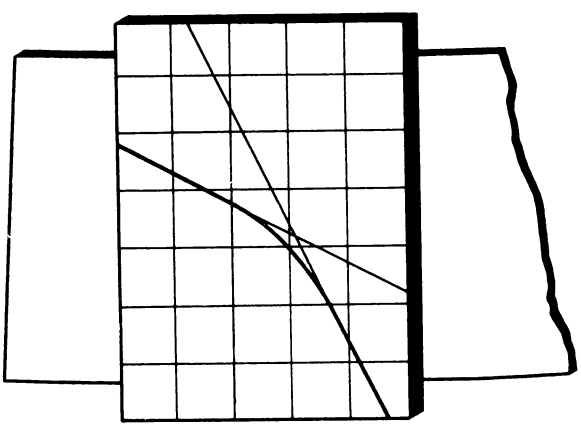
PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

ABSTRACTS



63RD ANNUAL MEETING

APRIL 30 — MAY 1, 1971

North Dakota State University
Fargo, North Dakota

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NORTH DAKOTA ACADEMY OF SCIENCE

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DIABETES-INDUCED ALTERATIONS IN THE RESPONSE OF PHOSPHOENOLPYRUVATE CARBOXYKINASE(PEPCK) TO TRYPTOPHAN AND ITS METABOLITES. F.L. Alvares and P.D. Ray. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

L-tryptophan(try) and various of its metabolites enroute to nicotinic acid have been found to inhibit gluconeogenesis in normal intact rats or perfused normal livers. The responsible metabolite, quinolinic acid(QA), inhibits conversion of oxalacetate to PEP even though it increases, paradoxically, the assayable specific activity of PEPCK. We now find that neither try nor 3-hydroxyanthranilic acid(3-HHA) significantly alters gluconeogenesis or the specific activity of PEPCK in intact alloxan diabetic rats. On the other hand, we find that try, 3-HHA and QA are apparently capable of inhibiting gluconeogenesis in the perfused diabetic liver although these compounds still exert relatively little effect on the specific activity of PEPCK. The inability of try or its metabolites to inhibit gluconeogenesis in the intact diabetic rat suggests the possible absence, in diabetes, of a mechanism important for normal regulation of glucose synthesis. Furthermore, the inability of try or its metabolites to affect the specific activity of PEPCK suggests that a factor normally capable of affecting the activity of the enzyme in the presence of QA, or perhaps even the enzyme itself, is altered in diabetes. Support by NIH AM 12705 and American Diabetes Association.

THE INFLUENCE OF METHALLIBURE AND ITS METABOLITES ON THYROID FUNCTION IN SWINE. T. J. Archbold, P. W. Aschbacher, and J. E. Tilton. Dept. Animal Science, NDSU, and USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D. 58102

Oral administration of methallibure (1-α-methylallylthiocarbamoyl-2-methylthiocarbamoylhydrazine) inhibits estrus and ovulation in swine. It has, however, been suggested that methallibure may have an inhibitory effect on normal thyroid function in rats. We report here on the effects of methallibure and two of its metabolites [metabolite A: 2,methylamino-5(methylallylamino) -1,3,4 thiadiazole, and metabolite B: 2,5,diamino-1,3,4 thiadiazole] on thyroid I^{125} uptake in gilts. Twelve crossbred gilts weighing from 73 to 88 kg were treated for 20 days with 2 mg/kg methallibure, 5 mg/kg metabolite A, 5 mg/kg metabolite B, or basal ration alone. Fifty-two hr. before sacrifice, the gilts were dosed with approximately 90 mCi I^{125} in a gelatin capsule. At autopsy, thyroids and reproductive tracts were removed and weighed. Gilts treated with methallibure and metabolite B showed significant decreases ($P<.05$) in I^{125} uptake by the thyroid when compared with controls. Uterine weight was significantly reduced ($P<.05$) in the methallibure group. No significant differences were observed in thyroid weight, ovarian weight, and I^{125} content in the blood among treatment groups.

GEOLOGY AND PALEONTOLOGY OF THE MOSBECK SITE, PENNINGTON COUNTY, MINNESOTA: Allan Ashworth, William B. Bickley, Jr., Lee Clayton, and G. H. Groenewald. Dept. of Geol. NDSU, Fargo, and Dept. of Geol., UND, Grand Forks, N. Dak.

The Mosbeck Site is a drainage ditch 0.25 mi east of the northwest corner of the SW¼ sec. 9, T. 152 N, R. 45 W, 2.0 mi west of the Campbell Scarp. The stratigraphic sequence is from bottom to top: (1) pebbly loam, (2) 2 in. of gravel, (3) 16 in. of fine sand, (4) 5 in. of peat, and (5) 54 in. of well-sorted, flatbedded, gravelly sand. The lower few inches of unit 5 is unoxidized and contains black spruce and tamarack driftwood that has been radiocarbon dated 9940±160 BP (I-3880). At least ten species of mollusks occur in units 3 and 4 and in the base of unit 5. Spruce pollen is abundant in unit 4. Abundant beetles (53 genera) occur in unit 4.

Unit 1 is late Wisconsinan glacial sediment. Unit 2 is a lag concentrate formed by wave action in Lake Agassiz I. Unit 3 is Lake Agassiz I shoreline sediment. Unit 4 is a rich swamp sediment deposited during the Lake Agassiz I/II interval. The Lake Agassiz plain at this time was covered by spruce forest, similar to that in east-central North America today. Unit 5 is shoreline sediment deposited as Lake Agassiz II rose, drowning the forest.

HALOGEN - OXIDATION OF URIC ACID; EFFECTS OF TEMPERATURE AND pH. La Quoc Bao and Franz H. Rathmann. (Dept. of Chemistry, No.Dak.State Univ., Fargo, No. Dakota.

The kinetics of the first step of the iodine-oxidation of uric acid, as described previously by Boerth, Benesh and Rathmann, for pH from 5 to 8 at 30° C(No.Dak.Acad.Sci., May 2, 1970) was studied at temperatures from 5°C to 35°C. The reaction presumably yields first the Behrend hydroxy-acetylene-diureidocarboxylic acid, as obtained in the H_2O_2 and $KMnO_4$ oxidations, which rearranges to uroxanic acid, or loses CO_2 and rearranges to allantoin (Bamberger, Helv. Chim.Acta 37, 643, 2216(1954). The consumption of iodine is 1.10±0.08 moles per mole of uric acid. The apparent rate constant, as calculated on the basis of a second order reaction, $-d(U)/dt = k \cdot (I_2) \cdot (U)$, where I_2 is the total available iodine and U the total uric acid, increases with the pH of the solution such that, $\log k = k'(pH) - b$, where $k' = 1.80$ and $b = 8.6$ for k in liter moles^{-1} sec^{-1}. The temperature coefficient per 10° C is 4.3 at pH=6, 5.1 at pH =7, and 6.6 at pH = 8. Reacting species appear to be urate ion and HIO.

PALEOCENE FRESHWATER MOLLUSCA FROM SOUTHERN WARD COUNTY, NORTH DAKOTA. David Bickel and Donald D. Hall. Dept. of Physical Sci., Minot State Coll., Minot, N. Dak.

During January, 1971, Mr. Fred Ballentyne of Sawyer, N. D. led the authors to an 18.5 ft. section of moderately consolidated and fossiliferous calcareous claystone, siltstone, and limestone in the Tongue River Fm. (Fort Union Group: Paleocene). The site is about 8 mi. S. of Sawyer, N. D. at an elevation of about 1800 ft. A preliminary list of the Mollusca includes freshwater mussels (Unionidae), Pisidium spp., Bicorbula mactriformis, Hydrobia spp., Campeloma nebrascensis, Lioplacodes limnaeformis, L. nebrascensis forms, Viviparus trochiformis, and V. retusus. The geographic location and presence of several forms of Pisidium and Hydrobia make the faunule especially interesting. Complete systematic study will probably reveal an assemblage of 13 to 15 molluscan species. Preservation is generally good and mussel specimens often have both values joined at the hinge. The sediments apparently accumulated in a small hard-water lake.

SEDIMENTATION IN SMALL PONDS IN THE MID-CONTINENT AREA DURING LATE QUATERNARY TIME: Wm. B. Bickley, Jr., and Lee Clayton, Dept. of Geology, UND, Grand Forks, N. Dak.

Five similar sequences of postglacial pond sediment have been studied or observed in areas of Saskatchewan, North Dakota, South Dakota, and Minnesota. The stratigraphy at all sites from bottom to top is: 1) pebbly, gray silt; 2) gray silt; 3) brown or green, organic silt; 4) calcareous mud; 5) dark-brown, sandy, clayey silt; 6) brown, silty sand; and 7) dark-brown, sandy, clayey silt.

This stratigraphy is interpreted to be the result of (1 and 2) rapid hillslope erosion immediately after deglaciation (late Wisconsinan); (3) increased hillslope stability with the area covered by a spruce-poplar woodland (late Wisconsinan-early Holocene); (4 and 5) continued hillslope stability with the area covered by grass sod (early Holocene); (6) decreased hillslope stability with the climate drier and warmer (middle Holocene); (7) increased hillslope stability with the modern prairie environment.

CORTISOL EXCRETION IN GUINEA PIGS EXPOSED TO HIGH PRESSURES (He-O_2). R.A. Bitter and T.W. Nielsen. Dept. of Physiology & Pharmacology, UND Sch. of Med. Grand Forks, North Dakota.

Cortisol is the principle adrenal cortical hormone in the guinea pig and is excreted in the urine in an unconjugated form. Increased excretion is used to show increased stress responses. Male guinea pigs (350-450 gm) were exposed in a high pressure chamber to He-O_2 (80-20% at 1 ATA), while control urine samples were collected for 24 hours. Animals were then exposed to He-O_2 mixtures at 10, 20, or 30 ATA for 14 hours and were then stage decompressed. The partial pressure of O_2 was kept between 150-275 mm Hg during experimental periods. Food and water were available ad lib. and chamber temperature was maintained within the He-O_2 comfort zone (29-30° C, 1 ATA). Cortisol was determined by the method of Mattingly (1964). Basal urinary cortisol values in room air were 87.3 (S.D.) $\pm$ 23.63 ug/day. Cortisol values obtained at 1 ATA He-O_2 were slightly less than room air controls while average cortisol excretion at 10, 20, and 30 ATA increased 23, 71, and 95%, respectively. Urine volumes did not increase significantly with pressure.

Supported in part by ONR Contract No. N00014-68-A-0499.

THE EFFICACY OF A BACTERIN IN PROTECTING CHINCHILLAS AGAINST ORAL CHALLENGES OF LISTERIA MONOCYTOGENES. F. M. Bolin, P. K. McIlwain and M. F. Andrews. Dept. of Veterinary Science N. Dak. State Univ., Fargo, N. Dak.

One hundred twenty-six (126) apparently normal chinchillas were used to study the efficacy of a bacterin in protecting against oral challenges of Listeria monocytogenes. The animals were divided into lots of 10 and the route of bacterin administration, number of inoculations, and environment varied. The time to death was longer for the animals receiving the bacterin than control animals and the gross pathology less significant. However, regardless of route of administration and vaccination regimen, the bacterin did not protect a sufficient number of chinchillas to be licensed under present federal regulations.

Studies on the Brain and Behavior of the Rat in a Hyperbaric Environment. J. A. Cromer, S. J. Brumleve, J. Carman, and E. S. Halas. University of North Dakota, Grand Forks, North Dakota.

Electroencephalograms (EEG) were recorded from chronically implanted electrodes in albino rats before, during and after compression and decompression, in order to determine the effect of high pressure (13 ATA) helium-oxygen or nitrogen-oxygen mixtures on the central nervous system. A comparison of EEG recordings suggest a state of light anesthesia in He-O_2 at 13 atmospheres.

The hypothesis was confirmed by a behavioral study using the conditioned anxiety learning paradigm. The conditioned anxiety was obtained by repeated presentation of a warning (a light) of an unavoidable electric shock which modified a stable ongoing operant performance (lever pressing) maintained by food reinforcement. The EEG changes and relationships to the behavioral pattern will be discussed.

Supported by ONR Grant No. N00014-68-A-0499, and PHS 1-SO1FR-5407-04.

PRESENT AND PAST MOLLUSKS OF THE FOREST RIVER, NORTH DAKOTA. *A. M. Cvancara, J. M. Erickson,* and *J. D. Delimata*. Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58201.

The Forest River in northeastern North Dakota was studied for living and fossil mollusks primarily during the summers of 1965 and 1966; additional data were gathered occasionally during 1967-1970. Twenty-two stations were sampled for living mollusks and fossil mollusks were collected at cutbank exposures at four sites. Twenty-one species of mollusks were found living in the river: 6 unionid bivalves (mussels), 5 pisidiid bivalves (fingernail clams) and 10 gastropods. Most of the mollusks occurred in the middle section of the river between Fordville and Minto. Unionids were found farthest downstream (few miles downstream from Minto), followed by pisidiids and gastropods. High chloride content (up to 1230 ppm) in the lower reaches of the river is perhaps the primary ecologic factor inhibiting the occurrence of mollusks. High turbidity, also in the lower reaches, may also be a limiting factor. Fossil assemblages indicate that the fossil fauna was very similar to that of the present, lacking in only five species or less and possessing no different forms. This suggests a similar regimen for the Forest River since the oldest sampled mollusks of that river lived in late (?) Holocene time. Supported by Univ. N. Dak. Faculty Res. Grant 4422-78 and N. Dak. WRRI (funds from U. S. Dept. Interior).

PALEOCENE FRESH-WATER FAUNA FROM SOUTHWESTERN NORTH DAKOTA. J. J. Delimata. Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dakota. 58201.

Nine molluscan species are known to occur in the Tongue River and Sentinel Butte sediments within parts of Billings, Golden Valley, and Slope Counties. The faunal list includes: two unionids, Plesielliptio priscus (Meek & Hayden) and Rhabdotophorus senectus (White); one corbulid, Bicorbula mactriformis (Meek & Hayden); and six viviparids, Viviparus retusus (Meek & Hayden), Viviparus trochiformis (Meek & Hayden), Campeloma nebrascensis (Meek & Hayden), Lioplacodes limnaeformis (Meek & Hayden), Lioplacodes nebrascensis (Meek & Hayden), and Lioplacodes tenuicarinata (Meek & Hayden).

Four of these mollusks are confined to the Tongue River Formation: R. senectus, B. mactriformis, L. limnaeformis, and L. tenuicarinata. Preliminary work indicates that these species may be of value as stratigraphic indicators in differentiating between sediments of the two formations.

The molluscan assemblage is typically one of fresh water with the exception of B. mactriformis. Modern corbulids are known to frequent brackish environments and the occurrence of this species may suggest minor brackish incursions at the time Tongue River sediments were deposited. Supported by N.D.G.S.

KARYOTYPES FOR TWO SPECIES OF NORTH DAKOTA CYPRINIDS. G. W. Dewald. Biol. Dept., Univ. of N. D., Grand Forks, N. D.

Karyotypes were established for Rhinichthyes atratulus Hermann (blacknose dace) and R. cataractae Valenciennes (longnose dace) based on six specimens of the former and seven of the latter. Excellent metaphase spreads were obtained using gill tissue extracted from colchicine injected specimens. Gill filaments were treated with .1 M KCN, triple distilled water and acid - alcohol fixative. Slides were prepared by smearing an epithelial cell monolayer followed by staining with carbol fuchsin. A modal diploid number of 50 was established for each species. R. atratulus consisted of four median, 10 submedian, eight subtelocentric and three telocentric chromosomes. R. cataractae was comprised of seven median, 15 submedian, two subtelocentric and two telocentric chromosomes. Thus, it would appear that these two species can be distinguished based on the differences in their karyotypes. Although there have been no other reports on karyotypes in this genus, it is generally accepted that most cyprinids are characterized by 44 to 50 chromosomes and those with 88 or more are polyploid. Therefore, the two species in question appear to be diploid.

VOLATILE FATTY ACIDS IN THE RUMEN INGESTA AS AFFECTED BY METHOD AND TIME OF SAMPLING. F. D. Deitz, D. O. Erickson, R. D. Gunderson and C. N. Haugse. Dept. of Animal Sci., North Dakota State University, Fargo, N. Dak.

Five combinations of alfalfa and corn were fed to 6 cattle and 6 sheep (3 of each fistulated) to determine the effect of ration, sampling time post-feeding, method of sampling and species on volatile fatty acid (VFA) concentrations and ratios. Samples were obtained from all animals at 2, 4, 6 and 8 hours after feeding via a suction strainer and via the cannula of fistulated animals. Samples were prepared according to standard procedures and the VFA's were analyzed using an Aerograph 660 gas chromatograph. The acetic to propionic and the acetic to propionic plus butyric acid ratios decrease ($P < .01$) as the level of corn increased in the diet. The acetic to propionic acid ratios widen ($P < .01$) with time after feeding, while the acetic to propionic plus butyric acid ratios widen ($P < .05$) with time. Ratios and total acid concentrations were different ($P < .01$) between species. Total acids decreased with time of sampling post-feeding ($P < .01$). The samples obtained via the cannula had higher ($P < .01$) concentrations of acids. The pH of samples obtained via the fistula were more acid ($P < .01$) than those obtained via the suction strainer. The pH was more acid ($P < .01$) in samples obtained from the sheep.

6-PHOSPHOGLUCONATE DEHYDROGENASE FROM SHEEP LIVER: KINETICS OF INHIBITION BY NUCLEOTIDES. R.E. D'Orazio and J.E.D. Dyson, Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks N. Dak. 58201

We have determined that di- and trinucleotides are potent inhibitors of the catalytic activity of sheep liver 6-phosphogluconate dehydrogenase. Mononucleotides are also somewhat inhibitory, but to a considerably lesser extent. The average K_i for all the di- and trinucleotides tested is approximately 6×10^{-4} M, and all K_i values lie within the range 2×10^{-4} M to 2×10^{-3} M. Thus it appears that slight differences in structure render some nucleotides more efficient than others as inhibitors of the enzyme. The inhibition is somewhat unusual in that it is competitive with respect to binding of both 6-phosphogluconate and NADP to the enzyme. As we have evidence from cysteine modification studies that the nucleotides are binding at the active site, it appears that these compounds are sufficiently large to block binding of both substrates. Further evidence for binding of the nucleotides at the enzyme active site and actually at the 6-phosphogluconate binding site, comes from a study of the effect of pH on the K_m for 6-phosphogluconate, and on the K_i for nucleotides. Both K_m and K_i follow a very similar pH-profile over the pH range 5.5 to 8.8. (Supported by NSF grant No.GB 12629)

THE USE AND EFFECTS OF VARIOUS PROTECTIVE AGENTS FOR LOW TEMPERATURE PRESERVATION AND STORAGE OF INSECT CELLS. P. E. Eide, J. M. Caldwell and M. M. Dockter. Met. and Rad. Res. Lab., USDA, State University Station, Fargo, North Dakota 58102.

The ability to preserve cells and tissues in a living state for long periods at low temperatures is of proven value. There have been few reports of insect cells or tissues being preserved in this manner. We examined the effects that various rates of cooling and several cryoprotective agents have on (a) Grace's Antheria cell line and (b) dispersed embryonic cells from Musca domestica. We found that the best cryoprotective agent used for the cell line is a poor agent for embryonic cells; the best cryoprotection was given by a combination of several agents. A 2 to 3 hr acclimation of the cells to the medium and a slow cooling rate gave the best survival. The cells were cooled to -30°C and either stored at that temperature or placed in liquid nitrogen at -196°C. After storing for 24 hr, no difference was noted between these temperatures. However, after long-term storage, neither the cell nor the embryonic cells showed good survival at -30°C, and a 60-day storage of the cell line in liquid nitrogen did not appear to affect cell multiplication after thawing. Embryonic cells showed a very poor survival in liquid nitrogen, with survival decreasing with time in storage.

PHENOBARBITAL EFFECTS ON DIELDRIN METABOLITES IN RATS. K. A. Engebretson and K. L. Davison. Dept. of Zoology, NDSU, and USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D. 58102

Phenobarbital is known to be a potent inducer of drug-metabolizing enzymes in the liver. We wished to determine some effects of phenobarbital feeding on products of dieldrin metabolism. Rats were fed diets containing 2 ppm dieldrin spiked with dieldrin-^{14}C for 6 weeks to build dieldrin residues in their tissues. During the 7th week, they were given a dieldrin-free diet containing 0 or 40 mg phenobarbital per kg body weight. Feces collected during the 7th week were extracted with petroleum ether and methanol to recover the dieldrin metabolites. With rats fed the 0-mg level of phenobarbital, 26, 72, and 15% of the ^{14}C were recovered in the petroleum ether and methanol extracts, and residue, respectively, while with rats fed the 40-mg level, 12, 51, and 52% were recovered in these fractions. Column chromatography with LH-20 and G-10 gels revealed further differences in relative distribution of ^{14}C among dieldrin and metabolites. Data collected to date indicate a shift in metabolite distribution to the more hydrophilic metabolites.

THE EFFECT OF POST-FEEDING SAMPLING TIME ON SOME PROXIMATE FRACTIONS OF RUMEN INGESTA. D. O. Erickson, F. D. Deitz, R. D. Gunderson and C. N. Haugse. Dept. of Animal Sci., North Dakota State University, Fargo.

Rumen ingesta samples were collected 2, 4, 6 and 8 hours post-feeding from 3 sheep and 3 cattle that were fitted with permanent rumen fistulae. Fourteen experiments were run using 5 levels each of oats, corn and barley fed along with alfalfa. The rumen ingesta dry matter decreased ($P < .01$) from 17% to 15% from 2 to 8 hours post-feeding. The protein content varied ($P < .01$) among rations and was lower ($P < .01$) in cattle than sheep, 15.5% and 18.6% respectively. Acid detergent fiber and lignin remained relatively constant from 4 to 8 hours post-feeding. Rumen acid detergent lignin to feed ratios can be used to estimate digestion in the rumen providing the samples are taken 4 or more hours post-feeding. Rumen ingesta in sheep contained more phosphorus ($P < .01$) than in cattle. Rumen ingesta became less acid ($P < .01$) with time post-feeding. Most fractions analyzed, varied ($P < .01$) with the individual rations fed.

BIOTIN, BOD, PHOSPHORUS, ALGAE AND OTHER RELATIONSHIPS IN AN OXIDATION LAGOON. G. M. Fillipi and J. W. Vennes, Dept. of Microbiol., UND, Grand Forks, N. D.

A previous report to the Academy indicated that biotin production in an overloaded oxidation lagoon was due primarily to *Aerobacter aerogenes*. Utilization of the vitamin was carried out by *Chlorella* and other algae as well as several purple-sulfur species. A study of several additional oxidation lagoons receiving domestic and some potato processing wastes was carried out during the summer of 1970. It was determined that biotin utilization during the summer treatment period was usually consistent with algal increases. The removal of phosphorus was minimal during this same period and was not directly related to BOD removal. Additionally, no direct relationship between biotin and BOD removal was apparent. *It thus becomes apparent that determinations for specific molecular and microbial species must be performed for each type of oxidation lagoon before one can assess specific treatment effects.*

METABOLISM OF 2, 4-DICHLOROPHENOXYACETIC ACID IN RED CURRANT. J. R. Fleeker. Dept. of Biochem., NDSU, Fargo, N. Dak.

Dealkylation of 2, 4-dichlorophenoxyacetic acid (2, 4-D) was studied in red currant (Ribes sativum Syme), a plant resistant to this herbicide. A ^{14}C-labeled, water-soluble metabolite was found 24 hours after red currant shoots were hydroponically fed ring-labeled 2, 4-D-^{14}C and was tentatively identified as a glycoside of 2, 4-dichlorophenol. Six hrs after shoots were fed 2, 4-D-1-^{14}C, glycine and glycolic acid contained ^{14}C predominantly in their carboxyl carbons. When 2, 4-D-2-^{14}C was fed under the same conditions, the ^{14}C-label was predominantly in carbon-2 of glycine and glycolic acid. A small amount of ^{14}C was found in aspartic acid when 2, 4-D-1-^{14}C was fed, with 66% of the ^{14}C found in carbon-1 of the amino acid. The data suggest a mechanism of 2, 4-D inactivation in which the side-chain carbons are removed intact, possibly as glyoxylate or glycolate.

SILVER COMPLEXES AS ADSORBENTS IN GAS-SOLID CHROMATOGRAPHY. S. H. Givand and L. E. Cook. Dept. of Chem., Univ. N. Dak., Grand Forks, N. Dak. 58201.

The use of Ag^+ complexes for several heterocyclic amines as adsorbents for gas chromatography was investigated. The complexes were coated on a support and placed in copper columns, 8 ft. x 1/8 in. O.D.. Chromatograms were obtained for several classes of compounds at a column temperature of 40°C and flow rate of carrier gas of 40 ml. per min.. A Beckman GC 4 equipped with a flame ionization detector was used throughout. Coatings prepared from complexes using 2, 3 and 4-methylpyridine ligands showed preferential adsorption for olefins over saturated compounds, similar to the behavior observed for $AgNO_3$. Larger ligands showed negligible interaction, probably due to steric hindrance to approach to the silver orbitals. Supported in part by NSF sponsored Faculty Research Grant No. 4522-87.

PERITONEAL FLUID ACCUMULATION IN RATS RESULTING FROM INJECTION OF POLYETHYLENE GLYCOL. D. J. Glatt and N. S. Jacobsen. Dept. of Zoology, N. D. State Univ., Fargo, N. Dak.

Organic solvents such as polyethylene glycol are frequently used as vehicles for the administration of drugs and other materials that are only slightly soluble in water. Its affects are often considered to be inconsequential. We have observed relatively large accumulations of fluid in the peritoneal cavity of rats following intraperitoneal administration of 0.1-0.3 ml of polyethylene glycol. As much as 5.6 mls of peritoneal fluid containing moderate amounts of protein has been recovered. In control animals given sham injections, less than 0.1 ml of fluid can be recovered. The fluid accumulation is greatest when 0.3 ml of solvent is injected, reaching a peak at two hours. This fluid shift is greater than fifty percent of the rat's plasma volume. Changes of this magnitude are enough to seriously affect experimental results. Supported in part by the Eagles Max Baer Heart Fund.

THE SULFHYDRYL GROUPS OF SHEEP LIVER 6-PHOSPHOGLUCONATE DEHYDROGENASE: PROTECTION AGAINST MODIFICATION BY SUBSTRATES AND INHIBITORS. W.H. Hanson and J.E.D. Dyson, Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

Modification studies of the sulfhydryl groups of sheep liver 6-phosphogluconate dehydrogenase have been carried out, employing as modifying reagents organic mercurial compounds (stoichiometric concentrations). Reaction of 6-phosphogluconate dehydrogenase with these reagents results in a progressive loss of activity which parallels the O.D. change due to reaction of the sulfhydryl groups with the reagent. The substrate, 6-phosphogluconate, and guanosine-5'-triphosphate and guanosine-5'-diphosphate (competitive with respect to both substrates), which are competitive inhibitors of the enzyme, provide excellent protection to the sulfhydryl groups against modification. The other substrate, NADP, and, rather surprisingly, the competitive inhibitor fructose-1,6-diphosphate (competitive with respect to binding of 6-phosphogluconate), provide little or no protection against modification. The results described above provide strong evidence for the involvement of a cysteine residue in the binding of 6-phosphogluconate to 6-phosphogluconate dehydrogenase, and for its possible participation in the catalytic process. (Supported by NSF grant No. GB 12629).

INTERACTION OF Ni-ACETYL ACETONATE WITH WATER VAPOR. W. S. Hnojewyj. Coll. of Chem. and Physics, N. D. S. U., Fargo, N. Dak.

Data are presented on the kinetics of H_2O Adsorption on Ni-acetyl acetonate at 25, 35, 45 and 55°C and preliminary sorption isotherms at 25 and 35°. The isotherms obtained are of similar geometric shapes. The total amount of H_2O adsorbed in the monolayer region at 25° is equal to about four moles per mole of Ni-acetyl acetonate. Only two moles of H_2O are strongly chemically bound. Data are discussed in relation to the possible molecular structure of Ni-acetyl acetonate.

PRELIMINARY STUDY OF HEAVY MINERALS OF THREE RED RIVER VALLEY TILLS. H. C. Hobbs and F. R. Karner, Dept. Geology, Univ. N. Dak., Grand Forks, N. Dak.

Heavy mineral suites of tills in the Red Lake Falls, Minn., area were studied by x-ray diffraction and optical methods. The upper two units (A and B) are probably upper Wisconsinan in age; the age of unit C is unknown.

The suites are similar, consisting primarily of hornblendes, garnet, pyroxene magnetite and epidote. Hornblende is always by far the most abundant. Unit B generally contains more garnet than the other two, which may reflect different proportions of material derived from different source areas.

Heavy liquid separations combined with microscopic analysis provided the most accurate and detailed data. Techniques using x-ray diffraction combined with magnetic separation may provide rapid methods for distinguishing different assemblages, once they are recognized and defined by optical methods.

INTRA-OVARIAN CONTROL MECHANISMS. J. S. Hunter. Dept. of Biol., Univ. N. Dak., Grand Forks, N. D.

Predictable intra-ovarian biochemical and morphological interactions play regulatory roles in the initiation and maintenance of pregnancy and lactation. Selected enzyme activities, pyridine nucleotides and ovarian venous progestins were measured and lutein cell structure analyzed cytologically.

Increases in ovarian glucose-6-phosphate dehydrogenase (G6PD) sp act and Δ^5-3β-hydroxysteroid dehydrogenase (3βHSD) are correlated with increases in progestin secretion rates during estrous cycle and suggest that G6PD and 3βHSD control rate-limiting steps in steroidogenesis during periods of follicular development. Lutein development is associated with increased ovarian NAD-kinase (NAD-K) sp act ppt and NADP and NADPH concentrations and with high progestin secretions in the first half of pregnancy. These results suggest that NAD-K plays a key role in the initiation of pregnancy. Changes in lutein cell structure indicate that there are significant changes in ovarian function on Day 12 of pregnancy which distinguish the first half of pregnancy from the second. Supported in part by USPHS (5-P01-CA-05007-13) Brown Univ., Providence, R. I.

SPIN-LATTICE RELAXATION OF PROTONS IN HYDRAZINIUM HYDROGEN OXALATE. Kurt Hyde(sponsored by J.W.Harrell,Jr.). Dept of Physics, Univ. of N.Dak., Grand Forks, N.Dak.

The spin-lattice relaxation time T_1 of protons in powdered hydrazinium hydrogen oxalate has been measured as a function of temperature from -60°C up to room temperature. The logarithm of T_1 varies roughly linearly with inverse absolute temperature from 2.6 sec at -60°C to 90 msec at room temperature and appears to be approaching a minimum above room temperature. The size and temperature dependence of T_1 indicates that the relaxation is due to dipole-dipole interactions between the protons. The activation energy for the relaxation process is 0.24 ev. The results for this compound are similar to results previously obtained for hydrazine sulfate[1] and lithium hydrazinium sulfate[2] except that the T_1 minimum occurs at a higher temperature.

[1] J.Harrell and F.Howell, to be published.

[2] W.D.MacClement, M.Pintar, and H.E.Petch, Can. J. Phys. 45, 3257(1967).

THIN-SECTION ANALYSIS OF THE FISH BED, SEIBOLD SITE, NORTH DAKOTA. A. F. Jacob, Dept. Geology, UND, Grand Forks, N. Dak.

Paleolake Seibold, located in SW¼ SW¼ 21 T141N R67W, Stutsman County, North Dakota, was the site of deposition of several sedimentary units during Wisconsinan and Holocene time. One of these units, the "fish bed", is an organic, laminated mud, ranging in thickness from 8.5 cm to more than 60 cm. Examination of oriented thin sections reveals the presence of microscopic laminae, each of which is rhythmic. The base of the typical lamina is lighter-colored and consists of coarser (silt-size) terrigenous material which grades up through fine (clay-size) material into a dark, organic-rich portion at the top. Pollen is concentrated either in the lower part of the clastic portion, or in the organic upper portion. Each lamina is interpreted as a varve.

Microscopic laminae range in thickness from less than .04 mm to more than .4 mm. They are grouped into megascopic laminae which range from a fraction of a millimeter to a few millimeters in thickness. The members of darker, organic-rich megascopic laminae are thinner than the members of lighter, organic-poor megascopic laminae. Measurements of microscopic laminae indicate a depositional rate for the fish bed on the order of 10 to 20 cm per 1000 years, which is consistent with the overall history of the lake.

OVERHEAD PROJECTION TO ILLUSTRATE A CONCEPT OF BIOCHEMICAL DYNAMICS. Francis A. Jacobs. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

Overhead projection can be used in place of the conventional chalk board by writing or drawing illustrations at the time a lecture is delivered. Overhead transparencies can be used which have been prepared previously as a skeletal concept to be completed at the time of presentation by filling in details directly or by placing additional fitted overlays upon the skeleton to fill in the detail. Thus a biochemical concept can be built up from a series of illustrations as overlays, each with its own chemical equation, drawn in such a way that a composite will form a sequence of reactions which develop the more elaborate concept. Intermediate overlays in color can be used for emphasis. The direction and dynamics of the chemistry is usually shown by arrows appropriately placed. These can be put into motion to reinforce the concepts being illustrated by the use of an additional overlay with special polarized patterns, set into "motion" by a simple change in the polarization of the projected image. Illustrations will be presented, and the materials used will be described.

AMINO ACID DISTRIBUTION IN TISSUES OF THE RAT FED ETHANOL. F.A. Jacobs and J.C. Crandall. Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak. 58201

A study was made of the effect of ethanol upon amino acid distribution in the intestinal mucosa, liver and blood plasma of young Sprague-Dawley origin rats. Two groups of rats were pair-fed synthetic liquid diets. Of the diets fed one contained 5% ethanol which isocalorically replaced sucrose in the control diet. Two additional groups of rats were fed Purina Rat Chow ad lib. with either water, or a solution containing 25% sucrose and 32% ethanol (wt./vol.) in water. Pooled tissue samples were analyzed by automatic column chromatography. Rats fed alcohol-containing liquid diets grew less well than their pair-fed controls, indicating, poorer dietary efficiency. These rats also developed fatty livers as determined by microscopic appearance and chemical analysis. The concentration of methionine, a lipotropic amino acid, was consistently higher in tissues of rats fed ethanol diets which indicates a decreased utilization of this amino acid despite high concentrations of fatty material in the liver. Urea levels were elevated 2 and 3 fold in tissues of rats fed alcohol-containing liquid diets indicating an increased catabolism of dietary amino acids. Supported in part by NIH research Grant MH-19235-01 NTN.

PATHOGENIC VARIABILITY IN SELFED LINES OF RACE 10 USTILAGO HORDEI AND RACE 6 USTILAGO NIGRA. L. L. Jensen, L. C. Darlington, and R. L. Kiesling. Plant Path. Dept., NDSU, Fargo, N. Dak.

The meiotic products from teliospores of Ustilago hordei, race 10, and U. nigra, race 6, both relatively virulent races, were selfed through 2 generations to develop lines that were pathogenically stable. The selfed lines were inoculated onto 9 differential barley varieties. The first selfed generation of each species showed variability both in per cent infected plants and in the number of varieties attacked. Progenies of the second self were virulent on fewer varieties than the parent races and showed the same degree of variability as the first selfed generation. No lines were developed that were pathogenically stable; therefore, selfing relatively virulent races of U. hordei or U. nigra was not the best approach to obtaining pathogenically stable lines.

IRRADIATION OF d,l-TROPIC ACID AND ITS METHYL ESTER. Deane L. Johnson and Virgil I. Stenberg. Dept. of Chem., Univ. N. Dak., Grand Forks, N. Dak. 58201.

The 253.7nm irradiations of d,l-tropic acid and its methyl ester were done in isopropanol solution under nitrogen, as part of a larger study of the photochemistry of atropine. Irradiation of tropic acid cleaved both C-C bonds β to the phenyl ring, with the preferred cleavage eliminating CO_2 and giving a 13% yield of phenylethanol. The cleavage of both bonds gave toluene in 6% yield, and the elimination of a carbinol radical gave a 1% yield of phenylacetic acid. Solvent adducts were also found. Methyl d,l-tropate behaved similarly, although the elimination of CO_2 gave only twice the yield of carbinol elimination. Deane L. Johnson gratefully acknowledges the financial assistance of the National Science Foundation through its Undergraduate Research Participation Program (GY-7415).

DDT INDUCED CHROMOSOMAL DAMAGE IN MICE. G. A. Johnson and S. M. Jalal. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

Thirty five mice (Mus musculus L.) in four groups were treated with 100 ppm (parts per million) to 400 ppm of DDT to determine its influence on chromosomes. A control of unexposed mice were included in every group. Intraperitoneal injection of a DDT peanut oil mixture was used. After three weeks of DDT administration, 250-350 C-metaphase bone marrow cells from each mouse were analyzed for chromosomal aberrations. Fragments, rings, and metacentric chromosomes were recorded as structural aberrations and chromosomal stickiness as a physiological abnormality. Chisquare values between control and treated animals for fragments were non-significant at 100-150 ppm but significant to highly significant at higher dosages. This relationship between control and stickiness displayed a significant to highly significant difference at 100 ppm and all the Chisquare values from 150-400 ppm were highly significant. These results indicate with clarity that DDT must be recognized as an agent capable of causing chromosomal damage; even at low dosages. Numberable reports of liver cirrhosis, nervous disorders, leukemia, sterility and other sub-lethal effects associated with DDT and other controversial chlorinated hydrocarbon insecticides can in part be explained by DDT's mutagenic properties as evidenced in this investigation.

PH STUDIES OF ALPHA-CHYMOTRYPSIN-L-TRYPTOPHAN ESTER SYSTEMS Phyllis E. Johnson & James A. Stewart Dept. of Chem., UND, Grand Forks, N.Dak.

Little is known of the chemistry of α-chymotrypsin and substrates with free α-amino groups. The pH-profiles for α-chymotrypsin-catlyzed hydrolysis of L-tryptophan benzyl and ethyl esters (TBE & TEE) were obtained by automatic titration. The profile for TBE gave three rate constants, indicative of three different ionic, reactive forms of the active site, and three acid-base equilibrium constants. The pKa's are those of the ionizable carboxyl and imidazoyl groups of the active site and of the free α-amino group in the substrate. The profile for TEE gave two rate constants and four pKa's, which represent carboxyl, imidazoyl, and α-amino groups at the active site, and the free α-amino group of the substrate. These results can be related to present knowledge of the chemistry of chymotrypsin.
Supported by USPHS, NIH (Grant GM-16167) and NSF (Grant GY-7415).

PRELIMINARY MODAL STUDY OF PORPHYRITIC QUARTZ SYENITE, OSSIPEE MOUNTAINS, NEW HAMPSHIRE. F. R. Karner and R. E. Bertram. Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak.

Bulk mineral compositions determined for this rock type by x-ray diffraction methods show relatively high quartz values. The quartz content ranges from about 10 to 35 percent but most values fall in the 15 to 25 percent range. Thus, the rocks range in composition from quartz syenite to granite and appear to be predominantly granite. This new information on the composition of the Ossipee ring dike suggests a closer relationship to the associated Moat Volcanics than previously suspected since the compositions of the two rock types may be nearly identical.

INTERPRETATION OF PARTIALLY-SMUTTED BARLEY PLANTS IN COVERED SMUT STUDIES. R. L. Kiesling and G. A. Peterson. Dept. of Plant Path. and Dept. of Agron., NDSU, Fargo, N. Dak.

The number of partially-smutted plants obtained in populations of inoculated barley crosses was affected by environmental factors, inoculation techniques, and genetics of host-parasite interactions. In studies of the inheritance of resistance to barley covered smut, Shands in 1956, Metcalfe in 1961, and Thomas and Person in 1965 classed all plants with one or more smutted heads as susceptible. Combining all smutted plants, regardless of the degree of infection, into a single class resulted in difficulty in establishing genetic ratios in some studies. When seed of F_3 families from partially-smutted F_2 plants was inoculated with covered smut, only a few families were found to be totally susceptible while many were segregating for their reaction to covered smut. Resistant, totally, and partially-smutted plants were recovered from F_3 families derived from partially-smutted F_2 plants. Seed from partially-smutted F_3 families frequently produced F_4 families that segregated for reaction to covered smut. It was concluded that the progeny from partially-smutted plants must be retested in order to interpret their reaction to covered smut.

CHROMOSOMAL ABERRATIONS AND FERTILITY INTERRELATIONSHIPS IN PRAIRIE BROMUS INERMIS LEYSS POPULATIONS. T. D. LaFleur and S. M. Jalal. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

Meiotic chromosomal aberrations were studied for 37 plants collected in the summers of 1969 and 1970 from three undisturbed Red River Valley prairie stations. Forty-five collections, from a total of 59, were analyzed for stainable pollen to ascertain the levels of fertility. Aberrations studied were unoriented univalents at metaphase-1, laggards at anaphase-telophase-1 and -2, micronuclei at dyad and quartet, dicentric bridges and translocation configurations. Excluding inversion bridges and translocation configurations, disturbances at all stages were correlated with one another and with pollen stainability. Correlations between aberrations of any two stages were significant to highly significant with the exception of a non-significant relationship between aberrations at metaphase-1 and anaphase-telophase-2. The assumption that the frequency of quartet micronuclei was an index of chromosomal aberrations was confirmed by the significant to highly significant associations with aberrations at earlier stages. A significant negative correlation existed between quartet micronuclei and stainable pollen; therefore, in the natural complex polyploid populations of bromegrass studied, chromosomal aberrations reduce fertility levels significantly.

EVALUATION OF ESTERASES FROM RUMEN BACTERIAL ISOLATE 53. Wayne W. Lanz and Phletus P. Williams. Dept. of Bacteriology, NDSU, and USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D. 58102

A rumen bacterial gram-negative curved rod culture (isolate No. 53) showed esterase activity with α-naphthyl acetate and fluorescein 3',6' diacetate. The reaction was measured spectrophotometrically. The enzymes were released by high frequency sonication from intact cells and were precipitated with ammonium sulphate at 30 to 60% (v/v) saturation. Esterase profiles obtained by starch and polyacrylamide gel electrophoresis indicated 5 to 7 esterase cathodally migrating bands. The enzymes were inhibited by pesticides (Bidrin, Mobam, and Phosdrin) and minerals (cobalt chloride and sodium fluoride) but were not inhibited by ethyl carbamate, acetyl choline chloride, potassium iodide, and iodine. Combinations of Phosdrin and Mobam, or Bidrin and Mobam showed an additive inhibition with esterases. Phosdrin and Bidrin in combination were not additive. Isolate 53 degraded tributyrin emulsions without lipase activity on olive oil emulsions.

SOME BACTERIAL, BENTHIC, CHEMICAL, AND PHYSICAL FACTORS IN THE RED RIVER AT FARGO, NORTH DAKOTA. D. R. Lerfald and D. R. Scoby, Div. Nat. Sci., NDSU, Fargo, North Dakota.

A study was conducted at five stations in the Red River near Fargo, N. Dak., to determine biological, chemical, and physical effects of major Fargo and Moorhead effluents on water quality. Bacteria, benthic organisms, water near the bottom, and bottom mud were sampled during the period of 8 June to 28 July, 1970. Estimates of numbers and kinds of oligochaetes, tendipedids, coliform bacteria, and total bacteria were made.

A multiple linear regression analysis indicated significant relationships for oligochaetes with sediment phosphates and sediment nitrates, and tendipedids with water nitrate nitrogen and total hardness.

Pollution indices were developed from the measurements of the biological, chemical, and physical factors. Values of these indices indicated stations 2 and 5 (after the Moorhead and Fargo sewage effluents, respectively) as having the highest increment over the preceding stations' index. Station 1 (before F-M effluents) had the least pollution as indicated by all three pollution indices, and the total pollution "points" at each subsequent station increased suggesting the cumulative effect of all effluents.

THE FILTERING RATE OF DIAPTOMUS LEPTOPUS OVER A 30 FOLD RANGE OF ALGAL CONCENTRATIONS. J. A. Levy and G. W. Comita, Dept. Zool., N. Dak. State Univ., Fargo, N. Dak.

The rate of filtration of a unialgal suspension of Chlamydomonas reinhardi by Diaptomus leptopus was measured under a wide (30 fold) range of algal concentration at 25°C. A Coulter Counter was used for making the algal counts. Four or five animals were suspended in each jar and allowed to feed for periods from 0.60 to 1.06 days. The algal concentrations were determined before and after. Allowing for exponential growth of the algae, the filtering rate was computed on a per animal basis as well as on a per mg of body weight basis. The rates obtained were 8.4 ml female^{-1} day^{-1} and 137.8 ml mg dry weight^{-1} day^{-1}. Analysis of variance indicates that on the per animal basis when the algal concentration was changed in repeated experiments, the mean filtering rate for each sample did not differ with varying initial algal concentrations at the ten percent level of significance and closely approached significance at the five percent level.

EFFECTS OF PLANT EXTRACTS ON DROSOPHILA MELANOGASTER CULTURE, Marvin Mattson, L. J. Schermeister, S. M. Quarishi, and P. C. Sandal. Dept. of Agron., N. Dak. State Univ., Fargo, N.D.

Water and alcohol extracts of leaves, stems, roots, flowers or fruits of wild plant species were incorporated into Drosophila culture media at an extract volume derived from one gram plant dry matter in 5 cc of media. Five paired wild-type fly mating cultures were evaluated per treatment along with checks. Egg production, larva and pupa development and fly emergence were observed. Second and third generation progenies of crosses were evaluated when available.

Some 285 extracts were assayed representing 64 genera among 26 families with 25 genera causing varying degrees of abberrant development. Extracts of 1) Euphorbia esula (root), Cynoglossum officinale (stem), Saguinaria canadensis (root), and Phryma leptostachya (root) inhibited pupation, 2) Asclepias syriaca (fruit, stem) and speciosa (stem), Menispermum canadense (stem), and Polygonum coccineum (flower) delayed pupation, 3) Astragalus caryocarpus (fruit) inhibited egg laying, 4) twelve genera greatly reduced emergence, and 5) twelve genera were toxic to parents on contact with media. (Research supported by NDSU Themis Project DADA 17-69-C-9023.)

CHACTERIZATION OF α-CHYMOTRYPSIN CATALYSIS USING pH-PROFILES. H. M. Ness and J. A Stewart. Dept. of Chem., Univ. of N.Dak., Grand Forks, North Dakota 58201.

Since the active site of α-chymotrypsin is highly ionic, pH-rate profiles are useful to characterize ionic groups involved in catalysis. The pH-rate profile for the α-chymotrypsin catalyzed hydrolysis of N-acetyl-L-tyrosine ethyl ester (ATEE) was studied in the pH range of 3.7 to 7.4 by automatic titration techniques. A sigmoidal curve was obtained from about pH 5 to 7.4 and is dependent upon an ionic group with a pK_a of about 7 and is attributed to His 57. A drop in rate was observed at lower pH values due to an ionic group (carboxyl) with a pK_a of about 4.3. The protonation of this group, which is free to ionize at higher pH values, was observed by a drop in the rate of hydrolysis. The carboxyl group is involved in deacylation, which is the rate-controlling step in the hydrolysis of ATEE. Salt studies on the catalyzed hydrolysis of ATEE exhibit a similar pH-rate profile and are also dependent on two ionic groups whose pK_a depends on the concentration of salt. At high pH, a positive salt effect was observed which increased the rate of hydrolysis while at low pH, a negative effect was observed which decreased the rate of hydrolysis. Supported in full by USPHS, (Grant No. GM-16167).

ANALYSIS OF A SALINE TALL GRASS PRAIRIE ECOSYSTEM. IV. PRELIMINARY INVESTIGATIONS ON SOIL ALGAE. R.N. NORDIN AND D.W. BLINN. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

Records of soil algae in the atate are lacking. Collections of soil were made monthly September through November 1970 at the University of North Dakota Oakville Prairie field station thirteen miles west of Grand Forks. Vertical soil profiles were obtained with a 14 cm vertical core and analyzed both qualitatively and quantitatively by using sub-samples at 2 cm depth intervals. Cultures were established using 5 gm of soil in Bold's Basal Medium with a two week incubation period. The greatest species diversity occurred in the uppermost soil samples. Species distribution through the profile was as follows: 13 in 0-2 cm; 10 in 4-6; 5 in 8-10; 3 in 12-14 cm. As expected, a trend in reduction of numbers of cells of most species was found as lower soil levels were cultured (i.e. Chlorococcum sp. occurred at 766 cells/ml at 0-2 cm and 26 cells/ml at 10-12 cm.). Seventeen species were identified which include: 6 Chlorophyceae, 6 Cyanophyceae and 5 Bacillariophyceae. Correlations between algal species and soil chemical data at various levels within the soil profile are considered.

METABOLISM OF PROPHAM (ISOPROPYL N-PHENYLCARBAMATE) IN THE CHICKEN. G. D. Paulson, M. V. Zehr, and M. M. Dockter. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D.

One group of mature Leghorn hens, surgically modified to facilitate separate collection of feces and urine, was given a single oral dose (10 mg/kg of body weight) of isopropyl-^{14}C N-phenylcarbamate (side chain label). A second group was given a single dose of isopropyl N-phenyl-^{14}C carbamate (ring label). Feces, urine and expiratory gases were collected 6, 12, 24, 36, and 48 hr. after dosing and analyzed for carbon-14. When the ring-labeled compound was given, 87 ± 4% (mean ± standard error) of the carbon-14 was excreted in the urine during the 48-hr. collection period; 80 ± 4% of carbon-14 given as the side-chain-labeled material was excreted in the urine. Seven ± 3% of the radioactivity given as the ring-labeled compound and 6 ± 1% given as the side-chain-labeled compound was excreted in the feces during the 48-hr. collection period. Carbon-14 in the expiratory gases accounted for 7 ± 5% of the activity given as the side-chain-labeled material and 0.2% of the activity given as the ring-labeled compound. Less than 2% of the carbon-14 was detected in the tissues 48 hr. after the dose was given. Preliminary investigations indicated that at least five radio-labeled metabolites were present in the urine.

SAMPLING SOLUTION OF MECHANICAL VIBRATION PROBLEMS, R.R. Power and K.W. Li (Sponsor), Mech. Engr. Dept., North Dakota State University, Fargo, North Dakota.

The hyperbolic or wave equation arises in the solution of many practical engineering problems. This equation describes the motion of various types of systems, including the transverse vibrations of flexible members in a stressed state, the torsional vibrations of cylindrical rods, the variation of voltage along a transmission line, and the transmission of sound in a column of air (the organ-pipe problem). However, even for the simple idealized problem, the analytical or exact solution requires a very rigorous mathematical background. The sampling technique introduced greatly simplifies the solution of the wave equation by using very simple prediction (algebraic) equations which can be readily solved using a digital computer. The accuracy of the solution is dependent on the size of the rectangular grid chosen in the space-time plane because the grid size affects the coefficient C in the prediction equations. As the value of C approaches 1, the sampling solution agrees exactly with the analytical solution.

STUDIES ON THE DIFFERENTIAL RESPONSES OF SYNTHETIC AND HYDROLYTIC ACTIVITIES OF LIVER MICROSOMAL GLUCOSE 6-PHOSPHATASE TO GLUCOCORTICOID THERAPY AND EXPERIMENTAL DIABETES. E.B. Proctor and R.C. Nordlie. Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak. 58201

Nordlie has reported previously responses of glucose-6-phosphatase to alloxan diabetes and cortisone administration (J.Biol. Chem., 240:2155,3479, 1965). This work has been extended by observing responses in cortisone-treated diabetic rats. Diabetes was induced by IP injection of alloxan (145 mg/kg). Seven days later diabetic and normal rats were given IM injections of cortisone (12.5 mg) twice daily for 4 days. Four hr. after the last injection rats were killed, liver homogenates prepared, and assays run in the presence and absence of detergent (0.2% Na deoxycholate). Glc-6-P phosphohydrolase, PPi-glc phosphotransferase, and inorganic pyrophosphatase exhibited the same responses: 1. Increased activity in diabetic and cortisone-treated (c.t.) rats. 2. Decreased detergent activation in c.t. animals. 3. Greatest increase in activity in c.t. diabetics with some diminished detergent activation apparent. Thus, diabetes results in induction of the enzyme while cortisone administration produces an activation of pre-existing enzyme. A combination of the two treatments results in superimposition of these two responses. Supported in part by NSF, NIH, and Am. Diab. Assn.

DEVELOPMENTAL METABOLISM OF MELANOPLUS SANGUINIPES. Dr. M. Wahid H. Qureshi. Dickinson State College, Dickinson, ND 58601

Respiratory use of oxygen was measured by an electrolytic respirometer for all post-embryonic stages of development of this grasshopper. The rate of use was highest for third instar nymph, and lowest for adults. First and Second instar nymphs consumed oxygen at a rate slightly lower than found for the third instar, and fourth and fifth instar rates were transitional toward the adult rate. The rates of oxygen consumption during development were expressed as a sequence of values reflecting changing metabolic patterns. There are indications that first instar and probably also the second is a transitional period between embryogenesis and free living growth and development. Sex related differences were first evident in third instar nymphs. Means of the last five hours of observation were considered to more accurately represent basal metabolism. Patterns of increase oxygen use were noted for both molting and egg deposition. The increase was abrupt for molting but more gradual for egg deposition.

THE EFFECTS OF BACILLUS THURINGIENSIS ON THE POST EMBRYONIC DEVELOPMENT OF GALLERIA MELLONELLA L..

Dr. M. Wahid H. Qureshi, Dickinson State College, Dickinson, ND 58601

The effects of B. Thuringiensis of inert material and starvations were observed on the development of the greater wax moth. Treatment observations were compared with a control series. The sublethal doses of inert material bring about an acceleration of the life history and reduce the rate of body weight gain. At .75 gram per 20 grams bees wax inert material most of the larvae died before pupation. A few were able to pupate but failed to emerge. The effect of the same quantities of the Bacillus was more pronounced than with the inert material. The .75 gram level of the Bacillus proved to be lethal concentration where all of the larvae died before pupating. Statistical analysis of data demonstrated a highly significant difference in the body weight gains and life span between various concentrations of inert material and the Bacillus. As the treatment concentration increased, life span was shortened and the body weight reduced. Stunting was more pronounced in those receiving the bacteria.

THE GEOGRAPHIC DISTRIBUTION OF SKUNK RABIES IN NORTH DAKOTA.

P. W. Rakowski, B.Sc. and M. F. Andrews, D.V.M. Dept. of Veterinary Science, North Dakota State Univ., Fargo, N. Dak.

Records of the North Dakota Veterinary Diagnostic Laboratory and the State Health Department from 1957 through 1970 were reviewed to determine the incidence of reported rabies in skunks by county of origin. Factors that appeared to at least partially influence the reported incidence of the disease were land use, human rural population density, soil type, and proximity to specimen collection centers. Reported incidence of rabies in North Dakota was highest in the Southeastern part of the state.

ISOXAZOLES: 1,4-PHENYLENE-bis-ISOXAZOLES. Franz H. Rathmann, North Dakota State University, Fargo, North Dakota, and Luis Jon Ravnaas, Turtle Lake High School, Turtle Lake, North Dakota.

1,4-Phenylene-bis-(3'-(5'-methyl-isoxazole-4'-carboxylic acid ethyl ester)) (III) was prepared by the general method described previously (N. Dak. Acad. Sci., Proc. 21: 208, 1967; N. Dak. Acad. Sci., Abstr., p. 16, 1968). Terephthal-aldehyde-dioxime (I) was chlorinated to form the dihydroximic acid dichloride (II), mpt. 174°C. Condensation of II with sodio-acetacetic ester gives the diester (III), mpt.169°C, in 90% yield. Hydrolysis of III yields the free dibasic acid (IV), mpt.289°. When IV is refluxed with thionyl chloride, the diacid dichloride (V), mpt.110°, is formed. Esterification of V with various alcohols gave the following dialkyl esters (VI): methyl, mpt.164°C; ethyl, 169°C; n-propyl, 119°; n-butyl, 93°; n-amyl, 114°; n-hexyl, 151°. Other derivatives obtained were the amide, 306°; anilide, 283°C. Structures of several of the compounds were confirmed by n m r spectroscopy.

PURIFICATION AND ISOLATION OF STREPTOMYCETE CELLULASES. L. J. Sikora, H. T. Tung and B. P. Sleeper. Dept. of Bacteriol., N. Dak. State Univ., Fargo, N. Dak.

Cellulases from the soil organism Streptomyces antibioticus have been purified by passage through an hydroxylapatite (HTP) column. A number of contaminating enzymes were removed by this treatment, resulting in a cellulase fraction with increased specific activity and five bands on analytical disc electrophoresis. The eluate from the HTP column was unaffected by p-chloromecuribenzoate and iodoacetic acid. When HTP column eluate was passed through CM Sephadex C-50 and DEAE-Sephadex A-50, three separate cellulase peaks were eluted but recovery of these peaks for further analysis of homogeneity has not been successful. Partial purification of HTP eluate has been achieved by preparative disc electrophoresis. Four peaks were obtained. One peak (A) showed a single major analytical disc electrophoresis band and a trace of the adjacent fraction. Another peak (D) from the prep-disc contained two bands. Studies performed on these fractions have shown that peak A has a single pH optimum at 5.7, and peak D has two optima, one at pH 5.6 and the other at pH 6.5. The two fractions also showed different specific activities toward carboxymethyl cellulase and no activity toward β-methyl-D-glucoside.

EFFECT OF ETHANOL INGESTION ON LIVER MITOCHONDRIAL AND MICROSOMAL PHOSPHATIDYL CHOLINE FRACTIONS. David N. Skurdal, M.F. Miller and W.E. Cornatzer. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

Female rats were fed a liquid diet for 2 and 6 weeks in which 36% of total calories was ethyl alcohol and contained vitamins, minerals and amino acids. Control animals were pair-fed the same diet containing sucrose. Total lipids were extracted from liver mitochondria and microsomes and total lecithins separated by TL-chromatography. Four phosphatidyl choline fractions were obtained by TL-chromatography on silica gel impregnated with $AgNO_3$. The total liver triglycerides of controls were 61 mgs and increased to 240 mgs in animals fed alcohol. Alcohol ingestion for 6 weeks decreased the % of total lecithin-P and increased the % phosphatidyl cholines of fractions 1 and 2 and decreased fraction 4 of liver mitochondria. Fraction 1 increased and fraction 4 decreased in liver microsomes. To further substantiate these lecithin changes in microsomes, the biosynthesis of the phosphatidyl choline fractions were measured by injecting ^{14}C-1,2 choline into rats fed the two diets for 2 weeks and doing a time uptake at 1, 3 and 4 hours following injection of the radioactive choline. (NIH Grant MH 19234).

A STUDY OF KIDNEY FUNCTION IN DOGS TREATED CHRONICALLY WITH LITHIUM CARBONATE. H. Spencer, A. Evan, T.W. Nielsen. Depts. Physiol. and Pharm. and Anat. UND Sch. of Med. Grand Forks, N.D.

Dogs (5-15 kg) maintained on lab chow and water ad lib. were dosed orally with 10, 30, and 60 mg/kg of Li_2CO_3. The 10 and 30 mg/kg animals showed no changes in water balance. Osmolarities of urine and plasma were normal. The 60 mg/kg animals had increased plasma osmolarities and decreased urine osmolarities. Polydipsia and polyuria were evident with water intake rising to over 7 L/24 hrs and urine volumes to over 6 L/24 hrs. Anorexia was transient, G.I. disturbances included vomiting and diarrhea. Kidneys were fixed by whole body perfusion with 1% glutaraldehyde in modified Tyrode buffer at pH 7.4. Tissues were post-osmicated in 1% OsO_4 in Tyrode buffer, and routinely processed for electron microscopy (EM). EM observations revealed mitochondrial swelling; dilatation of rough endoplasmic reticulum; karyolysis and karyorrhexis; and apical cytoplasmic swelling. Subcellular lesions were generally confined to the distal portions of the nephron. These areas function in urine dilution and concentration. The EM findings thus confirm and support the functional aberrations.

Supported by PHS GRS Grant No. 4314-04.

INHERITANCE OF PATHOGENICITY IN THE WHEAT LEAF RUST FUNGUS PUCCINIA RECONDITA. G. D. Statler and U. Maldonado. Dept. of Plant Path., NDSU, Fargo, N. Dak.

The inheritance of virulence in P. recondita was studied in cultures established from spermatizations of pycnia produced from teliospores collected in plots at Fargo. Several cultures were recovered from the crosses that differed widely in virulence from cultures collected at Fargo. Host genes Lr1 and Lr2 were attacked by a much higher percentage of isolates from the crosses than by field cultures. Five cultures recovered from the crosses were race UN13 virulent on host genes Lr1, Lr2, Lr2D, Lr3 and Lr10. One culture recovered from the crosses was avirulent on all isogenic lines tested. This wide difference in virulence indicated heterozygosity in the leaf rust population and the possible role of the alternate host as a source of new rust races.

THE PHYSIOLOGICAL EFFECTS OF NITROUS OXIDE AT VARIOUS PRESSURES ON THE MALE RAT. L.C. Stetzner and B. De Boer. Dept. of Physiol. and Pharm., Sch. of Medicine, UND, Grand Forks, No. Dak.

Nitrous oxide (N_2O) has been used for over a century as an analgesic and anesthetic. Studies have been confined primarily to semi closed systems with only relative success in controlling dosage. Our experiments were designed to gain information concerning heart rate and respiration rate (implanted silver ring electrodes via impedance pneumograph and polygraph), body temperature (YSI 402 probes and model 43 tele-thermometer), and group O_2 consumption (chromatograph) from male rats (400-450 gm) in a closed system. These studies are based upon 12 animals per group. Nitrogen (N_2) was the control gas and total pressure was maintained at 21 psi at 86° F throughout. The total exposure time was 4 hrs. Dosage levels of N_2O were 7 psi (with 14 psi of N_2), 14 psi (with 7 psi of N_2), and 21 psi. Results indicate only small changes at 7 psi N_2O. At 14 and 21 psi N_2O heart rate was elevated; respiration was irregular and elevated. Body temperature remained normal. O_2 consumption decreased, most observably at 21 psi. These studies indicate that further studies at higher pressures with helium and nitrogen should be important.

Supported by ONR Contract No. N00014-68-A-0499.

CAFFEINE CONCENTRATIONS IN BLOOD AND URINE OF ANIMALS SUBJECTED TO HIGH PRESSURE. D. A. Stites and B. De Boer. Dept. Physiol. and Pharm., Sch. of Medicine, UND, Grand Forks, N.Dak., 58201.

The effects of high pressure ($He-O_2$) upon the reaction of mice to caffeine injected intraperitoneally were studied. Previous investigations indicated that high pressure increased the mortality rate of mice pretreated with caffeine and that there was a considerable weight loss in such animals as compared to control animals under the same experimental conditions. Mice were pretreated with 100-200 mg/kg of caffeine and compressed to 30 atm with a $He-O_2$ mixture at the rate of 1 atm/min. After 6 hrs. the animals were rapidly decompressed and sacrificed. Blood samples from each animal were analyzed for caffeine content using a benzene-extraction procedure. Compared with $He-O_2$ controls at 1 atm, blood concentrations of caffeine decreased in mice exposed to 30 atm. Using pooled samples of urine from 15 mice, the total urinary output of animals compressed to 30 atm for 1 hour increased when compared to urine output of mice maintained under control conditions. However, caffeine content of urine of both experimental and control groups of animals remained unchanged. Supported by ONR Contract No. N00014-68-A-0499.

MERCURY LEVELS IN TISSUES OF DUCKS COLLECTED IN SOUTH CENTRAL NORTH DAKOTA. G. A. Swanson, G. L. Krapu and H. K. Nelson. Northern Prairie Wildlife Research Center, Jamestown, N. Dak.

Liver, kidney and breast muscle tissues of pintails (Anas acuta) that fed on uplands as well as in wetlands in south central North Dakota and shovelers (Spatula clypeata) that fed on aquatic invertebrates were analyzed for total mercury via neutron activation analysis. Tissues were obtained in 1969 and 1970 from adult birds during the spring and midsummer months and from young-of-the-year collected in the wild and others reared under penned conditions. A sample of food fed to the pen-reared birds was also examined for mercury.

Adult birds of both species contained individuals with mercury concentrations in their tissues that exceeded the 500 ppb standard. Mercury levels in the liver and kidney tissues were of a similar magnitude but breast muscle tissues were generally lower by a factor of 2 to 4 times. Mercury levels in wild young-of-the-year birds were lower than in adult birds but higher at the 0.05 probability level than pen-reared birds of similar age. Individual adult pintails reached higher maximum levels of mercury in liver tissues than did shovelers; however, a greater percentage (83 percent) of adult shoveler liver tissues exceeded 500 ppb than did pintails (32 percent).

USE OF REFLECTANCE OF DISPERSED VITRINITES AS A DIAGENETIC INDICATOR OF SEDIMENTS. Francis T.C. Ting. Dept. of Geology, Univ. of N. Dak., Grand Forks, N. Dak.

Reflectance measured from polished surfaces of vitrinite is a function of the degree of metamorphism or coalification of coal; i.e., the higher the reflectance the higher the rank of the coal. Vitrinite is derived from woody plant tissues that have undergone normal coalification at temperature and pressure conditions indigeneous to the depth of burial, geothermal condition, and general tectonic regime. Vitrinites are also found as dispersed particles in all sediments in small quantities. They can be concentrated by treating the sediments with strong acids, HF or HCl. More recently it has been established that temperature and geologic time are the major factors that control the upranking of vitrinite. Thus, the reflectance of vitrinite, together with the age of the sediments, reflects the maximum temperature condition that the host sediments have undergone and provides a means of measuring the thermal history of the sediments. Studies of deep drilled core-samples from Matagorda County, Texas (Frio Formation, Lower Miocene), indicate that an increase of reflectance of vitrinite by 0.1% corresponds to an increase in depth of burial of 1600 feet or to a temperature increase of 65°F. Thanks are due to the ESSO Research Production Company for providing the core samples and temperature data.

THE AGE AND RATE OF GROWTH OF WALLEYE (STIZOSTEDION VITREUM) AND SAUGER (STIZOSTEDION CANADENSE) IN LAKE SAKAKAWEA, NORTH DAKOTA, 1968-1969. C. H. Wahtola, Jr., D. E. Miller and J. B. Owen. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

The age and rate of growth of walleye (Stizostedion vitreum) and sauger (S. canadense) in Lake Sakakawea, North Dakota, were investigated during the summers of 1968 and 1969. A total of 236 walleye and 447 sauger were captured during the study. Scales were taken, and annual growth was back calculated. Ten and nine age classes were distinguished for 1968 and 1969 walleye respectively, while sauger exhibited only seven age classes during both years. The sex ratio for all walleye was 1.54 females per male, while sauger had a ratio of 1.14 males per female. The coefficient of condition (K-TL) computed for all walleye was .86, and the coefficient for sauger was .73. It was concluded that walleye and sauger grew well in Lake Sakakawea.

ANALYSIS OF A SALINE TALL GRASS PRAIRIE ECOSYSTEM.
III. A STUDY OF SOIL-PLANT-ANT INTERACTIONS.

M. K. Wali and P. B. Kannowski, Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota

The plant communities, ants and soils of a number of ant mounds in the eastern section of Oakville Prairie, 13 miles west of Grand Forks in eastern North Dakota were studied. The two major plant communities on these mounds are of Poa pratensis and Agropyron trachycaulum with lesser representation of communities of Poa pratensis-Agropyron trachycaulum, Spartina pectinata-Poa pratensis, Spartina pectinata and Bromus inermis. Three ant species, Lasius flavus, L. pallitarsis and L. umbratus are associated with these mounds. The volume of the mounds varied from 8.2 to 130.8 cu. dm. Soils are well drained and the physical characteristics show that bulk density values seldom exceed 1.0 gm/cm^3. Percent total porosity lies in the range of 64.5-76.7. Continuous faunopedoturbation seems to create rather uniform soil chemical characteristics. Out of 21 samples, only 2 show a pH exceeding 8.0 and 4 samples have a conductivity value above 1 millimho/cm. Replaceable Na in the samples averages 0.88 meq/100 gm. Other chemical analyses conducted include replaceable macro- and micronutrients and EDTA-extractable trace metals.

ANALYSIS OF A SALINE TALL GRASS PRAIRIE ECOSYSTEM.
II. VEGETATION-SOIL RELATIONSHIPS ALONG A TRANSECT.

M. K. Wali and S. M. Jalal, Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

Data from 20 sample plots was collected from a 1-mile transect in the eastern section of Oakville Prairie, 13 miles west of Grand Forks. Actual counts of standing shoots were made in each area. Dominant species are Koeleria cristata, Distichlis stricta, Muhlenbergia asperifolia and Spartina pectinata with lesser dominance of Hordeum jubatum, Agropyron dasystachum, A. repens and Poa pratensis. Community segregation along the transect is indistinct. Physical and chemical characteristics involving both major and trace metals have been determined for all samples collected from each genetic horizon in a soil pit. pH values lie in the range of 7.6 - 8.5. Conductivity values are as high as 11.5 millimhos/cm and there is at least 1 horizon in each soil pit with a conductivity >4 millimhos/cm. Generally soils under Spartina, Distichlis, Hordeum, Koeleria and Muhlenbergia show higher conductivity while surface soils under Agropyron trachycaulum and Poa pratensis show replaceable Na below 2 meq/100 gm. Species distributional patterns using actual counts and density, are shown along quantified gradients of water, replaceable Ca and Na, and some trace metals.

RESPONSE OF TISSUE CULTURE CELLS TO BIOTIN. J.R. Waller, J.J. Kelleher and R.J. Majerle. Dept. of Micro., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

LM tissue culture cells were grown at 35 C in suspension in medium 199 containing 153 or 38 ug of biotin per ml. Cultures were sampled, counted, harvested and analyzed for free and bound biotin at daily intervals. Free biotin content of the cells decreased dramatically when cultured in low biotin medium. Free biotin levels ranged from 10 to 45 fold greater in cells grown in high biotin medium. Bound biotin values in cells from the two media differed significantly only at 0 time and after 5 and 6 days incubation when cells from the high biotin medium contained twice as much bound biotin. Maximum bound biotin levels in cells from both media occurred at 2 days. From 2 to 6 days cells cultured in low biotin medium exhibited a steady decrease in bound biotin content while cells cultured in high biotin medium registered a decrease followed by an increase in both bound and free biotin.

EFFECTS OF HELIUM-OXYGEN ENVIRONMENTS AT 1 AND 11 ATMOSPHERES ABSOLUTE PRESSURE ON BACTERIAL GROWTH RESPONSES. James R. Waller and Carl A. Zogg. Depts. of Micro. and Phys., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

The growth responses of 12 organisms were measured in broth medium in environments consisting of air at 1 atm, helium-oxygen at 1 atm and helium-oxygen at 11 atms pressure. pO_2 values were adjusted to that of air. Maximum growth responses of all organisms after 3-5 days incubation in the three environments did not vary significantly. However, when growth was measured during active growth, significant differences in growth were observed except that Escherichia coli and Clostridium perfringens appeared to be unaffected by the environments. All other bacteria exhibited some degree of growth inhibition in the 1 atm helium-oxygen environment. The helium-oxygen environment at 11 atms appeared to stimulate the growth of Pseudomonas aeruginosa and the two lactobacilli tested and inhibit growth of Corynebacterium hoagii and the two staphylococci tested. It did not seem to affect the growth of Pseudomonas fluorescens or Bacillus subtilis. Proteus vulgaris was inhibited by the helium-oxygen environment at either pressure to the same degree.

ATTENUATION OF ATRAZINE INJURY IN PLANTS BY THE FUNGICIDE p-DIMETHYLAMINOBENZENEDIAZO SODIUM SULFONATE. W. C. Walsh and R. H. Shimabukuro. Metabolism and Radiation Research Lab, USDA, ARS, Fargo, N. D. 58102

The fungicide, (p-dimethylaminobenzenediazo sodium sulfonate) (I), was found to delay the onset of acute injury from the herbicide, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine), in intermediately susceptible pea (Pisum sativum L.) and susceptible soybean (Glycine max Merrill.). The addition of I did not significantly affect inhibition of the Hill reaction by atrazine in leaf discs. In intact plants treated through their roots with a solution of 0.5 μM atrazine and 50 μM of I, inhibition of the Hill reaction was not as severe as in plants treated with atrazine alone. However, the "protective" action of I was more effective in pea than in soybean. The attenuation of atrazine injury by I seems to be a secondary effect and not due to a direct herbicide-fungicide interaction. Addition of I decreased atrazine absorption by the roots and translocation to the shoots more in pea than in soybean. Total metabolism of atrazine was not significantly affected in both species, but N-dealkylation was slightly reduced in soybean but not in pea. The mechanism for the reduction in atrazine absorption by I is unknown.

CYTOTAXONOMIC STUDY OF THE SCIRPUS LACUSTRIS COMPLEX IN NORTH DAKOTA. Richard Ward and W. T. Barker. Dept. of Botany, North Dakota State Univ., Fargo, North Dakota.

Comparative chromosomal and morphological studies indicate that three species of Scirpus lacustris complex occur in North Dakota. These are S. acutus Muhl., S. heterochaetus Chase, and S. validus Vahl. Measurements of achenes, scales, spikelets and achene beaks have been made. It is possible to delineate the three species by correlating these measurements. Frequent hybridization in North Dakota reported by other authors has not been substantiated. Chromosome numbers have been determined for S. validus as n = 21, S. heterochaetus as n = 19, S. acutus as n = 19. Previous authors have reported S. validus as n = 21, S. heterochaetus as n = 20, and S. acutus as n = 18. Knowledge of distribution of S. acutus, S. heterochaetus, and S. validus has been increased through field studies over the 1968, 1969 and 1970 growing seasons.

ANALYSIS AND CONTROL OF FLOW NOISE AND SOUND REVERBERATIONS IN HYPERBARIC CHAMBERS. Dwight Wendschlag. Mech. Engr. Dept., University of N. Dak., Grand Forks, N. Dak.

Experiments with rats in hyperbaric chambers at pressures up to 600 psig indicated that they develop severe emotional stress and even death due to the noise from the circulating helium-oxygen environment. It became apparent that this noise level should be analyzed and controlled if other physiological experiments were to yield satisfactory results. Analysis was carried out with an audio spectrometer and a graphic level recorder, which yielded a graph of the noise amplitude vs its frequency. Noise levels above 120 db (re., 0.0002 microbar) were recorded. This exceeds the point of discomfort and approaches the region of pain. Methods of noise control attempted included: placing cotton in the inlet pipe, placing an external settling chamber filled with a sound absorbing material in the inlet pipe, and the addition of diffusers to the inlet pipe inside the chamber. All of these methods successfully reduced the noise levels and provided the animals with a more compatible environment.

VEGETATION OF THE FOREST RIVER BIOLOGY AREA IN RELATION TO SOME ENVIRONMENTAL GRADIENTS. D. A. Wikum and M. K. Wali. Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

This area, 45 miles northwest of Grand Forks, is ideal for a gradient analysis study because several large ravines and the river valley itself provide slopes of varying exposures and slope angles. Resulting diversity in environment has resulted in a rich vegetation within a small geographic area. Coverage values for about 80 woody and herbaceous species and basal area, frequency, density and mensuration data for 8 tree species were determined from 26 $10m^2$ plots. Three dominant tree species Quercus macrocarpa, Tilia americana, and Fraxinus pennsylvanica var. subintegerrima show the highest importance value (IV), being 128.3, 72.4 and 65.7 respectively. Soil samples collected from all genetic horizons from a pit in each plot have been analyzed for chemical and physical characteristics. Three types of gradients based on soil texture (% silt+clay), available water (1/3 - 15 bar), and replaceable nutrients have been established. The gradients have been corrected for bulk density and stoniness. Using coverage values, ecological modalities of many species are demonstrated along the quantified gradients. Generally trees show a bimodal, shrubs and herbs a unimodal distribution. (Supported in part by UND Faculty Res. Grant 4522-53).

VASCULAR FLORA OF PEMBINA COUNTY, NORTH DAKOTA. R. E. Willenbring and W. T. Barker. Dept. of Botany, North Dakota State Univ., Fargo, North Dakota.

Two years (1969-1970) of fieldwork combined with the compilation of data from herbarium specimens collected and deposited in the North Dakota State University Herbarium over the past 80 years has resulted in a checklist of 500 vascular plant species from Pembina County, North Dakota. Eighty-six plant families are represented in the Pembina County vascular plant flora. The three largest plant families are: the Compositae, Gramineae and Cyperaceae. Frequency and habitat information has been compiled for each species. Useful phenological data has been compiled for each species using herbarium specimens from the 39 northeastern North Dakota counties. Several interesting flowering and fruiting patterns that become evident are discussed. Approximately 3000 voucher specimens have been deposited in the North Dakota State University Herbarium as a result of this study.

ENUMERATION AND DIFFERENTIATION OF RUMEN MICROBIAL POPULATIONS OF GROWING CALVES IN ISOLATION. Phletus P. Williams. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D. 58102

Calves placed in isolation stalls 24-48 hr. after birth were studied for development of a rumen microflora free of ciliated protozoa. Differential rumen bacterial populations observed included coliforms, non-lactose and lactose fermenting bacteria, lactobacilli, hemolytics, lipolytics, and acidophilic-acidurics; and anaerobic-hemolytics and lipolytics. Counts of these microbes showed considerable variation in calves up to 50 days of age. Establishment of an anaerobic microflora within the first 7 days appears to be essential for development and stabilization of the rumen microflora. Calves 100 days of age showed a rumen microflora similar in composition to that of mature animals. However, calves free of ciliates showed, in general, a higher total anaerobic rumen bacterial population count. Inoculation of these calves with rumen ciliates resulted in a reduction in anaerobic bacterial count within 2 weeks.

PHYTOCHEMICAL STUDIES OF POISONOUS PLANTS OF NORTH DAKOTA--PART II. E.W. Wollmann and L.J. Schermeister. Dept. of Pharmacog., Col. of Pharm., N. Dak. State Univ., Fargo, N. Dak. and E.G. Schmiess. Sci. Ed., Univ. N. Dak., Grand Forks, N. Dak.

This phytochemical study is a continuation of the work of Sinha et al. Part I (Proceedings of the North Dakota Academy of Science 21:111, 1967) and provides additional phytochemical information on North Dakota plants. Thirty-six species of native plants were evaluated for the presence of alkaloids, saponins, tannins and flavanoids. These substances were semi-quantitatively determined using precipitation, hemolysis and gamma benzo-pyrone test methods. The results which are presented in tabular form, indicate that these chemical substances vary in amount and distribution. Most plant parts were positive for at least one of the chemical groups. Supported by N.S.F. (Grants GW-557 and GW-1723)

FLORISTIC SURVEY OF SOUTHWESTERN NORTH DAKOTA. N. K. Zaczkowski and W. T. Barker. Dept. of Botany, North Dakota State Univ., Fargo, North Dakota.

Intensive plant collecting in southwestern North Dakota (Billings, Bowman, Golden Valley, and Slope counties) during the 1969 and 1970 growing seasons has added to the knowledge of composition, habitat, and distribution of the native flora. Chief topographic features of the region include undulating plains, Badlands of the Little Missouri River, and prominent buttes; among them being White Butte, the highest elevation (3506 ft.) in the State. Mid-grass prairie, interrupted by stands of coniferous and deciduous trees and shrubs comprise the salient natural physiognomic groups. A preliminary checklist, compiled from the two-year collection shows that approximately 600 species of vascular plants occur in the 4500 sq. mile area. Seventy plant families are represented, with about one-third the species in the Compositae, Gramineae and Leguminosae. Of special interest is the genus Astragalus with 18 species, of which five are restricted to the extreme southwestern segment of the State. Many new entities have been discovered for the area and several state records recorded (Polypogon monspeliensis, Sporobolus airoides, Astragalus purshii, Gaura parviflora, Chaenactis douglasii).

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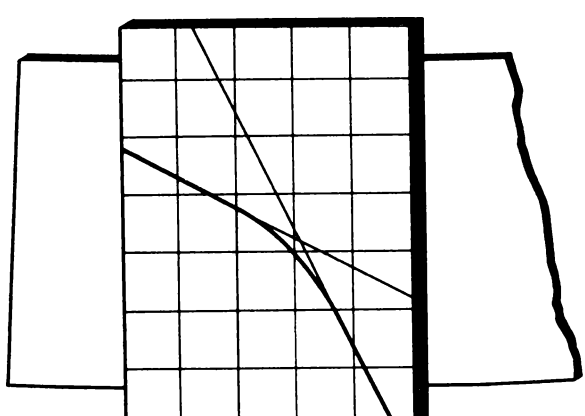

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INSTRUCTIONS TO AUTHORS FOR THE NORTH DAKOTA ACADEMY OF SCIENCE PROCEEDINGS

DEADLINES

Abstracts.—Both student competition and professional paper abstracts are due March 1 at the Office of the Secretary. 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 first week in May.

Papers.—Complete papers for student competition are due at the Office of the Secretary on April 1 so that time is available for judging. Complete professional papers are due at the time of oral presentation during the first week in May.

PRESENTATION OF MANUSCRIPT

General.—The general style for papers of the Proceedings will be that of *The Style Manual for Biological Journals* (Conference of Biological Editors, Committee on Form and Style, 1964. Style manual for biological journals. Second edition. American Institute of Biological Sciences, Washington, D.C. Available from: AIBS, 3900 Wisconsn 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 one copy (Xerox or similar copy), including illustrations.

A separate title page is to include authors names and their complete addresses (including zip code) and numbered as page one.

Each manuscript page, following the title page, is to be numbered consecutively as page two and following, and the number preceded by the author's name or the first author where several are involved.

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

Headings.—Major headings are centered and capitalized. Side headings 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 submitted on separate sheets ordinarily not exceeding the size of the manuscript page. Therefore larger drawings should be reduced photographically; if so, lines, lettering and symbols must be bold enough to stand the appropriate reduction.

Photographs must be unblurred and clearly show what is intended.

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

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

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

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

Tables.—Complex tables (those with vertical lines, characters on fractions 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 chymotrypsin

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

References.—References are to be listed at the end of the paper alphabetically and in the format of the *AIBS Style Manual*. Abbreviations of journals are also those suggested by the *Manual*. Examples of listing a book and journal are as follows:

Conference of Biological Editors, Committee on Form and Style. 1964. Style manual for biological journals. Second edition. Amer. Inst. Biol. Sci., Washington, D.C. 117 p.

Groenewold, G. H., and F. R. Karner. 1970. Preliminary classification of concretions and nodules in the Cretaceous Hell Creek Formation, North Dakota. N. Dak. Acad. Sci., Proc. 23 (II): 64-73.

Citations.—Citation of references in the text is by the name and year system. It may appear as Smith (1970, p. 21) or (Smith, 1970, p. 21). Figures and tables are also to be cited in the text. For example: In the second and later years females grew faster than males (Table 1, Figures 2-4).

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

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

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

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

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INTERPRETATION OF PARTIALLY-SMUTTED BARLEY PLANTS IN INHERITANCE STUDIES OF COVERED SMUT RESISTANCE[1]

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INTRODUCTION

Spring barley varieties susceptible to race 6, *Ustilago hordei* (Pers.) Lagerh. will produce sori in the leaves, heads and nodes when grown after inoculation under greenhouse conditions optimum for infection (Fischer and Holton, 1957 and Kiesling, 1962). All culms of a susceptible spring barley plant inoculated with the covered smut organism may not develop smutted organs (Kiesling, 1971). The term "partially-smutted plant" used in this paper refers to an inoculated barley plant which develops both healthy and smutted heads.

Chemical and physical factors of the environment, concentration of inoculum, and genetic composition of the host or parasite have been implicated as factors affecting the number of infected plants in an inoculated barley population (Fischer and Holton, 1957). Location of the inoculum on the coleoptile, spore concentration, and depth of seding also have been shown to affect the symptoms which develop on individual culms of an inoculated plant (Kiesling, 1962).

Interpretations of data from studies of the inheritance of resistance to *U. hordei* have been difficult because of highly variable results following inoculations of hybrid populations and their parental checks. In studies of the inheritance of resistance to barley covered smut, Shands (1956), Metcalfe (1962), and Thomas and Person (1965) classed all plants with one or more smutted heads as susceptible. Wells (1958) made no mention of partially-infected plants and apparently classed partially-infected plants as infected.

Odessa (C. I. 934) is highly susceptible and Pannier (C. I. 1330) is immune to race 6, *U. hordei* (Kiesling, 1962). Twenty-two lines of the cross, Odessa x Pannier and its reciprocal, were studied previously, and resistance to race 6, *U. hordei* was dominant in the F_1 seedlings (Kiesling, 1971). When F_2 seedlings were inoculated with race 6, *U. hordei* and grown to maturity, a number of partially-smutted plants developed (Table 1). The following study was undertaken to determine the genotype of partially-smutted F_2 plants.

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Table 1. Numbers of healthy, totally infected and partially infected plants in F_2 progeny of the barley cross, Odessa x Pannier and its reciprocal innoculated with race 6, U. *hordei*

Cross	Lines	Totally infected plants	Partially infected plants	Healthy plants
Odessa x Pannier	11	38	26	2365
Pannier x Odessa	11	26	16	2106

MATERIALS AND METHODS

Twelve F_3 lines from partially-smutted F_2 plants were selected from four F_2 crosses for further study. Partially-smutted F_2 plants frequently produced fewer kernels than healthy F_2 plants.

All of the seed was inoculated via the vacuum method of Tapke and Bever (1942). Inoculated seeds were removed from the vacuum, drained and incubated for 72 hr at 22 C. The inoculated seedlings were then planted in six-inch pots in the greenhouse to a depth of 1 -1.5 inches in a steam-sterilized, sand-soil-peat mixture. The plants were grown to maturity in a greenhouse with a temperature range of 20-24 C.

RESULTS AND DISCUSSION

Two F_3 lines, 5-2-5 and 5-2-33, from the F_2 cross 5-2 were segregating (Table 2). Three other F_3 lines, 5-2-32, 5-2-77, and 5-2-95, were probably homozygous for susceptibility. The genotype of F_3 line 5-2-85 was not determined since the F_3 population was too small to provide a reliable test. All three F_3 lines from the cross, 5-6 were probably segregating (Table 2). F_3 line 5-9-92 from the cross 5-9 was homozygous for susceptibility and line 5-9-188 was segregating. F_3 line 5-12-10 from the cross 5-12 was probably homozygous for susceptibility although the F_3 population was too small to provide a reliable test.

Table 2. Reaction of F_3 lines from partially infected F_2 plants of the barley cross, Odessa X Pannier, to inoculation with race 6, *U. hordei*

F_3 line	Totally infected plants	Partially infected plants	Healthy plants
5-2-5	7	9	32
5-2-32	4	0	1
5-2-33	0	1	10
5-2-77	3	2	0
5-2-85	2	1	4
5-2-95	20	3	3
5-6-1	9	6	26
5-6-147	3	13	48
5-6-152	4	7	22
5-9-92	23	2	5
5-9-188	4	6	16
5-12-10	4	1	2

The progeny of the single healthy plant in the F_3 line 5-2-32 were tested for a possible recessive gene (Table 3), but the F_4 and F_5 populations were predominantly susceptible.

Table 3. Reaction of F_3, F_4 and F_5 progeny of partially infected F_2 plants when inoculated with race 6, *U. hordei*

Line	Generation	No. of totally infected plants	No. of partially infected plants	No. of healthy plants
5-2-32	F_3	4	0	1
5-2-32-1	F_4	58	13	1
5-2-32-1-58	F_5	13	9	6
5-2-33	F_3	0	1	10
5-2-33-8	F_4	3	0	28

The F_3 line 5-2-33 contained a single partially-infected plant (Table 3). When the F_4 seedlings of this partially-infected F_3 plant were inoculated and grown to maturity, three totally infected and 28 healthy plants were obtained (Table 4). The reaction of F_3 line 5-2-33 and F_4 line 5-2-33-8 to these inoculations is similar to the response of barley varieties with an intermediate reaction to inoculation with race 6, *U. hordei* (Tapke, 1945).

Table 4. Reaction of F_3 and F_4 lines of a partially infected F_2 plant of the cross, Odessa x Pannier, to inoculation with race 6, *U hordei*

Generation and line		No. of plants totally infected	No. of plants partially infected	Number of healthy plants
F_3	5-2-5	7	9	32
F_4	5-2-5-1	10	6	21
F_4	5-2-5-2	0	0	37
F_4	5-2-5-5	1	3	32

In the F_3 test of line 5-2-5, F_3 plant 5-2-5-1 was partially-infected and F_3 plants 5-2-5-2 and 5-2-5-5 were healthy. The F_4 progenies of these three plants were inoculated with race 6, *U. hordei*. The F_4 data indicated that line 5-2-5-1 was probably segregating and 5-2-5-5 was intermediate in reaction (Table 4). No totally or partially-infected plants were found among the 37 inoculated seedlings of F_4 line 5-2-5-2. This line was, therefore, classified as resistant and was a resistant F_4 line selected from among the progeny of a partially-infected F_2 plant.

A total of 12 F_3 lines from partially-infected F_2 plants were inoculated (Table 5). The data show that the F_3 populations of five of these lines were segregating for reaction to race 6, *U. hordei*. One F_3 line, 5-2-33, possibly was homozygous resistant but with an intermediate type reaction to race 6 (Kiesling, 1962 and Tapke, 1945).

Table 5. Hypothesized genetic compositions of partially infected barley plants of the cross, Odessa x Pannier

Class	Number of F_3 lines from partially infected F_2 plants in each class
Homozygous susceptible	4
Heterozygous resistant	5
Homozygous resistant	1
Populations too small to interpret	2

Four F_3 lines were derived from susceptible F_2 plants, and two F_3 lines had populations too small to interpret accurately. Fifty per cent of the partially-infected F_2 plants whose F_3 progeny were tested for reaction to race 6, *U. hordei* were found to be either segregating for reaction to race 6 or, possibly, to be of an intermediate (resistant) type reaction.

If these partially-infected F_2 plants were classed as susceptible, then a skewed distribution of the susceptible class might be expected when attempting to establish a genetic hypothesis. When partially-smutted plants were classed as homozygous susceptible, a larger number of susceptible individuals was observed than was expected in fifteen of the twenty-two lines of the cross Odessa x Pannier and its reciprocal inoculated with race 6, *U. hordei* (Kiesling, 1971). It was concluded, therefore, that the genotypes of partially-smutted plants could not be accurately classified until tests of their progeny were carried out.

LITERATURE CITED

Fischer, G. W., and C. S. Holton. 1957. Biology and control of the smut fungi. The Ronald Press Co., N. Y. 622 p.

Kiesling, R. L. 1962. The effect of temperature and point of inoculation on the symptomology of barley covered smut. Phytopathology 52: 16-17. (Abstr.)

Kiesling, R. L. 1971. The inheritance of resistance to *Ustilago hordei* in spring barley. Barley Genetics II. Proceedings of Second International Barley Genetics Symposium. 500-507.

Metcalfe, D. R. 1962. Inheritance of resistance to loose smut, covered smut and false loose smut in the barley variety Jet. Can. J. Pl. Sci. 42: 176-189.

Shands, R. G. 1956. Inheritance of covered smut resistance in two barley crosses. Agron. J. 48:81-86.

Tapke, V. F. 1945. New physiologic races of *Ustilago hordei*. Phytopathology 35: 970-976.

Tapke. V. F., and W. M. Bever. 1942. Effective methods of inoculating seed barley with covered smut (*Ustilago hordei*). Phytopathology 32: 1015-1021.

Thomas, P. L., and C. Person. 1965. Genetic control of low virulence in *Ustilago*. Can. J. Genet. Cytol. 7: 583-588.

Wells, S. A. 1958. Inheritance of reaction to *Ustilago hordei* in cultivated barley. Can. J. Pl. Sci. 38: 45-60.

INHERITANCE OF PATHOGENICITY IN THE WHEAT LEAF RUST FUNGUS *PUCCINIA RECONDITA*[1]

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INTRODUCTION

Leaf rust of wheat, incited by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*, is a serious wheat disease in North America. Leaf rust resistant wheat varieties are used to control this disease. Resistant wheat varieties are attacked when virulent cultures of the leaf rust fungus develop. This occurs more frequently if a single gene-governing resistance is used in a breeding program (Anderson, 1961). The practice of using single genes for resistance allows the pathogen to adapt and attack many of the newly developed varieties once they are grown over large acreages.

Hybridization is one way that variation in pathogenicity of the leaf rust population occurs. Native species of *Thalictrum*, the alternate host of wheat leaf rust, are potential hybridization sites for the fungus (Young, Saari and Curtis, 1965). If a race of leaf rust heterozygous for pathogenicity hybridizes on *Thalictrum*, a number of genetic recombinants virulent on resistant varieties may develop. Several races of leaf rust were reported to be heterozygous for pathogenicity (Brown and Johnson, 1949; Dyck and Samborski, 1968; Samborski and Dyck, 1968). This study was made to determine if hybridization could account for changes in virulence in the leaf rust fungus.

MATERIALS AND METHODS

Thirty-four uredospore cultures of *P. recondita* were collected in plots of durum wheat (*Triticum durum* Desf.) and hard red spring wheat (*T. aestivum* L.) near Fargo in the summer of 1970. The cultures were increased on Little Club wheat and single pustuled. The virulence of the cultures derived from single pustules was tested on eight wheat lines with single genes for leaf rust resistance. The isogenic wheat lines contained genes *Lr*1, *Lr*2, *Lr*2D, *Lr*3, *Lr*10, *Lr*16, *Lr*17, and *Lr*18. Leaf rust races were identified based on resistance or susceptibility of host genes *Lr*1, *Lr*2, *Lr*2D and *Lr*3.

Teliospores were collected from the same durum plots used in uredial collections late in the summer of 1970. The dormancy of the teliospores was broken by several alternate wet-dry periods. Alter-

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nate wet-dry periods consisted of 24 hr at 100% relative humidity in an inoculation chamber followed by 24 hr on the greenhouse bench. When the teliospores began to germinate, they were placed over *Thalictrum speciosissimum* Loefl. (Meadow rue), the alternate host of wheat leaf rust. Numerous pycnial infections formed. Both reciprocal and mass spermatizations were made with a small brush when the pycniospores and nectar formed. Little Club wheat was inoculated with aeciospores from each fungal mating. The resulting 29 uredial cultures were single pustuled, and the virulence tested on the single gene lines. The virulence formulas and race determinations were compared to those of uredial cultures obtained from the same plots where the teliospores were collected.

RESULTS AND DISCUSSION

Ten of the 29 cultures derived from fungal matings had different virulence combinations than those found in durum or hard red spring plots at Fargo in 1970 (Table 1). Six of these were different from any of the 180 cultures collected in North Dakota in 1970 (Statler, 1971). Five of the cultures were more virulent and one was less virulent than any collected during the summer of 1970.

Table 1. Number of *P. recondita* cultures collected from plots of *T. aestivum* and *T. durum* compared to those derived from fungal matings that were virulent on 21 host gene combinations

Host genes	Number of virulent cultures		
	T. aestivum	*T. durum*	Fungal matings
0	0	0	1
3	4	0	1
3,10	7	7	16
3,18	0	0	1
1,2D,3	0	0	1
1,3,10	1	1	0
2,3,10,	1	0	0
2D,3,10	2	1	0
2D,3,16	1	0	0
2D,3,17	1	0	0
3,10,16	0	1	0
3,10,17	1	0	0
3,10,18	0	1	0
1,2,2D,10	0	0	1
1,2,3,16	0	0	1
2,2D,3,10	1	0	1
2D,3,10,17	1	0	0
2D,3,10,18	1	0	0
3,10,16,17	0	1	1
1,2,2D,3,10	0	0	5
3,10,16,17,18	1	0	0
Total	22	12	29

Segregation within the natural leaf rust population was demonstrated even when a relatively small number of cultures were derived by fungal crossing. Lines with host genes *Lr*1 and *Lr*2 were parasitized by a greater percentage of cultures from the crosses than from field collections (Table 2). Only one of the uredial cultures collected from the durum plots attacked *Lr*1. None attacked *Lr*2. In contrast, wheat lines with genes *Lr*17 and *Lr*18 were attacked by a larger percentage of cultures from field collections than from the cultures established from spermatizations.

Table 2. Number of *P. recondita* isolates from field collections and fungal matings on single gene host lines

	Virulence			
	Field collections		Fungal matings	
Host genes	No. of isolates	δ of total	No. of isolates	δ of total
Lr1	2	5.4	8	27.5
Lr2	2	5.4	8	27.5
Lr2D	9	24.3	8	27.5
Lr3	37	100.0	27	93.1
Lr10	31	83.8	24	88.8
Lr16	4	10.8	2	6.8
Lr17	5	13.5	1	3.4
Lr18	4	10.8	1	3.4
Total	37		29	

One culture recovered from the fungal crosses was not virulent on any of the isogenic lines tested. It was less virulent on Little Club (infection type 3) than any uredial culture collected in the summer of 1970 (Statler, 1971).

The variation in virulence of the cultures collected in field plots compared to those obtained in the hybridization study also is shown by UN race identification in Table 3. Race UN 2 represented the

Table 3. Frequency of UN races of *P. recondita* obtained from field plots and from fungal matings

UN race	*T. aestivum*	*T. durum*	Fungal matings
1	0	0	1
2	13	12	18
3	6	2	0
5	1	1	0
6	0	0	1
7	0	0	1
9	0	0	1
13	0	0	5
17	1	0	1
25	1	0	0

largest proportion of the race population collected from the field and from greenhouse crosses. UN races 1, 6, 7, 9 and 13 were identified from the crosses but not from the field plot collections. UN race 3 and 25 were identified from the field collections but not from the crosses. The most significant differences were the eight cultures of UN 3 collected from the field, but not identified from crosses, and conversely the five cultures of UN 13 recovered from the crosses, but not from field collections.

The fact that cultures resulting from spermatizations had a wider range of virulence than field cultures is evidence for heterozygosity in the natural leaf rust population. This wide range in virulence is also indicative of the role of the alternate host as a source of new rust races through hybridization in the natural leaf rust population. The general trend of segregation after hybridization was toward cultures more virulent on host genes *Lr*1 and *Lr*2.

LITERATURE CITED

Anderson, R. G. 1961. The inheritance of leaf rust resistance in seven varieties of common wheat. Can. Jour. Pl. Sci. 41: 342-359.

Brown, A. M., and T. Johnson. 1949. Studies on variation in pathogenicity in leaf rust of wheat, *Puccinia triticina* Erikss. Can. J. Res. 27: 191-202.

Dyck, P. L., and D. J. Samborski. 1968. Host-parasite interactions involving two genes for leaf rust resistance in wheat. Third International Wheat Genetics Symposium. Aust. Acad. Sci. Canberra. p. 245-250.

Samborski, D. J., and P. L. Dyck. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. Can. J. Genet. Cytol. 10: 24-32.

Statler, Glen D. 1971. Wheat leaf rust in North Dakota in 1970. Pl. Dis. Reptr. 55: 172-175.

Young, H. C., Jr., E. E. Saari, and B. C. Curtis. 1965. The potential function of native *Thalictrum* species as an alternate host of *Puccinia recondita* f. sp. *tritici*. Phytopathology 55: 502. (Abstr.)

ANALYSIS OF A SALINE TALLGRASS PRAIRIE ECOSYSTEM. IV. PRELIMINARY INVESTIGATIONS ON SOIL ALGAE

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ABSTRACT

Records of soil algae in the state are lacking. Collections of soil were made monthly September through November 1970 at the Uni-

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versity of North Dakota Oakville Prairie field station 13 miles west of Grand Forks. Vertical soil samples were obtained with a 14-cm vertical core and analyzed both qualitatively and quantitatively by using sub-samples at 2-cm depth intervals. Cultures were established using 5 gm of soil in Bold's Basal Medium with a two week incubation period. Twenty-one species were identified which include: 8 Chlorophyceae, 7 Cyanophyceae and 6 Bacillariophyceae. As expected, fewer algal cells of most species were found at lower soil levels (i.e. 32,851 cells/ml at 0-2 cm; 6779 cells/ml at 6-8 cm and 406 cells/ml at 12-14 cm). Greatest total cell numbers of Chlorophyceae were measured at subsurface levels (4-6 cm) as was similarly true for Bacillariophyceae. Cyanophyceae reached highest levels at the immediate surface and diminished with depth.

INTRODUCTION

Algae are predominantly aquatic, but there exists a rich soil flora which is an important factor in the ecology of the soil. Algae often act as primary colonizers of soil substrates, and help bind the soil together Booth (1941). Members of the Cyanophyceae are important because of their nitrogen fixation property (Fogg, 1956). The species present and their distribution in any particular soil seem to be dependent on several factors: soil texture, soil chemistry, temperature, light, and moisture. Shields and Durell (1964) give an excellent review of these and other influencing factors.

There exists little, if any, literature on the soil microflora of North Dakota or adjacent states. The only study in direct proximity to North Dakota is a soil algae survey made in Manitoba by Lowe and Mayse (1934).

Information on vertical distribution of algae in the soil in North American literature is extremely limited. Smith (1944) and Morre and Carter (1926) noted the phenomenon of decrease in numbers of cells with increased soil depth. Wilson and Forest (1957) took 6-inch vertical cores and cultured top, middle and bottom thirds and noted only that the flora was similar throughout the profile though usually more luxuriant at the surface. Little work has been done on quantifying the algae in a soil profile.

In Britain, John (1942) sampled the upper 4 cm and noted that certain species were confined to or excluded from the upper half or lower half of the profile.

Therefore it was the intent of this preliminary study to: 1) record the microalgae present in saline soils from several sites on a tall grass prairie ecosystem in North Dakota and 2) to quantify the "reproductive potential" of soil algae under selected conditions at various vertical levels in these soils.

MATERIALS AND METHODS

Collections of soil were made monthly September through November 1970 at the University of North Dakota Oakville Prairie field station 13 miles west of Grand Forks. Samples were taken with a vertical core to a depth of 14 cm, the cores removed and divided at 2-cm depth intervals and placed in sterile collecting bags,

and held in storage until air dried. The coring apparatus was cleaned and rinsed with ethanol between samples to avoid contamination. Soil pH and specific conductivity were measured from a 1:2.5 soil: water paste.

In analyzing the vertical distribution of soil algae, two series of duplicate cultures were established using 5 gm of soil from each 2-cm interval with 50 ml of Bold Basal Media (Deason and Bold, 1960) in 125-ml erlenmyer flasks. Cultures were incubated for two weeks in a controlled environmental chamber at a temperature of 20±2°C and a light intensity of 4300 lux on a 16 hr light/8 hr dark cycle. The cultures were examined, species identified and cells enumerated using a Sedwick-Rafter counting cell. Cultures at alternate 2-cm intervals (i.e. 0-2 cm, 4-6 cm, etc.) were enumerated in the second series for comparison. The authorities used for identification were: for Bacillariophyceae, Hustedt (1930) and Lund (1946); for Cyanophyceae, Geitler (1932) and Kantz and Bold (1969); and for Chlorophyceae, Groover and Bold (1969), Mattox and Bold 1962), and Fott and Novakova (1969).

Cultures were also established to examine growth, species composition and succession after the normal 2-week interval. Selected species were isolated into unialgal culture either BBM or a Bristol's agar preparation (Bold, 1949) to aid in identification.

RESULTS

Examination of cultures after a 2-week incubation period revealed several characteristic patterns. The total number of cells and the total number of species in cultures decreased noticeably with lower soil profiles (Table 1). After 2 weeks of incubation at the conditions described, total algal cell numbers in the uppermost layer (0-2 cm) averaged 32.851/ml as compared to 6778/ml at intermediate levels (6-8 cm) and 406/ml at lower levels (12-14 cm). This trend was also evident in the macroscopic appearance of the flasks after 2 weeks. The growth ranged from obvious visible growth in cultures of the upper layers (0-6 cm), to what appeared to be negligible growth in cultures of the lower soil levels (10-14 cm). In considering individual species, the same trend was exhibited. Most species showed a decline in cell numbers in lower soil levels, the most indicative being the genera *Chlorococcum* and *Oscillatoria* (Figures 1 and 2).

Several other phenomenon were noted. Certain species grew in greater numbers at subsurface levels (2-6 cm) than at the immediate surface (i.e. *Ulothrix minuta* and *Surirella ovata*) or only at subsurface levels (i.e. *Chroococcus*) This was also illustrated by *Oscillatoria* and *Chlorococcum* which show a curious increase in cell numbers between 6-10 cm (Figure 1 and 2). In considering vertical distributional patterns of major algal classes, after 2 week incubation, the greatest number of cells of Chlorophyceae were measured at subsurface levels (4-6 cm) with a similar but less pronounced trend apparent with Bacillariophyceae at 2-4 cm (Figure 3). Cyanophyceae reached highest levels at the immediate surface, diminishing with depth, but again exhibited a slight increase in cell numbers at the 6-8 cm level.

Table 1. Number of algal cells/ml of BBM at 2-cm depth intervals after 2-week incubation period

Species	0-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-14 cm
Anabaena sphaerica	95 *798	219					
Chroococcus sp.				38 *209			
Nostoc commune	1,055 *1,069						
Oscillatoria spp.	29,916 *30,795	1,985	1,254 *1,577	6,223	381 *361	171	380 *375
Chlorococcum sp.	766 *1,054	1,026	839 *1,615	437	808 *247	147	26 *27
Gonium sp.			627				
Protococcus viridis	26 *38						
Ulothrix minuta	409		3,667	74			
Hantzschia amphyoxis	399 *467	323	9 *10	6	9		
Navicula mutica	26 *26	19	2				
Navicula seminulum	9 *22	76		10	5		
Nitzschia ignorata	95 *29	9	35 *10				
Surirella ovata	57 *26	190	154 *21	26			

*Indicates second series

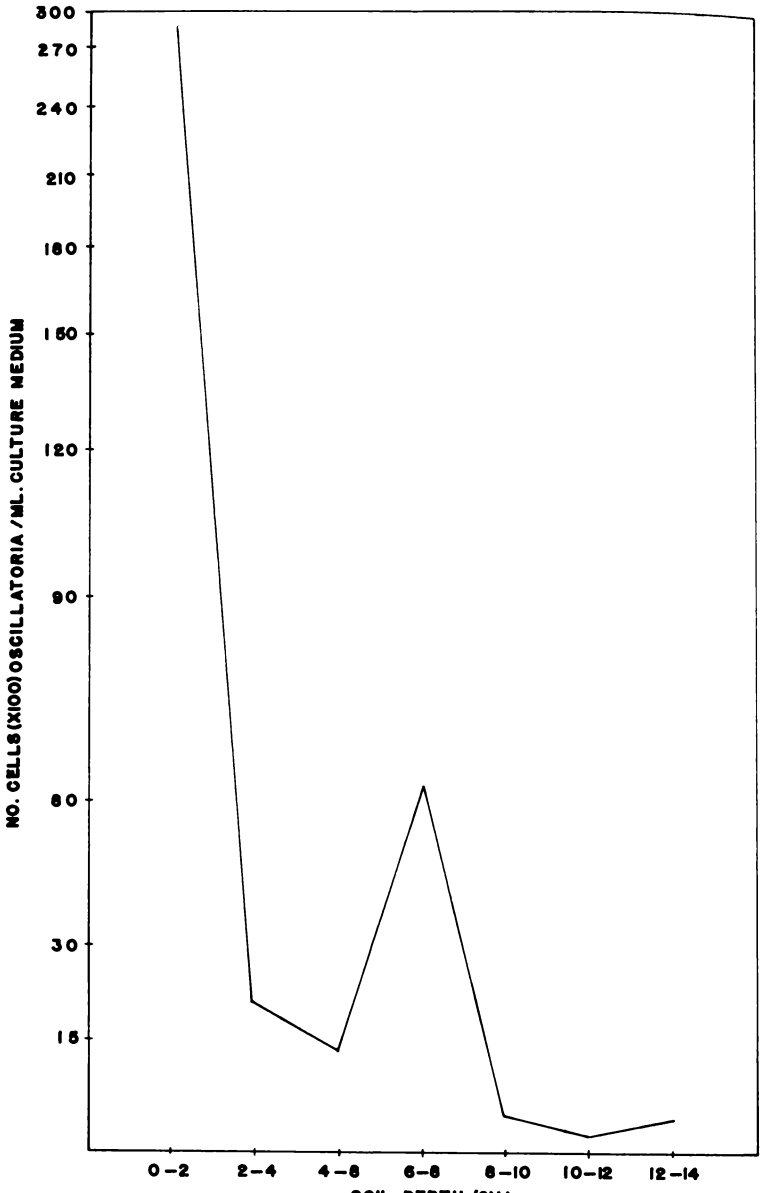

Figure 1. Vertical distribution of *Oscillatoria* spp. with soil layer (0-14 cm) measured at 2-cm intervals. Cell counts were made after two weeks incubation in BBM.

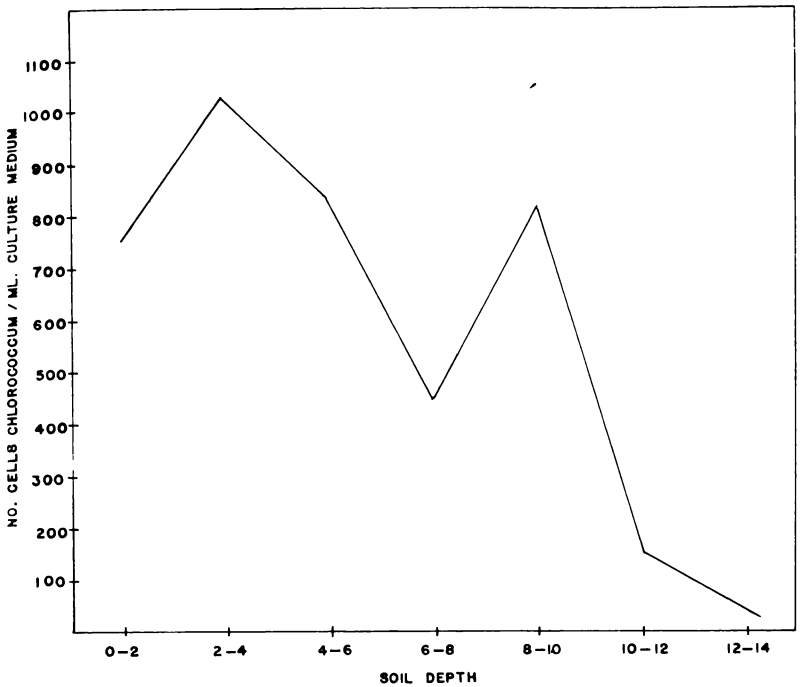

Figure 2. Vertical distribution of *Chlorococcum* sp. with soil layer (0-14 cm) measured at 2-cm intervals. Cell counts made after two weeks incubation in BBM.

Most species were restricted to the top 8 cm and only two forms, *Chlorococcum* and *Oscillatoria*, were present throughout all depths though at much reduced numbers at the lower levels.

A total of 21 species (Table 2) were identified during the course of the study. Several species were not encountered until the cultures had reached an age of several weeks (e.g. *Stigonema minutum* and *Cylindrospermum stagnale)*. This successional pattern developed in flasks of the top 6 cm such that at 2 weeks the dominant organisms were *Oscillatoria* and *Chlorococcum;* after 3-4 weeks cultures were dominated by *Ulothrix minuta* and Cyanophyceae (i.e. *Nostoc commune, Oscillatoria* spp., and *Anabaena sphaerica)*, and after 5-6 weeks a dominance of Cyanophyceae was established *(Nostoc commune, Anabaena sphaerica, Cylindrospermum* and *Stigonema minutum)*. There seemed to be a correlation between the number of species present and the age of the culture. Relatively young cultures (2 weeks) consisted of many species (14) whereas older cultures were dominated by only a few species, predominantly blue-greens (i.e. 3 weeks 13 species, 4 weeks 6 species and 5 weeks 5 species).

The pH of soil samples in this study was 8.1-8.3; specific conductivity was 3.4 millimhos/ cm.

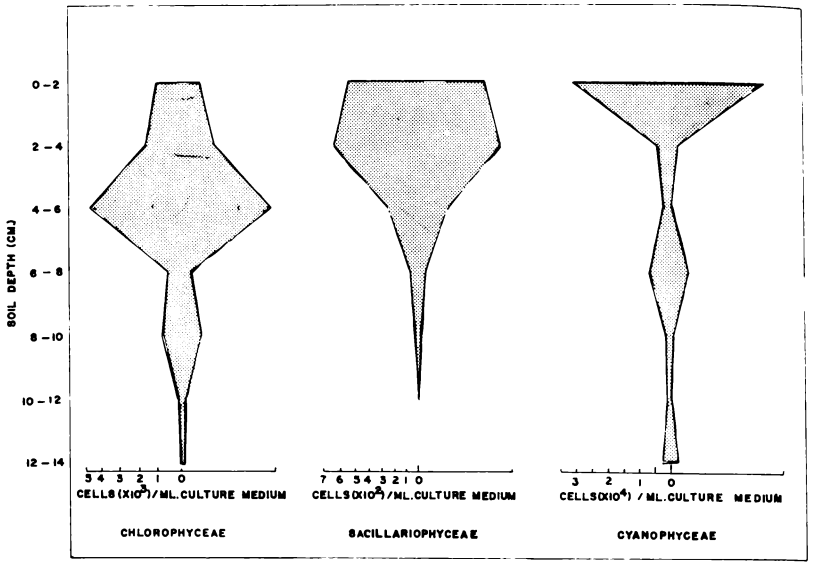

Figure 3. Distribution of Chlorophyceae, Bacillariophyceae and Cyanophyceae with soil layer (0-14 cm) showing abundance of each group at 2-cm depth intervals.

DISCUSSION

The number of individuals and species encountered in each culture is the result of the growth of not only the organisms growing there naturally but also of spores and encysted stages present in the soil. The patterns that were observed in this study are probably not a reflection of the actual vertical growth pattern of algae in the soil, but approximates the potential under ideal growing conditions. The presence of spores at lower levels due to leaching by rain or by animal transport may be misleading as far as the vertical distribution is concerned. Most authors feel that actively growing and reproducing cells are limited to the top few centimeters of the soil; however, Moore and Karrer (1919) found species to a depth of 1 m. Parker, Bold and Deason (1961) and Lewin and Lewin (1960) have demonstrated the heterotrophic ability of pigmented algae, thus giving credibility to the actively growing algae at depth in the soil.

Quantification of microalgae at various levels within the soil profile is extremely controversial. One must examine the reproductive potential of individual species in the laboratory by observing the number of reproductive cells (i.e. zoospores, gametes) released by dormant stages. Also, the environmental conditions influencing the release of these reproductive cells must be examined before estimation of algal numbers within the soil profile can be fully realized. However, the present study lends some insight to the "potential productivity" under the conditions described which can be further investigated with laboratory studies of individual species.

Table 2. Species of algae collected from Oakville Prairie, North Dakota

Cyanophyceae
Anabaena sphaerica Born. & Flah.
Chroococcus sp.
Cylindrospermum stagnale Kuetz.
Lyngbya sp.
Nostoc commune Vaucher
Oscillatoria spp.
Stigonema minutum (Ag.) Hassal
Chlorophyceae
Chlamydomonas sp.
Chlorella vulgaris Beijerinck
Chlorococcum sp.
Chlorosareinopsis pseudominor Groover & Bold
Fritschiella tuberosa Iyeng.
Gonium sp.
Protococcus viridis Agardh.
Ulothri minuta Mattov & Bold
Bacillariophyceae
Fragilaria sp.
Hantzschia amphyoxis (Ehr.) Grun.
Navicula mutica Kutz.
Navicula seminulum Grev.
Nitzschia ignorata Krasske
Surirella ovata Kutz.

Finding certain species at subsurface levels that were not present at the immediate surface is at variance with Peterson's (1932) observation that "no algal species [are present] at depth [that are] not found at the surface." The phenomenon of more prolific growth at subsurface levels has been observed by Copeland (1932) who stated that "at times of unfavorable growth more algae may be present at 2-6 inches than at the surface". This phenomenon was also noted in the present study (Figure 3). Explanations for such a trend can only come from critical laboratory investigations of individual species coupled with measurements of physico-chemical conditions with exist at each evel. Such factors as nutrient availabiliy at these subsurface levels due to leaching processes, among others, may well explain such occurrences.

Bacillariophyceae as a group seemed generally to occupy the upper soil layer whereas Cyanophyceae and Chlorophyceae were spread throughout the sampling area, with certain species showing affinities for specific levels.

One of the primary problems of a study of this type is taxonomy. Many of the soil algal forms are poorly described and little work has been done on them. The approach to taxonomy is microbiological rather than morphological as is normally used for aquatic algae (Bold, 1970). Many forms require isolation and complete life history studies before they can be identified to species. Another difficulty

is that seasonal succession may exist. Certain species were observed in cultures from samples collected in the early fall that were not present in the later samples, and vice versa.

The species identified are all new state records for soil algae since no other work has been conducted in this area, and the list (Table 2) represents a partial compilation of the algal microflora of the upper 14-cm layer of an alkaline virgin prairie grassland soil.

REFERENCES CITED

Bold, H. C. 1949. The morphology of *Chlamydomonas chlamydogama* sp. nov. Bull. Torrey Bot. Club 76: 101-108.

Bold, H. C. 1970. Some aspects of the taxonomy of soil algae. Ann. N.Y. Acad. Sci. 175: 601-616.

Booth, W. E. 1941. Algae as pioneers in plant succession and their importance in erosion control. Ecol. 22:38-46.

Copeland, J.J. 1932. Nitrogen fixation by Myxophyceae. Am. J. Bot. 19: 884.

Deason, T. R., and H. C. Bold. 1960. Phycological Studies I. Exploratory studies of Texas soil algae. Univ. of Texas Publ. No. 6022, Austin Tex.

Fogg, G. E. 1956. Nitrogen fixation by photosynthetic organisms. Ann. Rev. Plant Phys. 7: 51-70.

Fott, B., and M. Novakova. 1969. A monograph of the genus *Chlorella* the freshwater species. *In* Fott, B. [ed.] Studies in Phychology. E. Schweizerbart'schue Verlagsbuchhandlung., Stuttgart.

Geitler, L. 1932. Cyanophyceae. Kryptogamen-Flora von Deutechlands, Osterreich und der Schweiz. ed. L. Rabenhorst. Akademische Verlagsgesellschaft.

Groover, R. D., and H. C. Bold. 1969. Phychological Studies VIII. The taxonomy and comparative physiology of the Chlorosarcinales and certain other edaphic algae. Univ. of Texas Publ. No. 6907. Austin, Texas.

Hustedt, F. 1930. Bacillariophyta. *In* Pascher, A [ed.] Die Susswasserflora Mitteleuropas. Vol. 10, Jena: G. Fischer.

John, R. . 1942. An ecological and taxonomic study of the algae of British soils. I. The distribution of the surface growing algae. Ann. Bot. n.s. 6: 323-349.

Kantz, T., and H. C. Bold. 1969. Phychological Studies IX. Morphological and taxonomic investigations of *Nostoc* and *Anabaena* in culture. Univ. of Texas Publ. No. 6924. Austin, Texas.

Lewin, J.C., and R. A. Lewin. 1960. Auxotrophy and heterotrophy, in marine littoral diatoms. Can. J. Microbiol. 6: 127-134.

Lowe, C. W., and A. V. Mayse. 1935. An investigation of some Manitoba soils for the presence of soil algae. Trans. Roy. Soc. Can. 28 -V: 119-153.

Lund, J. W. G. 1946. Observations of soil algae. I. The ecology, size and taxonomy of British soil diatoms. New Phytol. 45: 56-110.

Mattox, K. R., and H. C. Bold. 1962. Phychological studies III. The taxonomy of certain ulotrichacean algae. Univ. Texas Pub. No. 6222.

Moore, G. T., and J. L. Karrer. 1919. A subterranean algal flora. Ann. Mo. Bot. Gard. 6:281-307.

Moore, G. T., and N. Carter. 1926. Further studies on the subterranean algal flora of the Missouri Botanical Garden. Ann. Mo. Bot. Gard. 13: 101-117.

Parker, B. C., H. C. Bold, and T. R. Deason. 1961. Facultative heterotrophy in some Chloroccacean algae. Sci. 133: 761-763.
Peterson, J. B. 1932. The algal vegetation of Hammer Bakker. Bot. Tidskr. 42: 1-48.
Shields, L. M., and L. W .Durrell. 1964. Algae in relation to soil fertility. Bot. Rev. 30: 92-128.
Smith, F. B. 1944. Occurrence and distribution of algae in soils. Proc. Fla. Acad. Sci. 7: 44-49.
Willson, D. and H. S. Forest. 1957. An exploratory study on soil algae. Ecol. 38: 309-313.

PHYTOCHEMICAL STUDIES OF POISONOUS PLANTS OF NORTH DAKOTA. PART II

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ABSTRACT

This phytochemical study is a continuation of the work of Sinha et al. (1967) and provides additional phytochemical information on North Dakota plants. Thirty-six species of native plants were evaluated for the presence of alkaloids, saponins, tannins and flavonoids. These substances were semi-quantitatively determined using precipitation, hemolysis and gamma benzopyrone test methods. The results indicate that these chemical substances vary in amount and distribution. Most plant parts were positive for at least one of the chemical groups.

INTRODUCTION

The toxicity of plants has been known since ancient times. A complete knowledge of their toxic components has become increasingly important. The plant kingdom according to Raffauf (1960, p. 276) is the source of an extensive variety of chemical compounds ranging in complexity from the simple, but extremely toxic, potassium fluoracetates found in spurges to the complicated pyrrole derivatives in chlorophyll. The alkaloid, saponin, flavonoid and tannin contents of some temperate and tropical plants of North America have been included in the recent works of Dominquez et al. (1960, p. 158) and Farnsworth et al. (1966, p. 101).

The flora of North Dakota (Stevens, 1963, p. 324) includes approximately 139 species which have been considered by Pammel (1911, p. 977), Kingsbury (1964, p. 626), and Muenscher (1940, p. 327) to be chemically poisonous to animals and man. Phytochemical surveys of 85 poisonous North Dakota plant species were recently conducted by McCracken and Schermeister (1970, p. 19), Nadodwalla

(1967, p. 30), and Sinha (1966, p. 25). This phytochemical study is a continuation of the work of Sinha et al. (1967) to provide more complete phytochemical information on North Dakota plants.

MATERIALS AND METHODS

Collection and preparation of plant material. — The plant material was collected by the Department of Pharmacognosy from native and naturalized plants throughout the state of North Dakota. The plants were identified by Dr. O. A. Stevens and voucher specimens of each species were retained by the Department of Pharmacognosy at North Dakota State University. Nomenclature follows that of Stevens (1963).

The fresh plant material was air-dried at room temperature and separated into various plant parts wherever feasible. The material was then ground to a number 40 powder and stored in tight containers till needed.

To prepare the alcohol extracts, 5 g of the powdered sample were minced in a Waring blender for 5 minutes with 40 ml of 80% ethanol. The ground material was then placed in a reflux flask and refluxed for 2 hrs, filtered with Whatman 1 filter paper and washed with sufficient 80% ethanol to bring the filtrate to 50 ml.

To prepare the water extracts, 5 g of the powdered sample were minced in a Waring blender for 5 minutes with 50 ml of water. The material was then filtered and washed with sufficient water to bring the filtrate to 50 ml. Ten ml of the filtrate were removed for the saponin, tannin and flavonoid tests. The remainder of the filtrate was evaporated under a vacuum to 20 ml and used for the alkaloid test.

Identification of chemical groups. — The plants under investigation were tested for the presence of alkaloids, saponins, flavonoids, and tannins. The methods used for the extraction and indentification of the chemical groups were those of Persinos et al. (1964, p. 329).

Alkaloids: Four 10-ml portions of the alcohol extract were evaporated to dryness by heating in a water bath. The residue was then dissolved in 5 ml of hot acidified water (1.5% hydrochloric acid) and filtered. The filtrate was tested with Mayer's, Valser's, Bertrand's, and Kraut's reagents. The 20 ml of evaporated water extract was divided into four equal portions and also tested with Mayer's, Valser's, Bertrand's and Kraut's reagents.

The precipitates produced by the alkaloidal reagent were semiquantitatively estimated with equal volumes of sample solution and standard alkaloid solution (atropine dissolved in water), and ranged in concentration from a trace amount (0.5 mg/ml) as (1+) to large amounts (50 mg/ml) or over (4+). All precipitates were evaluated with the eye. It is inferred that no precipitate means the absence of alkaloids. A positive test for alkaloids was reported only if the precipitate was obtained with both the Mayer and Bertrand reagents. Consistent precipitate with all reagents should be interpreted as suggesting the presence of one or more alkaloids or alkaloidal substances.

Saponins: The saponins usually hemolyze red blood cells in addition to frothing in aqueous media. Therefore, the test for hemolysis was used for the detection of saponins. Ten ml of whole

human blood was suspended in 100 ml of physiological saline solution. The mixture was then centrifuged, and the supernatant fluid was decanted. The red blood cells were washed twice more with saline and the volume was brought back to 100 ml. Ten ml of this red blood cell suspension was standardized by mixing with 1 ml of 0.1% alcohol-digitonin solution and allowed to stand for 5 minutes to obtain complete hemolysis.

To test the extracts for the presence of saponins, 1 ml of the ethanolic extract of water extract was added to 10 ml of the standardized red blood cell suspension and the time for hemolysis was recorded. Complete hemolysis within 1 minute was taken as the presence of large amounts of saponin (4+) whereas hemolysis after 5 minutes was considered the result of trace amounts (1+).

Flavonoids: A distinctive color reaction occurs when these compounds are reduced in alcoholic solutions with hydrochloric acid in the presence of sodium amalgam or magnesium. This reaction is due to the presence of the gamma benzopyrone nucleus which is common to all flavonoids. To test for the presence of flavonoids in the plant extracts, 1 ml of 80% ethanolic extract or water was tested with 0.5 ml of 10% hydrochloric acid and a few magnesium turnings.

The intensity of color reactions given by the test reagent was semi-quantitatively estimated with equal volumes of sample solution and standard flavonoid solution (quercitrin dissolved in alcohol), and ranged in concentration from trace amounts (0.15 mg/ml as (1+) to large amounts (5 mg/ml or over) as (4+). All precipitates were evaluated with the eye.

Tannins: These substances are detected by the precipitate formed with the gelatin-salt reagent and also from the colors produced with ferric chloride solution. The colors range from blue-black to green-black. To test for tannins, 4 ml of the alcohol extract were evaporated to dryness, dissolved in 5 ml of distilled water and filtered. For the water extract, the remaining 4 ml of water extract were filtered and tested. One-half of each extract was then treated with a few drops of gelatin-salt reagent. This reagent was made by soaking 1 g of gelatin in 100 ml of distilled water at 40°C until dissolved. Ten g of sodium chloride was then added and stirred until dissolved.

The precipitate formed by the gelatin-salt reagent was semi-quantitatively estimated with equal volumes of reagent solution and standard tannin solution (tannic acid in water), ranging in concentration from trace amounts (0.15 mg/ml) as (1+) to large amounts (1.25 mg/ml or over) as (4+).

The same procedure was used for the ferric chloride test. This reagent was made by dissolving 9 g of ferric chloride in sufficient water to make 100 ml. All precipitates were evaluated with the eye. Unless precipitates were found in both the gelatin-salt and fabric chloride reagents, the presence of tannins was interpreted as doubtful.

RESULTS AND DISCUSSIONS

Ninety-six plant parts from 36 species of 17 families of North Dakota plants were evaluated for the presence of alkaloids, saponins, tannins and flavonoids. The results of these studies are presented in Tables 1 and 2.

Table 1. Alkaloid, saponin, tannin, and flavonoid content of alcoholic extracts of North Dakota plants

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	FeCl	Flavonoids
Amorpha nana Nutt. (Fabaceae)	S	0	0	1+	1+	0	0	1+	0
	L	0	0	1+	1+	0	1+	2+*	3+
	Fl	1+	1+	1+	1+**	0	0	2+	0
Artemisia longifolia Nutt. (Asteraceae)	R	0	0	1+	0	0	0	0	0
	S	0	0	0	2+	0	0	0	0
	L	2+	1+	1+	3+**	0	0	0	0
	Sd	1+	1+	2+	3+**	0	0	1+	0
Asclepias incarnata L. (Asclepiadaceae)	R	1+	1+	2+	1+*	4+	0	0	0
	S	0	0	0	0	0	0	0	0
	L	1+	1+	0	2+*	0	0	3+	0
	Fl	0	0	1+	2+	0	0	2+	3+
Asclepias verticillata L. (Asclepiadaceae)	R	0	1+	2+	1+**	0	0	0	0
	S	1+	1+	3+	1+**	0	0	0	0
	L	1+	1+	2+	2+**	0	0	2+	1+
	Fl	1+	1+	2+	2+**	0	0	0	1+
Asparagus officinalis L. (Liliaceae)	S	0	1+	1+	0	0	0	0	0
Atriplex nuttallii S. Wats. (Chenopodiaceae)	R	0	0	1+	0	1+	0	1+	0
	S	0	0	1+	0	1+	0	0	0
Carduus nutans L. (Asteraceae)	R	1+	1+	1+	1+**	0	0	0	0
	S	0	0	0	0	1+	0	0	0
	L	0	0	1+	1+	0	0	1+	1+
	Fl	0	0	1+	1+	0	0	3+	1+
Cynoglossum officinale L. (Boraginaceae)	R	0	0	1+	0	0	0	0	0
	S	0	0	1+	2+	0	0	0	0
	L	0	0	1+	2+	0	0	1+	0
Equisetum arvense L. (Equisetaceae)	WP	0	0	0	1+	0	0	3+	2+
Equisetum fluviatile L. (Equisetaceae)	WP	0	0	0	0	0	0	3+	1+
Equuisetum laevigatum A. Br. (Equisetaceae)	WP	0	0	0	0	0	0	1+	0

Table 1 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonolds
Equisetum pratense Ehrh. (Equisetaceae)	WP	0	0	0	0	0	0	3+	1+
Eupatorium purpureum L. (Asteraceae)	R	0	0	0	0	0	0	1+	0
	S	0	0	0	0	0	0	1+	0
	L	0	1+	1+	1+**	0	0	2+	2+
	Fl	0	0	1+	1+	0	0	3+	2+
Eupatorium rugosum Houtt. (Asteraceae)	R	0	0	0	0	0	0	1+	0
Gutierrezia sarothrae (Pursh) Britt & Rusby (Asteraceae)	R	0	0	0	0	1+	0	1+	0
	S	0	0	1+	0	0	1+	3+*	1+
	L	0	0	1+	0	0	1+	4+*	2+
Hedysarum boreale Nutt. (Fabaceae)	S	0	0	1+	1+	1+	0	0	0
	L	0	0	0	0	0	0	2+	2+
	Fl	0	0	0	0	1+	0	2+	2+
Kuhnia eupatorioides L. (Asteraceae)	S	0	1+	0	1+	0	0	1+	1+
	L	1+	1+	1+	1+**	0	0	2+	3+
	Fl	1+	1+	1+	1+**	0	0	1+	1+
Laportea canadensis (L) Gaud. (Urticaceae)	S	0	1+	0	1+	0	0	1+	0
	L	2+	2+	2+	2+**	0	0	2+	0
Lathyrus venosus Muhl. (Fabaceae)	Fr	2+	3+	3+	4+**	0	0	2+	0
	S	1+	3+	3+	3+**	0	0	1+	0
	L	2+	3+	3+	4+**	0	0	3+	1+
Lepidium densiflorum Schrad. (Brassicaceae)	S	1+	1+	1+	1+**	1+	0	1+	0
	L	1+	2+	2+	2+**	0	0	2+	1+
	Fr	1+	1+	1+	2+**	0	0	2+	1+
Opuntia fragilis (Nutt.) Haw. (Cactaceae)	WP	1+	1+	1+	1+**	0	0	1+	1+
Oxytropis splendens Dougl. (Fabaceae)	R	0	1+	1+	1+**	1+	0	0	0
	S	0	0	0	1+	0	0	0	0
	L	0	1+	1+	3+**	0	0	2+	1+
	Fr	1+	1+	1+	2+**	0	0	1+	0

Table 1 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonoids
Penstemon	R	1+	1+	1+	1+**	0	0	1+	0
augustifolius Pursh.	S	1+	2+	2+	3+**	0	0	2+	0
(Scrophulariaceae)	Fr	0	1+	1+	1+**	1+	0	0	0
Petalostemum	R	0	0	0	0	2+	0	0	0
vilosum Nutt.	S	0	0	0	0	0	0	1+	0
(Fabaceae)	L	0	0	0	0	0	2+	2+*	1+
	Fr	0	0	0	1+	0	3+	3+*	0
Plantago	R	2+	3+	2+	3+**	0	0	1+	0
eriopoda Torr.	L	1+	1+	1+	2+**	0	0	1+	0
(Plantaginaceae)									
Plantago	S&R	1+	1+	1+	1+**	0	0	2+	2+
purshii R. & S.	L	0	0	0	1+	0	0	2+	1+
(Plantaginaceae)	Fr	0	0	0	0	0	0	2+	0
Psoralea	R	0	0	1+	1+	0	0	1+	0
lanceolata Pursh.	S	1+	1+	1+	2+**	0	0	1+	0
(Fabaceae)	L	0	1+	1+	1+**	0	0	2+	0
Ranunculus	R	0	0	0	0	0	0	1+	0
pennsylvanicus L.	L	0	0	0	0	0	0	2+	0
(Ranunculaceae)									
Ratibida	S	0	0	0	0	0	0	1+	1+
columnifera Wooten & Standl.	L	1+	3+	2+	3+**	0	0	3+	2+
(Asteraceae)									
Rumex	R	0	0	0	1+	0	0	1+	0
occidentalis S. Wats.	S	0	0	0	1+	0	0	1+	0
(Polygonaceae)	L	0	0	0	1+	0	1+	3+*	1+
	Fr	0	0	0	0	0	3+	3+*	1+
Salvia	R	0	0	0	1+	1+	0	2+	0
sylvestris L.	S	0	0	0	1+	1+	0	1+	0
(Lamiaceae)	L	0	0	0	1+	0	1+	3+*	1+
Saponaria	L	2+	3+	4+	3+**	4+	0	2+	1+
officinalis L.	Fl	0	0	4+	1+	4+	0	2+	1+
(Caryophyllaceae)									
Similax	L	1+	1+	1+	2+**	0	0	2+	0
herbacea L.									
(Liliaceae)									
Verbena	S	1+	1+	1+	1+**	0	0	1+	0
bracteosa Mich.	L	1+	1+	1+	1+**	0	0	0	0
(Verbenaceae)									

Table 1 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	FeCl$_3$	Flavonolds
Verbena	R	0	0	0	0	0	0	1+	0
urticifolia L.	S	1+	1+	1+	2+ **	0	0	1+	0
(Verbenaceae)	L	1+	1+	1+	1+ **	0	0	1+	0
	Fr	0	0	1+	1+	0	0	2+	0
Verbena	R	0	0	0	0	0	0	2+	0
stricta Vert.	S	1+	1+	1+	2+ **	0	0	2+	0
(Verbenaceae)	L	1+	1+	1+	2+ **	0	0	2+	0
	Fr	1+	1+	1+	1+ **	0	0	2+	0

*High probability of tannins
**High probability of alkaloids

Abbreviations and symbols used in Tables 1 and 2:

Alkaloidal reagents:
- M—Mayer's
- V—Valer's
- K—Kraut's
- B—Bertrand's

Plant parts:
- R—Roots
- S—Stems
- L—Leaves
- Fl—Flowers
- Fr—Fruits
- Sd—Seed
- WP—Whole Plant

Quantities of chemicals present:
- 1+—Trace
- 2+—Low
- 3+—Medium
- 4+—Large

Table 2. Alkaloid, saponin, tannin and flavonoid content of water extracts of North Dakota plants

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonoids
Amorpha	R	1+	1+	1+	1+**	0	0	2+	0
nana Nutt.	S	0	0	0	0	0	0	0	0
(Fabaceae)	L	0	0	1+	1+	0	1+	1+*	2+
	Fl	0	0	2+	2+	0	2+	3+*	0
Artemisia	R	0	0	0	0	0	0	0	0
longifolia Nutt.	S	0	0	0	0	0	0	1+	0
(Asteraceae)	L	1+	1+	2+	1+**	0	0	1+	0
	Sd	0	0	0	0	0	0	1+	0
Asclepias	R	1+	0	1+	1+**	0	0	0	0
incarnata L.	S	0	0	0	0	0	0	0	0
(Asclepiadaceae)	L	0	0	0	0	0	0	2+	2+
	Fl	0	0	0	0	0	0	2+	3+
Asclepias	R	1+	0	1+	1+**	0	0	0	0
verticillata L.	S	1+	1+	1+	0**	0	0	0	0
(Asclepiadaceae)	L	1+	0	1+	1+**	0	0	0	1+
	Fl	1+	1+	1+	1+**	0	0	1+	1+
Asparagus	S	0	0	1+	1+	0	0	0	0
officinalis L.									
(Liliaceae)									
Atriplex	R	0	0	2+	0	0	0	0	0
nuttallii S. Wats.	S	0	0	2+	0	0	0	0	0
(Chenopodiaceae)									
Carduus	R	0	0	2+	0	0	0	2+	0
nutans L.	S	0	0	0	0	0	0	1+	0
(Asteraceae)	L	0	0	0	0	0	0	2+	0
	Fl	0	0	0	0	0	0	1+	1+
Cynoglossum	R	0	0	1+	0	0	0	2+	0
officinale L.	S	0	0	1+	0	0	0	1+	0
(Boraginaceae)	L	0	0	1+	0	0	0	2+	0
Equisetum	WP	0	0	3+	0	0	0	2+	0
arvense L.									
(Equisetaceae)									
Equisetum	WP	0	0	2+	0	0	0	1+	0
fluviatile L.									
(Equisetaceae)									

Table 2 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonoids
Equisetum laevigatum A. Br. (Equisetaceae)	WP	0	0	0	0	0	0	1+	0
Equisetum pratense Ehrh. (Equisetaceae)	WP	0	0	3+	0	0	0	2+	0
Eupatorium purpureum L. (Asteraceae)	R	0	0	1+	0	0	0	0	0
	S	0	0	1+	0	0	0	1+	0
Eupatorium rugosum Houtt. (Asteraceae)	R	0	0	1+	0	0	0	1+	0
Gutierrezia sarothrae (Pursh) Britt & Rusby. (Asteraceae)	R	0	0	1+	0	0	0	1+	0
	S	0	0	1+	0	0	0	3+	0
	L	0	0	2+	0	0	0	3+	0
Hedysarum boreale Nutt. (Fabaceae)	R	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	1+	0
	L	0	0	1+	0	0	1+	2+*	1+
Kuhnia eupatorioides L. (Asteraceae)	S	0	0	1+	0	0	0	1+	0
	L	0	0	3+	0	0	0	2+	0
	Fl	0	0	2+	2+	0	0	1+	0
Laportea canadensis L. (Urticaceae)	S	0	0	0	0	0	0	1+	0
	L	0	0	3+	0	0	0	3+	0
Lathyrus venosus Muhl. (Fabaceae)	S	0	0	0	0	0	0	0	0
	L	0	0	2+	0	0	0	2+	0
	Fr	0	0	2+	1+	0	0	1+	0
Lepidium densiflorum Schrad. (Brassicaceae)	S	0	0	1+	0	0	1+	2+*	0
	L	0	0	1+	0	0	0	2+	0
	Fr	0	0	2+	0	0	0	1+	1+
Opuntia fragilis (Nutt.) Haw. (Cactaceae)	WP	0	0	0	0	0	0	1+	0
Oxytropis splendens Dougl. (Fabaceae)	R	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	1+	0
	Fr	0	0	1+	0	0	0	1+	0

Table 2 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonoids
Penstemon augustifolius Pursh. (Scrophulariaceae)	R	0	0	0	0	0	0	0	0
	S	1+	1+	1+	1+**	0	0	2+	0
	Fr	1+	1+	1+	1+**	0	0	1+	0
Petalostemum villosum Nutt. (Fabaceae)	R	0	0	0	0	0	0	0	0
	S	0	0	1+	0	0	0	0	0
	L	0	0	2+	0	0	2+	2+*	0
	Fr	0	0	4+	0	0	3+	3+*	0
Plantago eriopoda Torr. (Plantaginaceae)	R	0	0	0	0	0	0	0	0
	L	0	0	1+	0	0	0	1+	0
Plantago purshii R. & S. (Plantaginaceae)	S&R	0	0	2+	0	0	0	2+	0
	L	0	0	1+	0	0	0	2+	0
	Fr	0	0	0	0	0	0	0	0
Psoralea lanceolata Pursh. (Fabaceae)	R	0	0	1+	0	0	0	1+	0
	S	0	0	1+	0	0	0	2+	0
	L	0	0	3+	0	0	0	2+	1+
Ranunculus pennsylvanicus L. F. (Ranunuculaceae)	R	0	0	1+	0	0	0	1+	0
	L	0	0	1+	1+	0	0	2+	0
Ratibida columnifera Nutt. (Wooten & Standl.) (Asteraceae)	S	0	0	1+	0	0	0	2+	0
	L	0	0	2+	0	0	0	2+	0
Rumex occidentalis S. Wats. (Polygonaceae)	R	0	0	1+	0	0	0	1+	0
	S	0	0	2+	0	0	0	1+	0
	L	0	0	2+	0	0	0	2+	0
	Fr	0	0	2+	0	0	0	2+	0
Salvia sylvestris L. (Lamiaceae)	R	0	0	2+	0	0	0	2+	0
	S	0	0	2+	0	0	0	2+	0
	L	0	0	2+	0	0	0	3+	0
Saponaria officinalis L. (Caryophyllaceae)	L	0	0	2+	0	3+	0	3+	0
	Fl	0	0	2+	0	4+	0	2+	0
Smilax hebacea L. (Liliaceae)	L	0	0	2+	2+	0	0	2+	0

Table 2 (Cont.)

Scientific name	Plant part	Chemical groups tested: Alkaloids Reagents used M	V	K	B	Saponins	Tannins Reagents used Gel-Salt	$FeCl_3$	Flavonolds
Verbena	S	0	0	0	0	0	0	1+	0
bracteosa Mich.	L	0	0	0	0	0	0	1+	0
(Verbenaceae)									
Verbena	R	0	0	1	0	0	0	0	0
urticifolia L.	S	0	0	1+	0	0	0	0	0
(Verbenaceae)	L	0	0	1+	0	0	0	1+	0
	Fr	0	0	1+	0	0	0	1+	0
Verbena	R	0	0	1+	0	0	0	1+	0
stricta Vent.	S	0	0	1+	0	0	0	2+	0
(Verbenaceae)	L	0	0	2+	0	0	0	2+	0
	Fr	0	0	1+	0	0	0	2+	0

*High probability of tannins
**High probability of alkaloids

It is evident from the results that individual plant parts varied in type of chemical as well as in the amount of that substance present. Non-alkaloids such as proteins, betaines and tannins may give precipitates with alkaloidal reagents under the condition used in these tests. In general, however, it may be assumed that the spot tests employed are reasonably satisfactory guides to the presence of alkaloids in the species tested. Negative tests are generally certain.

Alkaloids, as indicated in a previous study by Sinha et. al. (1967), were found to be more widely distributed in certain North Dakota plants than all other chemical groups tested. Of the 96 plant parts tested, 43 parts had high probability of containing alkaloids. The distribution of the alkaloids in the various plant parts is relatively the same. The following seven families include species which contain appreciable quantities of alkaloids or alkaloidal substances. The numerator of the fraction (in parentheses) refers to the number of "positive' 'species; the denominator represents the number of species tested in the family.

Asclepiadaceae	(2/2)	Asteraceae	(5/7)
Brassicaceae	(1/1)	Fabaceae	(4/6)
Plantagianceae	(2/2)	Scrophulariaceae	(1/1)
Urticariaceae	(1/1)		

The saponins were absent from most plants investigated, occurring in only 15 plant parts. Only two plants, *Saponaria officinalis* and *Asclepias incarnata* contained appreciable quantities.

Tannins were found in 13 of 96 plant parts in sufficient quantities to give a positive test at the 0.15 mg/ml level. A greater prevalence of tannins in leaf tissue is consistent with the widely recognized higher concentration of tannin in leaf tissue. It should also be noted

that tannins, as determined from other studies, although they were found frequently in more than one member of a family such as the Fabaceae, they were not present in detectable quantities in all members of that family. In interpreting the data (Tables 1 and 2) it should be kept in mind that a false positive for the ferric chloride test could be a result of the presence of plant phenols and phenolic acids which are quite common in plant tissues. It should also be remembered that flavonoids are phenolic in nature. False positive tests can be given with the gelatin-salt reagent by the presence of alkaloids.

Flavonoids showed a wide distribution among the plants tested. A positive flavonoid test was found in 36 of the 96 plant parts tested. This coincides with their recognized role as coloring agents for flowers, leaves, and sometimes stems. Since most roots are not highly colored, the absence of flavanoids in roots was not unexpected.

ACKNOWLEDGMENTS

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LITERATURE CITED

Raffauf, R. F. 1960. Plants as a source of new drugs. Econ. Bot. 14: 276-279.

Dominguez, A. X., P. Rojas, V. Collins, and M. D. R. Morales. 1960. A phytochemical study of eight Mexican plants. Econ. Bot. 14: 157-159.

Farnsworth, N. R., L. K. Henry, G. H. Svoboda, R. N. Blomster, M. J. Yates, and K. L. Euler. 1966. Biological and phytochemical evaluation of plants. Lloydia 29: 111-122.

Stevens, O. A. 1963. Handbook of North Dakota plants. N. Dak. Inst. Regional Studies, Fargo, N. Dak.

Pammel, L. H. 1911. A manual of poisonous plants. The Torch Press, Iowa.

Kingsbury, J. M. 1964. Poisonous plants of the United States and Canada. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Muenscher, W. C. 1940. Poisonous plants of the United States. The Macmillan Co., New York.

McCracken, D. S., and L. J. Schermeister. 1970. Phytochemical and cytotoxic evaluation of several *Astragalus* species of North Dakota. Lloydia 33: 19.

Nadodwalla, K. S. 1967. Evaluation of North Dakota plants for cytotoxicity and the preliminary isolation of active constituents. Thesis. N. Dak. State Univ.

Sinha, J. R. 1966. Phytochemical survey and preliminary separation of alkaloids of certain poisonous plants of North Dakota. Thesis. N. Dak. State Univ.

———, K. S. Nadodwalla, D. S. McCracken, L. J. Schermeister, and W. H. Bhatti. 1969. Phytochemical studies of poisonous plants of North Dakota—Part I. N. Dak. Acad. Sci., Proc. 21: 111-120.

Persinos, G. J., M. W. Quimby, and J. W. Schermerborn. 1964. Preliminary pharmacognostical study of ten Nigerian plants. Econ. Bot. 18: 329.

THIN-SECTION ANALYSIS OF THE FISH BED, SEIBOLD SITE, NORTH DAKOTA

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ABSTRACT

Paleolake Seibold, located in Stutsman County, North Dakota, was the site of deposition of several sedimentary units during Wisconsinan and Holocene time. One of these units, the fish bed, which is an organic, laminated mud, is 8.5-60 cm thick where exposed. Rhythmic microcopic laminae are visible in oriented thin sections. The base of a typical lamina is light-colored, silt-size, terrigenous sediment. It grades up to dark clay, rich in organic material at the top. Pollen is concentrated either in the lower part of the terrigenous layer or in the organic upper layer. Each lamina is interpreted to be a varve.

The microscopic laminae are 0.06-0.66 mm thick. They are grouped into megascopic laminae that range from a fraction of a millimeter to a few millimeters in thickness. The microscopic laminae in the darker megascopic laminae are thinner than the microscopic laminae in the lighter megascopic laminae. Measurements of microscopic laminae indicate a depositional rate for the fish bed of 6-66 cm per 1,000 years. The highest rates are the most reasonable.

INTRODUCTION

In the fall of 1969 a richly fossiliferous, late Quaternary lacustrine deposit was discovered in SW¼ NW¼ sec. 21, T. 141 N., R. 67 W., Stutsman County, southeastern North Dakota. The deposit was exposed in a man-made excavation (Seibold Site) in a depression called the Seibold Slough (Cvancara et al., 1971). The depression, approximately 100 m in diameter, is one of many located on the Missouri Coteau. It resulted from the presence of stagnant ice which existed in the area about 9,000-13,000 years ago (Clayton, 1967, p. 28). As the ice melted, enclosed debris was concentrated at the surface. It settled irregularly, producing small depressions and non-integrated drainage. Paleolake Seibold formed in one of these depressions.

Other small late Quaternary pond deposits have been studied in the midcontinent area of the United States. All of these, including the Seibold deposit, show similar stratigraphic sequences which reflect the late Quaternary history of the region (Bickley and Clayton, 1972). The basic sequence, from bottom to top, consists of (1) pebbly, gray silt, (2) gray silt, (3) brown, gray or green organic mud (fish bed), 4) calcareous mud, (5) dark-brown, sandy, silty clay, (6) brown, silty sand, and (7) dark brown, sandy, silty clay. These sediments all are generally unconsolidated to semi-consolidated.

MATERIALS AND METHODS

A petrographic analysis of unit 3 was made by microscopically examining thin sections. This unit has been called the "fish bed" because of the large numbers of extremely well-preserved fish fos-

sils that it contains. Other types of well-preserved organic remains are also present, including beaver-gnawed wood, frogs, and delicately preserved insects.

Where exposed, the fish bed is 8.5-60 cm thick. A single block of the entire unit, 32 cm thick, was collected by William B. Bickley, Jr., University of North Dakota, from the Seibold Slough about 2 m west of measured section B (Bickley et al., 1971, Figure 1). A vertical strip a few centimeters wide was cut from the block. The strip was then cut into 16 oriented chips, each the size of a petrographic thin-section slide, and allowed to dry at room temperature. Some distortion and splitting of the chips occurred during drying. The chips were then impregnated with Elvacite 2045, an acrylic resin manufactured by DuPont. The following procedure was used:

1. Following drying, the sample was heated on a hot plate to approximately 250 F.
2. The Elvacite solution was prepared by dissolving about 1 part of Elvacite in 3 or 4 parts of acetone. (Mixing for a considerable length of time allowed the resin to dissolve in the acetone.)
3. Enough of the solution was poured into a small dish to cover the sample. (Aluminum foil dishes are excellent, because the resin will not adhere to the dish, which may be bent away to remove the hardened sample.)
4. The heated chip was removed from the hot plate and plunged into the Elvacite solution. Effervescence occurred when air was expelled during absorption of the solution.
5. The material was allowed to harden for about one week. Oriented thin sections of each chip were then prepared.

RESULTS AND DISCUSSION

Mineralogy.—Organic matter is the most abundant constituent visible in the slides, comprising perhaps 50-60% of the sediment. The most abundant type is non-opaque, structureless, translucent, and light to dark brown or greenish-brown. This is either dispersed throughout the sediment or occurs in disinct laminae, which average about 0.01-0.02 mm in thickness, but may be as thick as 0.1 mm or more. The greenish color apparently is due to the presence of an unknown organic pigment which is probably the agent responsible for the overall greenish color of the fish bed. Structureless, oqaque, organic matter may also occur as distinct silt-size and clay-size grains, or as definite opaque laminae.

Calcite is the second most abundant constituent, making up perhaps 20-30% of the sediment. It occurs mostly as micrite, which is either dispersed throughout the slides or is concentrated in blebs. These blebs are of two general sizes. The largest are elongate (about 0.5-1 mm long and 0.1 mm wide) and are concentrated along bedding planes, almost entirely in the parts of the slides rich in organic matter. The blebs probably are closs-sections of stems and other fragments of Charophyceae. Unidentifiable organic structures are present in some places. Rarely the blebs are silicified. The smaller blebs of micrite are subrounded to angular and average about 0.05

mm in diameter, although their size is quite variable. They probably are pieces of Charophyceae and are the most abundant form of calcite in the samples.

Silt-size carbonate rhombs are present. Possibly some of them are dolomite crystals resulting from recrystallization of magnesian micrite, or they may be terrigenous grains washed into the basin. Coarsely crystalline, silt-size carbonate fragments are also present. Since they are hydraulic equivalents of non-calcareous clastic fragments, they too are probably clastic grains derived from outside of the basin.

Fine-sand-size, silt-size and clay-size terrigenous fragments occur throughout the unit. They consist of orthoclase, plagioclase, quartz, and sedimentary, metamorphic, and igneous rock fragments. In some places the coarser terrigenous fragments are concentrated into small lenses that show slightly scoured bases, suggesting transportation by bottom currents.

Organisms.—Diatoms are the most abundant fossil in the samples. Identification was not attempted, but several forms are present. Preservation is excellent, and identification in a disaggregated hand sample should not be difficult. Unidentified pollen grains are the second most abundant fossil. In the lower part of the fish bed they are spheres 0.01 mm in diameter. The spheres are generally clear, but in places they show internal structure. Approximately 24 cm above the base of the fish bed, finer-grained pollen grains appear. They average about 0.004 mm in diameter and are less clear than the larger forms. Results of a detailed study of the pollen of the fish bed are presented by Cvancara et al. (1971).

Other organic structures include siliceous sponge spicules, fish bone fragments, ostracode carapaces, and mollusk shell fragments. Large, hollow, unidentified spheres are alo present, but are uncommon. They are about 0.3-1.8 mm in diameter .They consist of a thin shell made of calcite needles arranged radially. One distorted sphere and several fragments were found directly associated with a cluster of fish bones. Various types of plant tissue are also visible in places.

Megascopic laminae.—Laminae in the fish bed occur within two general size ranges: megascopic and microscopic. The megascopic laminae (Figure 1) range in thickness from a fraction of a mm to approximately 3 mm. They are distinguished on the basis of color, both in thin section and in hand sample, the lighter part being poorer in organic matter. The thicker megascopic laminae generally consist of perhaps 10-20 sets of microscopic laminae, which are rhythmic. These microscopic sets are very difficult to distinguish in places.

Each microscopic set consists of a layer rich in organic matter and a layer poor in organic matter but within certain megascopic laminae the layers rich in organic matter are not well developed. These megascopic laminae are light colored. They may represent periods during which environmental factors (climate, nutrient availability) were unfavorable enough to reduce production of organic material. This is also suggested by the reduced number of

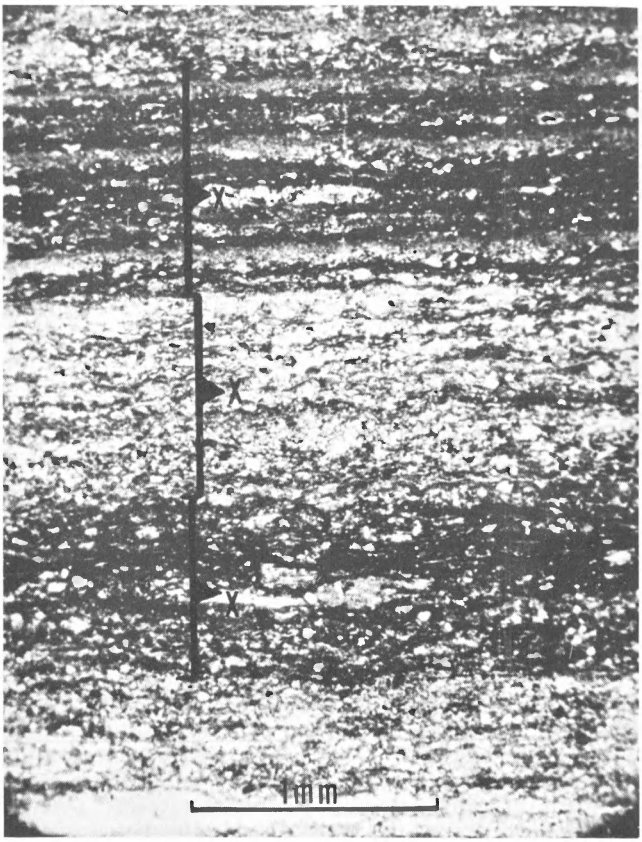

Figure 1. Photomicrograph of fish bed 12 cm above base. Megascopic laminae (x) consist of groups of microscopic laminae. Plane light.

whole Charophyceae fragments in zones containing little organic matter.

Darker megascopic laminae contain abundant organic matter, which may be present in great enough quantities to partially obscure microscopic sets. These darker megascopic layers may have formed during periods favorable for the production of larger amounts of organic matter. Thinner megascopic laminae may consist of a single layer, which is either rich or poor in organic matter.

Microscopic laminae. — Microscopic laminae, observably only in thin section, are 0.06-0.66 mm thick and average 0.18 mm. The typical lamina consists of a clastic lower portion and an organic upper part (Figures 2, 3). The clastic lower part of a typical lamina, in turn, consists of two parts, a coarser-grained lower part overlain by a

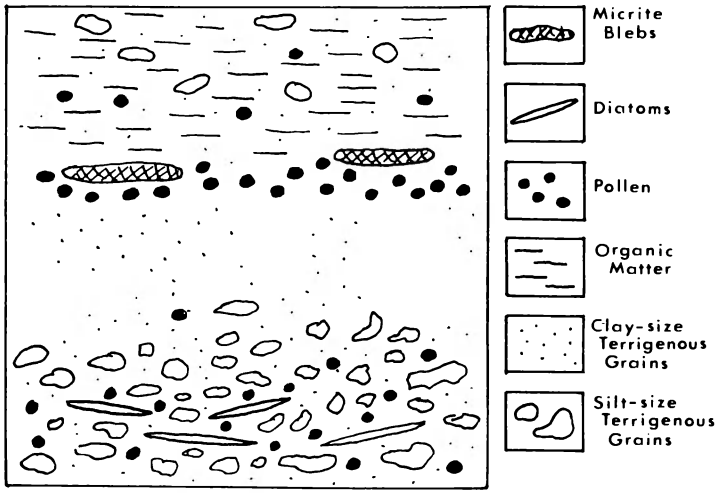

Figure 2. Diagram of an ideal microscopic lamina (varve).

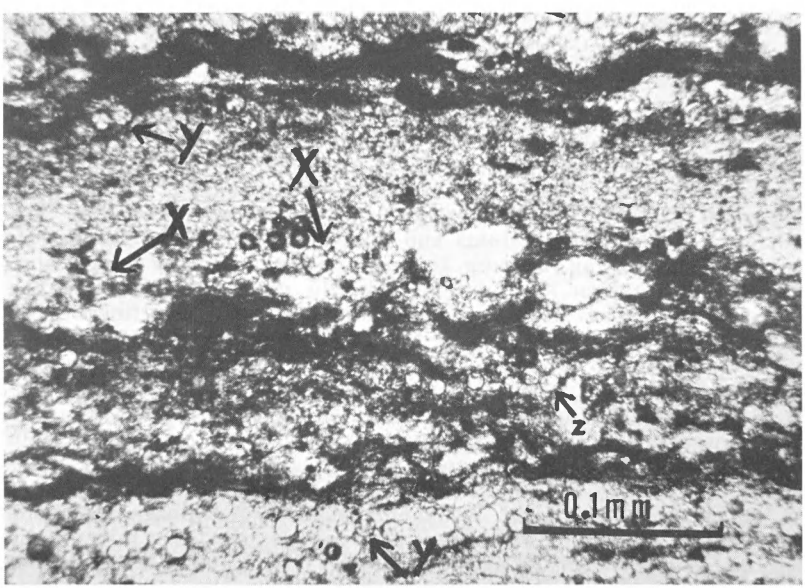

Figure 3. Photomicrograph of microscopic laminae in plane light. Pollen grains are concentrated within the lower, coarser-grained portions of the clastic members (x), at the base of organic-rich members (y), and within the organic-rich members (z). Diatoms and micrite blebs are not present. Terrigenous grains are white or grey, and organic matter is gray or black.

finer-grained upper part. The contact between these may be gradational, in which case the entire clastic layer shows graded bedding. In many places, however, the contact between the coarser-grained and finer-grained clastic zones may be quite sharp. The lower coarser-grained portion of the clastic member consists of mixed silt and clay, although medium and fine sand grains also occur in places. Clastic fragments observed include quartz, orthoclase, plagioclase, and igneous, metamorphic and sedimentary rock fragments, and particles of fine to coarsely crystalline carbonate. The finely crystalline carbonate probably formed biochemically within the pond, and the coarsely crystalline carbonate was probably derived from outside the basin.

Diatoms and pollen are concentrated mostly within the coarser clastic layers. In many places the coarser-grained layers are absent, in which case their position may be occupied by the diatoms, pollen, or both. Pollen may also be concentrated just below the organic member, as may be Charophyceae fragments. Rarely, pollen may be found within the finer-grained zone of the clastic unit.

Microscopic laminae are most easily observed where there is an intermediate amount of organic matter. Where considerable organic matter is present the laminae are obscured, and where little is present the laminae are not easily seen. In places they are not well developed even where the correct ratio exists between organic and inorganic matter. This may be the result of disturbance of the bottom by currents.

Origin of the microscopic laminae.—The microscopic laminae are interpreted as varves. During spring run-off the coarser-grained lower part of a clastic unit probably originated by means of bottom-current transport. The coarser material was brought to the lake by more rapidly flowing streams swollen by spring floods. The presence of abundant diatoms and pollen in this layer also suggests origin in the spring, because this is the season during which both diatoms and pollen are most abundantly dispersed. Later in the summer, as stream flow into the lake decreased, the finer upper part of the clastic zone was deposited from suspension. Concentrations of pollen at the top of this layer in places suggests another period of increased pollen dispersal late in the summer.

During and following freezing of the lake, phytoplankton and other organic debris settled from suspension to create the thin upper organic layer. Occurrence of the large Charophyceae fragments at the bases of organic layers may reflect higher settling rates of the calcareous material. Fragments of fossil fish observed in one thin section appear to be concentrated within part of a microscopic lamina, which is rich in organic matter. This may be explained as the result of a fish-kill due to oxygen depletion while the lake was frozen over. At the Seibold Site large concentrations of fish fossils are found on single bedding surfaces, which are the organic parts of microscopic laminae.

Figure 4 shows the vertical distribution of varve thickness within the fish bed. The thickness of the varves increases upward, which indicates a higher rate of sedimentation toward the end of deposition

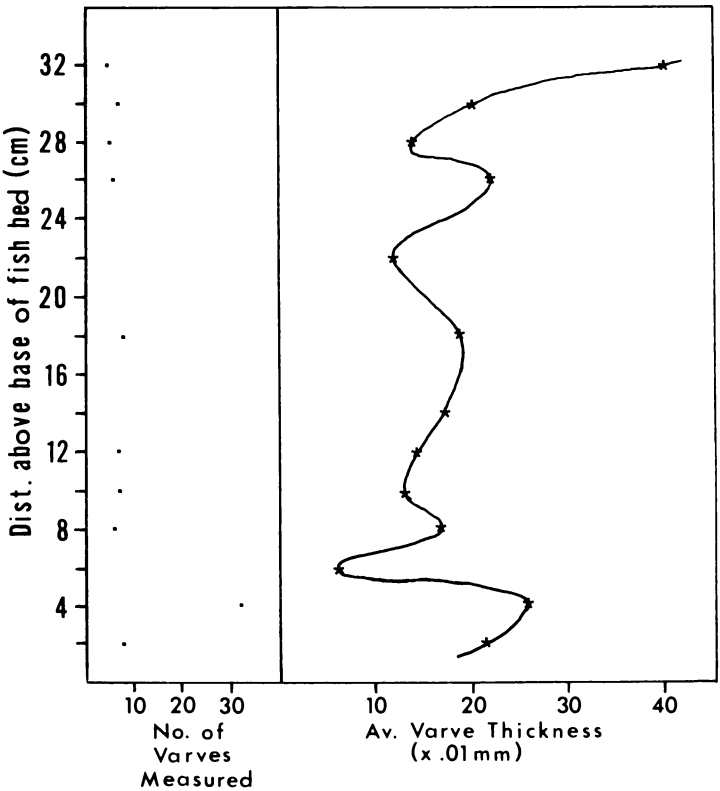

Figure 4. Average thickness of varves within the fish bed. Data based on 139 measurements in thin sections at a single locality.

of the unit. A carbon date of 9,750 ± 140 years B. P. was obtained about 10 cm above the base of the fish bed at the horizon where prairie (with tree groves) replaced spruce forest (Cvancara et al., 1971). This date agrees very well with the work of others (Ogden, 1967; Ritchie and Lichti-Federovich, 1968; and Watts and Bright, 1968), which indicates that prairie replaced forest between 10,500 and 9,500 years ago.

Each varve includes the amount of sediment deposited in one year. The varves are 0.06-0.66 mm thick and average 0.18 mm. So the depositional rate for the fish bed is 6-66 cm per 1,000 years and averages 18 cm per 1,000 years.

The fish bed is 32 cm thick where the samples for this study were collected. Using the depositional rate of 18 cm per 1,000 years the total period of deposition of the fish bed was 1,780 years. The base of the fish bed was deposited during the last several hundred years of the Wisconsinan. So the rest of the fish bed was deposited during more than the first 1,000 years of the early Holocene. This allows

only about the last few hundred years of the early Holocene for deposition of units 4 and 5. This is not an unreasonable period, but it does suggest a somewhat shorter period of deposition of the fish bed than that calculated using average thickness of varves. Perhaps multiple periods of freezing and thawing caused deposition of several microscopic laminae during one year in some cases.

REFERENCES CITED

Bickley, William B., Jr., and Lee Clayton. 1971. Sedimentation in small sloughs in the Mid-continent area during late Quaternary time. N. Dak. Acad. Sci., Proc. 25 (I):36-42.

Bickley, W. B., Jr., Lee Clayton, and Alan M. Cvancara. 1971. Seibold Site: Comparison with other late Quaternary fossil sites in North Dakota. N. Dak. Acad. Sci., Proc. 24 (II): 73-79.

Clayton, Lee. 1967. Stagnant-glacier features of the Missouri Coteau in North Dakota, p. 25-46. *In* L. Clayton and T. F. Freers [eds.] Glacial geology of the Missouri Coteau. N. Dak. Geol. Surv. Misc. Ser. 30.

Cvancara, Alan M., Lee Clayton, William B. Bickley, Jr., Arthur F. Jacob, Allan C. Ashworth, John A. Brophy, C. T. Shay, L. Denis Delorme, and George E. Lammers. 1971. Paleolimnology of late Quaternary deposits: Seibold Site, North Dakota. Science 171: 172-74.

Ogden, J. G. 1967. Radiocarbon and pollen evidence for a sudden change in climate in the Great Lakes region approximately 10,000 years ago, p. 117-127. *In* E. J. Cushing and H. E. Wright [eds.] Quaternary paleoecology. Yale Univ. Press, New Haven.

Ritchie, J. C., and Sigrid Lichti-Federovich. 1968. Holocene pollen assemblages from the Tiger Hills, Manitoba. Can. J. Earth Sci. 5: 873-880.

Watts, W. A., and R. C. Bright. 1968. Pollen, seed and mollusk analysis of a sediment core from Pickerel Lake, northeastern South Dakota. Geol. Soc. Amer. Bull. 79: 855-876.

SEDIMENTATION IN SMALL SLOUGHS IN THE MID-CONTINENT AREA DURING LATE QUATERNARY TIME

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ABSTRACT

Four similar sequences of postglacial slough sediment have been observed in areas that were glaciated in late Wisconsinan time in Saskatchewan, North Dakota, and Minnesota. The stratigraphy at these sites is, from bottom to top: (1) gray, unlaminated, pebbly, sandy silt with few fossils, (2) gray, laminated silt with few fossils, (3) brown or green, well laminated, organic silt (trojanite) with ex-

cellently preserved fossil fish, insects, mollusks, amphipods, ostracods, diatoms, frogs, muskrats, beaver, poplar leaves, and spruce needles, (4) brown, poorly laminated, calcareous mud with abundant fish bones, mollusks, and ostracods, (5) dark brown, unlaminated, clayey silt with abundant fossil mollusks and ostracods, (6) brown, unlaminated, silty sand with few fossils, and (7) dark brown, unlaminated, clayey silt with abundant fossil mollusks and ostracods.

This stratigraphy is interpreted to be the result of the following series of wide-spread environmental changes: (1 and 2) rapid introduction of slopewash sediment into meltwater-filled ponds immediately after deglaciation (late Wisconsinan); (3) increased hillslope stability with the area covered by a spruce-poplar woodland (late Wisconsinan and early Holocene); (4 and 5) continued hillslope stability with gradually lowering water levels and the area covered by grass sod (early Holocene); (6) decreased hillslope stability with the climate dry and warm and the watertable frequently below the slough bottoms; and (7) increased hillslope stability and a return to cooler and more moist modern prairie environment.

INTRODUCTION

Four similar postglacial stratigraphic sequences in small sloughs (a few hundred feet wide) along a transect (Figure 1) from southern Saskatchewan to southwestern Minnesota have provided some insight into the climatic and environmental changes during the 13,000 years since the area was glaciated.

The general stratigraphic sequence at these sites is, from bottom to top: (1) gray, poorly laminated, pebbly, sandy silt with few fossils, (2) gray, laminated silt with few fossils, (3) trojanite, a

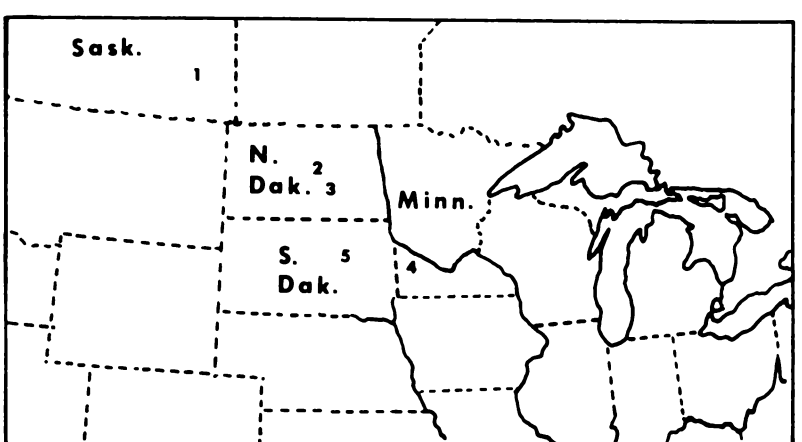

Figure 1. Location of the Lillestrom Site (1), the Prophets Mountain Site (2), the Seibold Site (3), the Swan Site 4), and the Ree Hills Site (5).

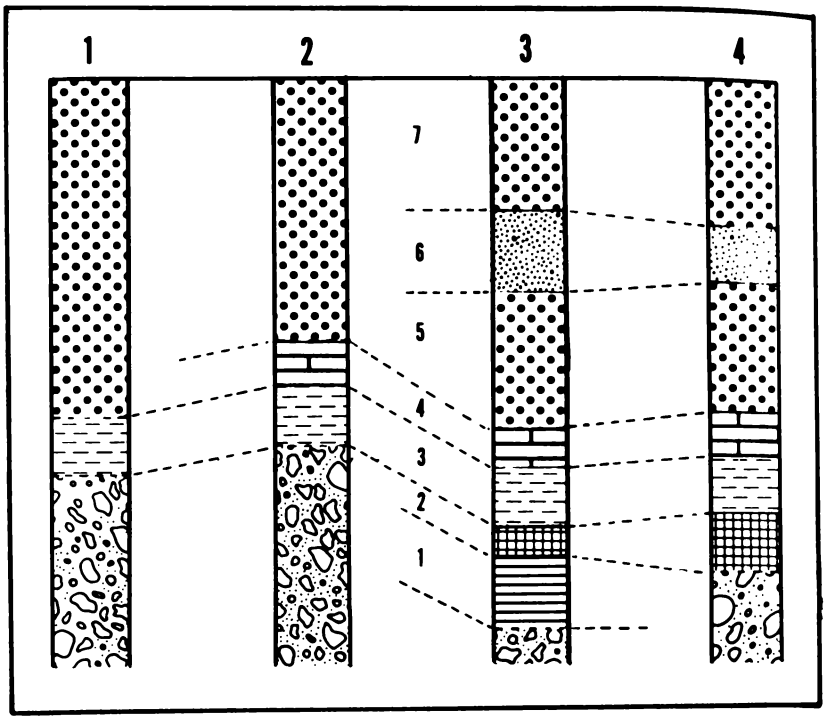

Figure 2. Generalized stratigraphic sequences at the Lillestrom Site 1), the Prophets Mountain Site (2), the Seibold Site (3), and the Swan Site (4). Each section is about 15 feet thick. The designations for each stratigraphic unit correspond to those given in the text. The lower most unit shown is glacial drift.

brown to green, flaccid, well laminated organic silt with excellently preserved fossil fish, insects, mollusks, (especially branchiate snails), ostracods, amphipods, diatoms, frogs, muskrats, beaver, and the leaves, wood, and seeds of spruce, poplar, and other trees, shrubs, herbs, and aquatic plants, (4) brown, poorly laminated, calcareous mud with abundant fossil mollusks and ostracods, (5) dark brown, unlaminated , clayey silt with abundant fossil mollusks and ostracods, (6) brown, unlaminated, silty sand with few fossils, and (7) dark brown, unlaminated clayey silt with abundant fossil mollusks and ostracods (Figure 2).

The upper three units have been differentiated at the Swan Site in southwestern Minnesota (Roger Reede, Geology Department, Marshall State College; conversation, November 1970) and the Seibold Site in North Dakota (Bickley, 1970; Cvancara and others, 1971), but have not been definitely differentiated at the Prophets Mountain Site in North Dakota (Sherrod, 1963) and the Lillestrom Site in

southern Saskatchewan (Uyeno and Miller, 1963). Unit 4 (calcareous mud) is present at both North Dakota sites and the Swan Site, but not known to be present at the Lillestrom Site. Unit 3 (trojanite) is present at all four sites. Unit 2 (gray silt) is known to be present at the Swan and Seibold iStes. Unit 1 (pebbly, sandy silt) is known to be present only at the Seibold Site, but is probably present also at the other sites. Glacial drift probably underlies unit 1 at all four sites. A fifth site, the Ree Hills Site in South Dakota (Ossian, 1970), has a similar, though more complex stratigraphic sequence; it is dominated by trojanite intercalated with silty layers.

ENVIRONMENTAL INTERPRETATIONS

Units 1 and 2 are interpreted to be sediment washed into the ponds from adjacent hillslopes that were still unstable immediately after deglaciation, which occurred in late Wisconsinan time, about 13,000 B.P., at all four sites. The ponds contained meltwater and had little life; aquatic fossils are scarce in units 1 and 2. However, trees grew on the hillslopes surrounding the ponds because some spruce needles occur in the sediment at most of the sites.

Unit 3 was deposited in eutrophic ponds resembling those in present-day north-central Minnesota; it is finely laminated and contains large amounts of organic material. Benthic organisms were generally absent and the bottom was below wave base because the fine laminations of unit 3 have not been disrupted and have no cross-bedding . A striking change in fossil pollen and plant microfossils in unit 3 indicate that the vegetation on the surrounding hillslopes changed rather rapidly from a spruce-poplar woodland to prairie. This change occured just after 9750±140 B.P., the age of a beaver-gnawed branch from the Seibold Site (Cvancara and others, 1971). The vegetation stabilized the hillslopes, preventing any large amount of detrital sediment from reaching the ponds.

Unit 4 was deposited in ponds only a few feet deep. The unit is calcareous because of the abundance of lime-secreting algae, pulmonate snails, and ostracods. Wave action and benthonic organisms prevented the development of laminations such as are present in unit 3. Fish were present, indicating that the ponds probably did not freeze to the bottom or dry up.

Units 5 and 7 are nearly identical and were deposited in ephemeral sloughs. Deposition of unit 7 continues today. Grassland vegetation similar to that present in the area today was present throughout most of the time units 5 and 7 were being deposited.

Unit 6 was deposited in depressions that rarely contained water. A poor sod cover existed on adjacent hillslopes, and large amounts of coarse sediment was washed into the depressions.

PALEOCLIMATOLOGY

All four sites are in morainic depressions lacking outlets; nearly all of the sediment deposited in the depressions during Holocene time was washed off of adjacent hillslopes. According to Schumm (1965), variations in hillslope stability are largely controlled by variations in vegetation and overland flow, which are controlled by precipitation and temperature. As shown in Figure 3, sediment yield from

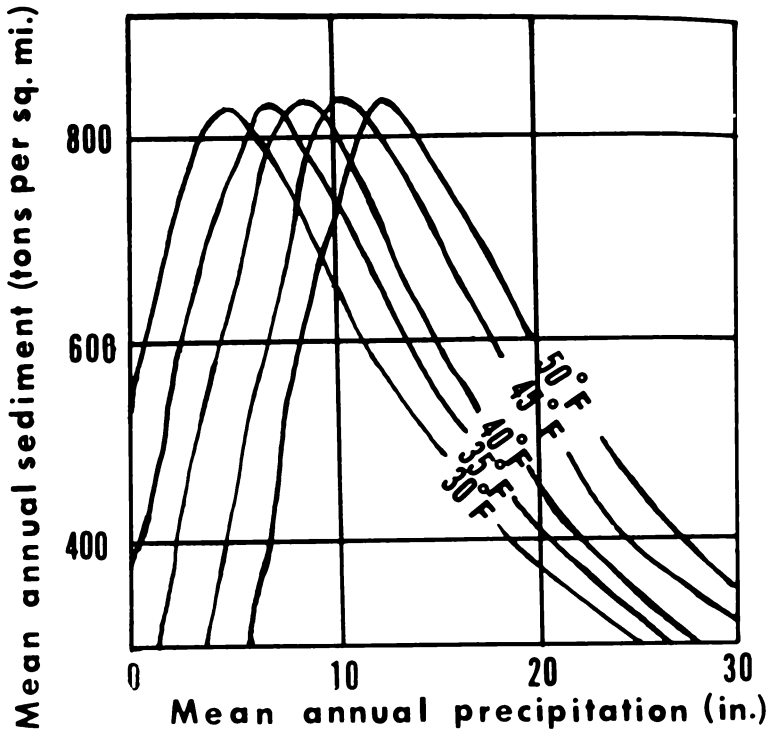

Figure 3. Relation of sediment yield from hillslopes to mean annual temperature and mean annual precipitation (Schumm, 1965).

hillslopes increases rapidly as effective precipitation increases in warm, dry areas because of an increase in overland flow; not enough moisture is available, however, to support a protective vegetative cover. In contrast, sediment yield rapidly decreases as precipitation increases in cool, moist areas because of an increase in the vegetative cover. Thus, a drought should cause increased sediment yield in a relatively cool, moist area like North Dakota (the present mean annual temperature is about 40° F and the mean annual precipitation is about 18 inches) and decreased sediment yield in a warm, dry area like Arizona. Thus, in North Dakota, warmer, drier episodes should be characterized by the deposition of large amounts of coarse, nonorganic sediment in sloughs, whereas cool, more moist episodes should be characterized by the deposition of small amounts of fine, organic-rich sediment.

These relationships seem to be born out by the sedimentological evidence at the four sites. The paleontology of unit 3 indicates that the climate was cool and moist (a few degrees cooler and a few inches more annual precipitation than at present); as a result, hillslopes were stable and little clastic sediment was washed into the

depressions. The climate began to become somewhat drier and warmer as spruce-poplar woodland was replaced by prairie and the water levels dropped during the deposition of unit 4; hillslopes were somewhat less stable and more clastic sediment was washed into the depressions. The paleontology and the increased amounts of coarser clastic sediment indicate that the climate continued to become warmer and drier and the watertable dropped during the deposition of unit 5 until the relatively arid times when unit 6 was deposited. During the deposition of unit 7 (modern times) the climate returned to somewhat cooler and more moist conditions that existed during the deposition of unit 5.

The generalizations of Figure 3 are in conflict with the paleontological evidence in units 1 and 2. Much clastic sediment was washed into the depressions then, even though the climate was cool and moist, because the hillslopes were still unstable as a result of the recent deglaciation.

REGIONAL CORRELATION

The glacial drift at the base of the sequence is late Wisconsinan in age (about 13,000 B.P.). Units 1 and 2 are immediately postglacial (between about 13,000 and 11,000 B.P.). Unit 3 straddles the change from woodland to prairie that is known to have occurred about 10,000 B.P. throughout the Northern Plains (McAndrews, 1966). Unit 4 is earliest Holocene; unit 5 is early Holocene; unit 6 is middle Holocene; and unit 7 is late Holocene.

Units 1 and 2 and the lower part of unit 3 probably chronologically correlate with McAndrew's (1966, Plate 1) *Picea-Populus* Assemblage Zone in northwestern Minnesota. The upper part of unit 3 and units 4 and 5 probably chronologically correlate with his *Pinus banksiana/resinosa-Pteridium* Assemblage Zone. Unit 6 probably chronologically correlates with his *Gramineae-Artemisia* Assemblage Zone. Unit 7 probably chronologically correlates with his *Ostrya-Ulmus* Subzone.

Units 1 and 2 correlate lithologically and probably chronologically with Walker's (1966, Table 16) "lower silt zone" in north-central Iowa. Units 3, 4, and 5 correlate lithologically and probably chronologically with his "lower muck zone." Unit 6 correlates lithologically and chronologically with his "upper silt zone." Unit 7 correlates lithologically and chronologically with his "upper muck zone."

Units 1, 2, and the lower part of 3 may chronologically correlate with Bryson, Baerreis, and Wendland's (1968) "Late Glacial Episode." The upper part of unit 3 and unit 4 correlate chronologically with their "Pre-Boreal Episode." Unit 5 correlates chronologically with their "Boreal Episode." Unit 6 correlates chronologically with their "Sub-Boreal," "Sub-Atlantic," "Neo-Atlantic," "Pacific," and "Neo-Boreal Episodes."

REFERENCES CITED

Bickley, W. B., Jr. 1970. Paleoenvironmental reconstructions of late Quaternary lacustrine sediments (Seibold Site) in southeastern North Dakota. M.S. Thesis. Univ. North Dakota. 88 p.

Bryson, R. A., D. A. Baerreis, and W. M. Wendland. 1968. The character of late- and post-glacial climatic changes. (dittoed) Univ. Wisconsin Dept. of Meteorology Center for Climatic Research. 26 p.

Cvancara, A. M., Lee Clayton, W. B. Bickley, Jr., A. F. Jacob, A. C. Ashworth, J. A. Brophy, C. T. Shay, L. D. Delorme, and G. E. Lammers. 1971. Paleolimnology of late Quaternary deposits: Seibold Site, North Dakota. Science 171: 172-174.

McAndrews, J. H. 1966. Postglacial history of prairie, savanna, and forest in northwestern Minnesota. Torrey Botan. Club Mem. 22(2): 1-72.

Ossian, C. R. 1970. Fishes of a Pleistocene lake in South Dakota. M.S. Thesis. Michigan State Univ. 69 p.

Sherrod, Neil. 1963. Late Pleistocene fish from the lake sediments in Sheridan County, North Dakota, N. Dak. Acad. Sci., Proc. 17: 32-36.

Shumm, S. A. 1965. Quaternary paleohydrology, p. 783 to 794. *In* H. E. Wright, Jr., and J. C. Frye [ed.] The Quaternary of the United States. Univ. Princeton Press, Princeton.

Uyeno, Ternya, and R. R. Miller. 1963. Summary of late Cenozoic fish records for North America. Univ. Michigan, Mus. of Zool. Occ. Pap. 631. 34 p.

Walker, P. H. 1966. Post glacial environments in relation to landscape and soils on the Cary drift. Iowa. Agri. and Home Econ. Exper. Stat. Res. Bull. 549: 838-875.

PRELIMINARY STUDY OF HEAVY MINERALS OF THREE RED RIVER VALLEY TILLS

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ABSTRACT

Heavy mineral suites of three tills in the Red Lake Falls, Minnesota area were studied by x-ray diffraction and optical methods. The age of the lower member, the Marcoux, is unknown; the upper two members, the Red Lake Falls and Huot, are probably Late Wisconsinan in age.

The suites are similar, consisting primarily of clinoamphibole, garnet, pyroxene, magnetite and epidote. Clinoamphibole (predominantly hornblende) is by far the most abundant. The Red Lake Falls member generally contains more garnet than the other two units, which may reflect a different proportion of bedrock types in the source area of this member.

Heavy liquid separations combined with microscopic analysis provided the most accurate detailed data. X-ray diffraction combined with magnetic separation may provide rapid methods of distinguishing different assemblages once they are recognized and defined by optical methods.

INTRODUCTION

Heavy mineral assemblages in tills have been studied as a correlation tool (Sitler, 1968). The present study was done to see whether three Red River Valley tills had any noticeable differences in their heavy mineral assemblages which could later be studied more systematically and in greater detail.

The localities sampled were all sites along the bluffs of the Red Lake River in northwestern Minnesota. (Table 1). The units are informally known (from bottom to top) as the Marcoux, Red Lake Falls, and Huot members (Lee Clayton, Univ. N. Dakota) of the Coleharbor Formation of Bluemle (1971). The age of the lowest till is not known. The uppermost and middle tills are probably Late Wisconsinan in age (S. Moran. N. Dak. Geol. Survey, personal communication).

METHODS

Samples of about 100 g were gently broken with a mortar and pestle, mixed with water and deflocculant, and wet-sieved. Generally only grains ranging from 125-250 microns were utilized. The heavy minerals were separated with bromoform using a funnel. One sample was separated into three magnetic fractions with a Frantz magnetic separator at 0.4, 0.8 and 1.2 amp. The heavy fraction was generally about 5% of the total fine sand fraction. Grains were cleaned with concentrated HCl.

For optical study, several hundred grains were poured onto a drop of artifical Canada balsam on a glass slide and covered with a cover glass. Five hundred heavy mineral grains were counted for each sample except 290 grains were counted for the Huot member from the Red Lake Falls site. For x-ray diffraction study, about 0.05 g were ground about 5 minutes by mortar and pestle and dusted onto a cardboard-supported cover glass with a thin film of wet, clear-brushing lacquer. One hundred grains of each slide were counted to determine the relative proportions of heavy minerals, light minerals, and rock fragments. Grains altered too extensively to be identified were counted as rock fragments.

RESULTS AND DISCUSSION

Optical data are summarized in Table 1 and Figure 1 and X-ray data in Figure 2. Clinoamphibole (predominantly hornblende) was the most abundant heavy mineral in all samples but shows little consistent variation. Abundance of garnet is relatively high in the Red Lake Falls member, low in the Marcoux and intermediate in the Huot. Magnetite is relatively lower in the Red Lake Falls. All other minerals are present in amounts of 10% or less. A small proportion (usually less than 10%) of most mineral grains are rounded, and probably came from sedimentary rocks. Rounding is almost universal in sphene and zircon, but is rarer in pyroxenes than in any other group.

The ratios of hornblende, garnet and clinopyroxene of the tills were compared using optical and x-ray methods (Figures 1 and 2). This ratio was used because the three mineral groups usually have high, easy-to-read peaks on the x-ray diffractometer record. The

Table 1. Percent frequency of heavy mineral grains in three Red River Valley tills

Mineral	Huot member		Red Lake Falls member			Marcoux member	
	SC**	F	SC	F	PN	F	PN
Clinoamphibole*	45	46	46	39	51	51	46
Garnet	13	9	14	16	20	8	7
Magnetite	11	9	7	12	4	13	12
Clinopyroxene	6	5	4	4	4	8	10
Orthopyroxene	3	8	4	3	5	4	2
Epidote	6	9	7	5	5	8	9
Biotite	2	1	3	3	2	4	1
Sphene	3	4	2	2	2	2	2
Zircon	1	1	4	3	1	2	3
Chlorite	1	1	1	1	4	4	4
Ilmenite	1	—	4	2	4	—	1
Tourmaline	1	1	4	1	1	1	1
Dolomite	2	3	1	1	—	4	1
Pyrite	3	—	6	4	—	—	—
Topaz	—	4	4	4	1	—	4

*Predominantly hornblende

**SC—Snake Curve Section (SE¼ sec. 13, T. 151 N., R. 45 W.; Moran et al., 1971)

F—Red Lake Falls Section (NE¼ sec. 22, T. 151., R. 44 W.)

PN—Red Lake County—Pennington County line section (NW¼, sec. 29, T. 152 N., R. 43 W.)

ratio also has some genetic significance: garnet is derived predominantly from metamorphic rocks, clinopyroxene from (basic) igneous rocks, and hornblende is very common in both kinds of rocks. Samples from the same stratigraphic position tend to group together, especially on the optical grain plot.

Dolomite, pyrite and biotite are not useful for correlation; an unknown amount of dolomite is dissolved in the acid bath, pyrite is easily oxidized on the outcrop, and some biotite is lost in the wet-sieving process. The amounts of all other minerals are probably mainly determined by provenance, the areas across which the ice moved while it was picking up its load. In each of the tills the ice apparently advanced over somewhat different terrain.

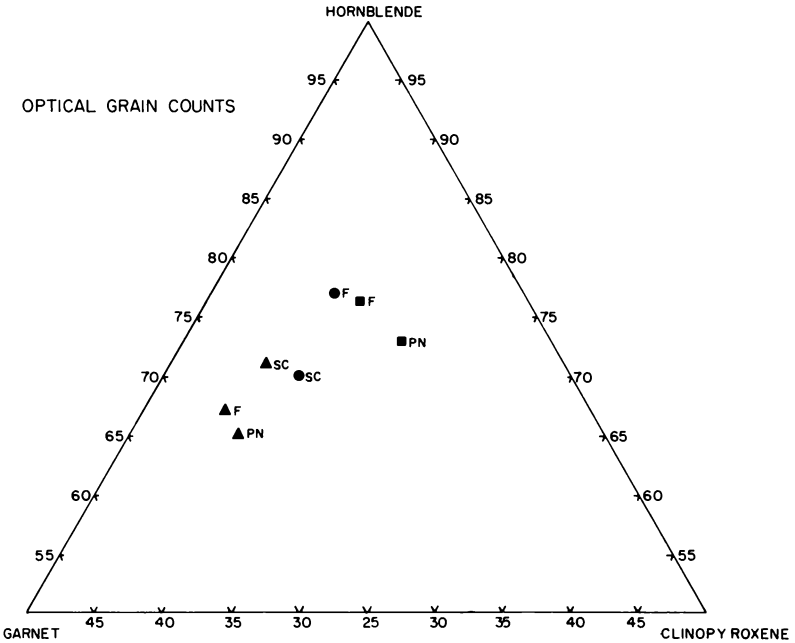

Figure 1. Hornblende, garnet, and clinopyroxene percentage ratios as determined by optical grain counts. Squares indicate the Marcoux member, triangles the Red Lake Falls member, and circles the Huot member. Letters indicate sample locations as explained in Table 1.

X-ray peak interpretations were hampered by large amounts of rock fragments and light minerals which contaminated many samples, producing a high background and many unwanted peaks. The fractions separated at 0.4 amp and 0.8 amp on the magnetic separator are fairly free of contamination and are relatively easy to use. A combination of x-ray and magnetic separation techniques may provide a rapid method of recognizing heavy mineral suites once they have been defined by the slower and more detailed microscopic technique.

REFERENCES CITED

Bluemle, John P. 1971. Geology of McLean County, North Dakota. N. Dak. Geol. Surv. Bull. 60 (2).

Moran, Stephen R., Lee Clayton, and Alan M. Cvancara. 1971. New sedimentological and paleontological evidence for history of Lake Agassiz: Snake Curve Section, Red Lake County, Minnesota. N. Dak. Acad. Sci., Proc. 24(II): 61-73.

Sitler, Robert F. 1968. Glacial till in oriented thin section. Internat. Geol. Cong. Rept. 23rd 8: 283-95.

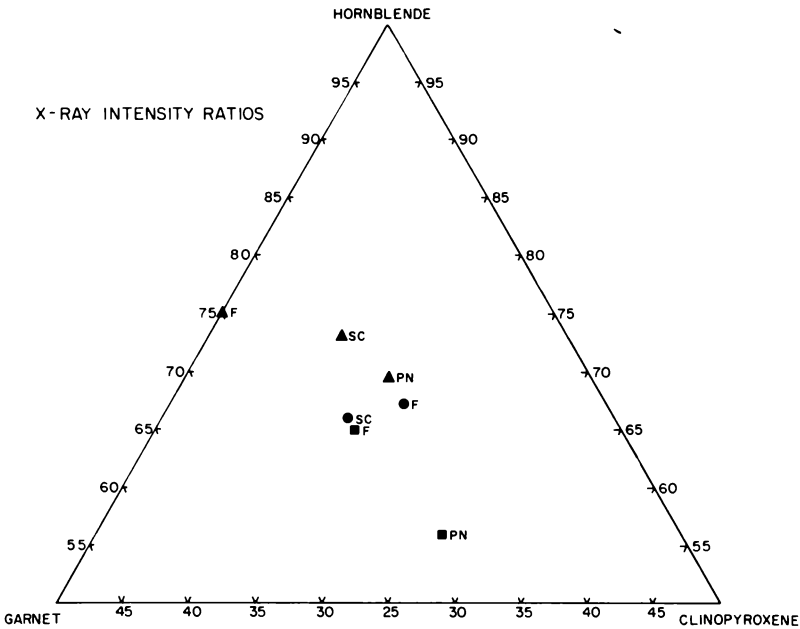

Figure 2. X-ray intensity ratios for hornblende, garnet and clinopyroxene peaks. Squares indicate the Marcoux member, triangles the Red Lake Falls member, and circles the Huot member. Letters indicate sample locations (Table 1). X-ray reflections (2θ for CuK alpha radiation) are hornblende, 10.4°; clinopyroxene, 30.3°; and garnet, 34.7°.

PRELIMINARY MODAL STUDY OF PORPHYRITIC QUARTZ SYENITE, OSSIPEE MOUNTAINS, NEW HAMPSHIRE

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ABSTRACT

Bulk mineral compositions of rocks mapped as porphyritic quartz syenite in the Ossipee Mountains area, New Hampshire, show relatively high quartz values. The range of quartz contents as determined by x-ray diffraction is 15-34% with most values 20-25%. Moat volcanics and intermediate rocks account for many values outside the 20-25% range. This study suggests that the analyzed rocks are typically granitic in composition and probably should not be included in the porphyritic quartz syenite unit of the White Mountain plutonic-volcanic series.

INTRODUCTION

This study is part of a program to determine quantitative mineralogic (modal) data for the rock units of the Jurassic-Cretaceous White Mountain plutonic-volcanic series of New England. General geology of the series is summarized by Billings (1956) and Chapman (1968). The Ossipee Mountains are one of the major areas of exposure of the porphyritic quartz syenite unit of the series (Billings, 1956). Kingsley (1931) mapped a continuous, 360-degree, ring-dike of this unit in the Ossipee region and Wilson (1969) has remapped the northeast part of the ring dike in the Ossipee Lake Quadrangle. Billings (1956) suggested that typical porphyritic quartz syenite contains 12-18% quartz. Our study suggests a considerably higher value for the majority of rocks of this type in the Ossipee region.

METHODS

Detailed, systematic sampling was accomplished by the following plan during the summer of 1970: (1) A grid was overlaid on the geologic map (Figure 1) of porphyritic quartz syenite dividing it into ½km x ½km sampling areas; 2) Regions of high probability of outcrops, as determined by preliminary study of pre-existing geologic maps and reports, were sampled in every ½km x ½km sampling area in which they occurred; and (3) Regions of low probability of outcrops were searched for outcrops only in every fourth ½km x ½km sampling area. Sample locations for rocks analyzed in this study are shown in Figure 1.

Mineralogical compositions were determined by x-ray diffraction procedures suggested by Alexander and Klug (1948), Leroux and others (1953), and described fully by Karner (1968). The method utilizes peak-height data for the 26.7° and 50.2° 2θ ($CuK\alpha$) quartz reflections corrected for variation of mass absorption coefficients. The quartz standard was taken from sample 1-15-7-1-4. Reliability of quartz determinations for similar methods is generally found to be better than about 10% of the amount present (Niskanen, 1964; Tatlock, 1966). We are currently evaluating precision by repeated analysis of individual samples, and accuracy by comparison with data determined by optical methods. Preliminary results suggest precision is high and that quartz contents determined by x-ray methods are generally within 2-3% of contents determined by optical methods.

RESULTS AND DISCUSSION

Quartz contents are given in Table 1 and illustrated in Figure 2. Rocks mapped as porphyritic quartz syenite have been divided into porphyritic quartz syenite and Moat volcanics (Kingsley, 1931; Billings, 1956; and Wilson, 1969). Intermediate rocks in Table 1 and Figure 2 may be varieties of either or both of the above. Results show (1) quartz-contents of 18-28% (median 22%) for the porphyritic quartz syenite and (2) quartz contents generally above or below this range for the Moat vocanics and intermediate rocks.

These preliminary data indicate that, in the Ossipee area, the rocks mapped as porphyritic quartz syenite have quartz contents which are too high for the rock type described by Billings (1956).

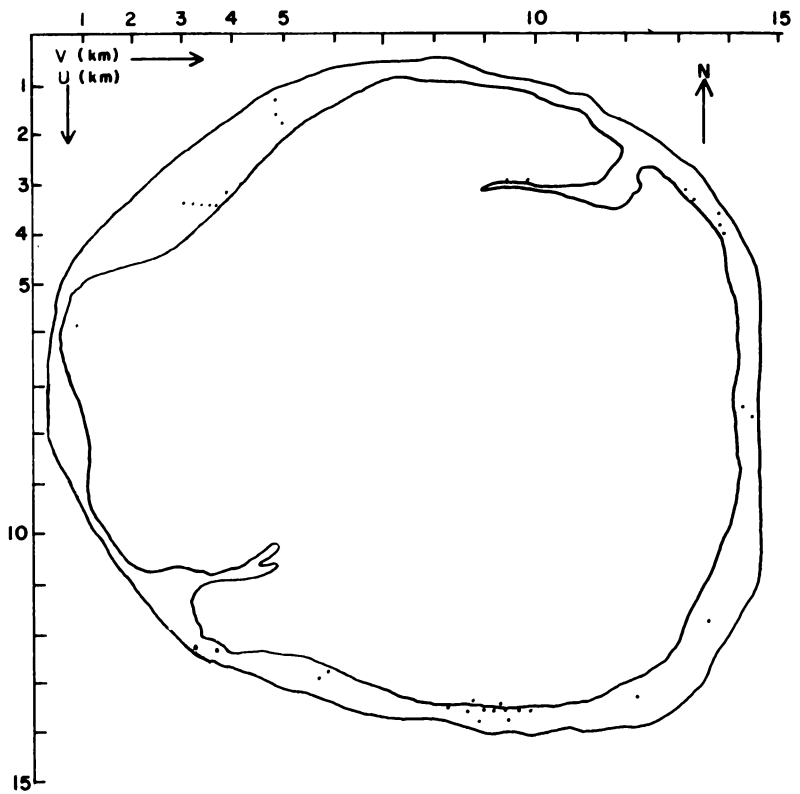

Figure 1. Exposure area of porphyritic quartz syenite in the Ossipee region as mapped by Kingsley (1931). Samples used in this study are located by dots and are identified by V and U coordinates in Table 1.

However, they agree more closely with the 17 and 19% quartz values which Wilson (1969) obtained in the Ossipee region. Completion of this study may show that the Ossipee rocks are typically granitic in composition and should not be included in the quartz syenite unit of the White Mountain series.

Other areas of exposure of porphyritic quartz syenite are also being studied to see if they qualify as quartz syenites or whether they are compositionally more comparable to the granitic rock types of the White Mountain series.

ACKNOWLEDGMENTS

This study was supported by National Science Foundation Undergraduate Research Participation Grant GY 7619.

Table 1. Weight percentages of quartz in analyzed rocks of the Ossipee region

Sample*	Rock type**	Quartz content	Sample**	Rock type**	Quartz content
1-1-5-4-0	MV	28.3	1-9-13-2-4	PQS	20.8
1-4-3-1-0	PQS	23.3	1-9-13-4-2	I	33.6
1-4-3-1-0-A	PQS	23.0	1-10-2-3-0	I	29.6
1-4-3-1-0-B	PQS	24.2	1-10-2-4-0	PQS	21.9
1-4-3-1-0-C	PQS	23.2	1-10-13-1-0	MV	15.3
1-4-3-1-0-D	PQS	23.2	1-10-13-1-3	PQS	21.9
1-4-3-1-0-E	PQS	24.7	1-10-13-1-3-A	PQS	22.2
1-4-12-1-0	I	30.0	1-10-13-1-4	PQS	21.3
1-4-12-2-0	PQS	26.7	1-10-13-2-0	MV	14.7
1-5-1-2-0	PQS	19.8	1-10-13-2-3	PQS	18.4
1-5-1-4-4	PQS	20.0	1-13-13-1-1	PQS	20.5
1-5-1-4-4-A	PQS	20.3	1-14-3-1-0	PQS	20.2
1-6-12-4-0	PQS	24.0	1-14-3-1-1	I	18.5
1-6-12-4-2-A	PQS	23.6	1-14-3-4-0	PQS	27.9
1-9-13-1-0	PQS	22.5	1-14-11-4-1	PQS	23.7
1-9-13-2-0	PQS	23.1	1-15-7-1-0	PQS	23.5
1-9-13-2-3	PQS	19.4	1-15-7-1-4	PQS	20.0

*The first number is an index to the Ossipee region. The second and third numbers are V and U coordinates of a 1 km^2 area on Figure 1. The fourth number subdivides the 1-km^2 area into quarters with numbering from left to right and top to bottom. The fifth number subdivides the quarter into four more parts with numbering from left to right and top to bottom.

**PQS—Porphyritic quartz syenite

MV—Moat volcanics

I—Intermediate rocks

REFERENCES CITED

Alexander, L., and H. P. Klug. 1948. Basic aspects of x-ray absorption in quantitative diffraction anlysis of powder mixtures. Anal. Chem. 20: 886-889.

Billings, M. P. 1956. The geology of New Hampshire. Pt. II, Bedrock geology. Concord, N.H., N.H. State Plan. and Devel. Comm., 203 p.

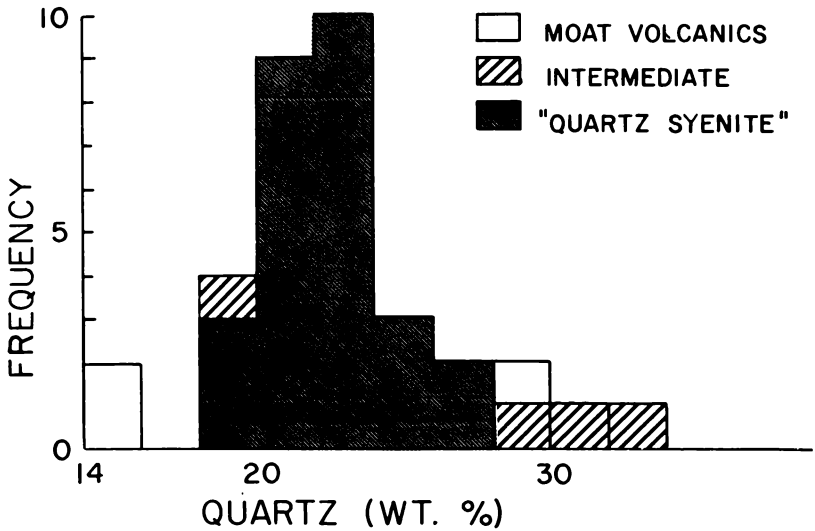

Figure 2. Frequency distribution of quartz in analyzed rocks of the Ossipee region.

Chapman, C. A. 1968. A comparison of the Maine coastal plutons and the magmatic central complexes of New Hampshire, p.385 to 396. *In* E-an Zen, W. S. White, J. B. Hadley, and J. B. Thompson [eds.] Study of Appalachian geology: northern and maritime. John Wiley & Sons. New York.

Karner, F. R. 1968. X-ray analysis of cement-rock cores, p. 41 to 55. *In* S. B. Anderson and H. C. Haraldson. Cement-rock possibilities in Paleozoic rocks of eastern North Dakota. N. D. Geol. Surv. Rep. of Invest. 48.

Kingsley, L. 1931. Cauldron-subsidence of the Ossipee Mountains. Am. Jour. Sci. 22: 139-168.

Leroux, J., D. H. Lennox, and K. Kay. 1953. Direct quantitative x-ray analysis by diffraction-absorption technique. Anal. Chem. 25: 740-743.

Niskanen, E. 1964. Quantitative mineral analysis of kaolin-bearing rocks by x-ray diffraction. Anal. Chem. 36: 1268-1271.

Tatlock, D. B. 1966. Rapid modal analysis of some felsic rocks from calibrated x-ray diffraction patterns. U.S. Geol. Surv. Bull. 1209. 41 p.

Wilson. J. R. 1969. The geology of the Ossipee Lake Quadrangle New Hampshire. N.H. Dept. of Resources and Econ. Devel. Bull. 3. 116 p.

PERITONEAL FLUID ACCUMULATION IN RATS AFTER INJECTION OF POLYETHYLENE GLYCOL

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Second Place Winner
A. Roger Denison Student Research Competition

ABSTRACT

Polyethylene glycols (PEG) are polymers of ethylene oxide often used as solvents or bases for water insoluble therapeutic compounds. We have observed an accumulation of peritoneal fluid in rats following the intraperitoneal administration of this solvent. Forty-four rats were randomly divided into a control group of eight animals and three experimental groups of twelve animals each. Control animals received sham injections and groups 1, 2 and 3 received intraperitoneal injections of 0.1, 0.2 and 0.3 ml, respectively, of PEG-400 (m.w.-400). At 0.5, 1.0, 2.0 and 4.0 hours post injection, two animals from the control group and three animals from each experimental group were sacrificed and examined for peritoneal fluid accumulation, hematocrit, peritoneal fluid protein and plasma protein. Peritoneal fluid volumes of less than 0.1 ml were measured in control animals whereas volumes as high as 6.6 ml were measured in experimental animals. Significant differences were demonstrated between all dose levels, the highest occurring between the controls and the 0.3 ml group (P 0.001). Hematocrits ranged from 42.5-60.0%. Plasma protein concentrations ranged from 6.48-9.12 g% and peritoneal fluid protein from 0.32-2.10 g%. Results indicated that the mechanism for this fluid accumulation is by increased capillary permeability with associated vasodilation. In light of the magnitude of these accumulations and the variance observed in all parameters, caution should be exercised in the interpretation of experiments where polyethylene glycol is used.

INTRODUCTION

Polyethylene glycols (PEG), polymers of ethylene oxide, are available in varying molecular weights (Smyth, Carpenter and Weil, 1950). The lower molecular weight polyethylene glycols (less than 1000) are moderately viscous, colorless, somewhat hygroscopic liquids. Molecular weights above 1000 exist as white, wavy solids. Polyethylene glycols do not hydrolze or deteriorate and dissolve in water in all proportions to form clear solutions (Union Carbide Chemicals Company, 1959 and Carpenter and Shaffer, 1952). They are frequently used as solvents or bases for water insoluble therapeutic compounds. The literature abounds in papers on the oral and topical toxicities of PEG but there is a paucity of literature on its parenteral effects (Smyth, Carpenter and Shaffer, 1945; Shaffer and Critchfield, 1947; Tusing, Elsea and Sauveur, 1954 and Smyth, Carpenter and Weil, 1955). The 300 and 400 molecular weight PEGs are the most often used in parenteral products and their intraperitoneal LD-50s are 19, 125 and 17,000 mg/kg rat body weight, respectively. PEG-400

does not elicit foreign body reactions in animals (Smyth et al., 1950). When PEG was used as a solvent for intraperitoneal injection of carbamate pesticides, we observed an excessive accumulation of peritoneal fluid. This investigation was designed to determine if an intraperitoneal injection of PEG alone could cause this abdominal fluid accumulation. This paper reports the results of those investigations.

METHODS AND MATERIALS

Male, 350-gram rats (Holzman Co., Madison, Wisc.) given Purina Lab Chow and water *ad libidum* were used. Forty-four sodium pentobarbital-anesthetized rats were randomly divided into four groups. A control group received sham injections and experimental groups 1, 2, and 3 received intraperitoneal injections of 0.1, 0.2 and 0.3 ml respectively, of PEG-400 (Matheson, Coleman and Bell, Northwood, Ohio). At 0.5, 1.0, and 2.0 and 4.0 hours post injection, two animals from the control group and three animals from each experimental group were sacrificed by skull fracture and examined for peritoneal fluid accumulation. Fluid was aspirated by pipet and measured in a 10 ml graduated cylinder. Blood was drawn per cardiac puncture into heparinized syringes and hematocrits were immediately determined. Cells were separated and samples of plasma and peritoneal fluid were frozen for later protein determination. Data were analyzed by analysis of variance and Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS

Less than 0.1 ml of peritoneal fluid was recovered from any control animal whereas experimental animals had up to 6.6 ml (Figure 1). Significant differences in volumes of fluid recovered were found between all dose levels (P 0.05), the greatest difference occurring between the controls and the 0.3 ml group (P 0.001). In appearance, peritoneal fluids ranged from lightly straw colored to dark red; mesenteric veins were dilated. All peritoneal fluid was observed to gel shortly after aspiration from the animal. Although hematocrit values from blood drawn per cardiac puncture ranged from 42.5-68.0%, there was no significant difference between groups. Peritoneal fluid protein ranged from 0.32-2.10 g% and plasma protein ranged from 6.48-9.12 g%. No significant differences occurred between the control and experimental groups for any of the protein values. It was notable however, that in some instances the plasma protein concentration decreased as the peritoneal fluid protein increased. This was most evident in the 0.3 ml group where an opposite and nearly equal change occurred between the plasma and peritoneal fluid protein. Maximum mean fluid accumulations were 2.8 ml at 2 hrs for the 0.1 ml group, 4.2 ml at 2 hrs for the 0.2 group and 5.5 ml at 1 hr for the 0.3 ml group (Figure 1). By the fourth hour the mean values of fluid recovered were lower for all experimental groups. The fourth hour post injection volume for group 1 was 2.1 ml, for group 2, 0.9 ml and 4.1 ml for group 3. All fluid volumes, it appears, were returning to normal.

DISCUSSION

The largest volume of fluid recovered was 6.6 ml 1 hr after injec-

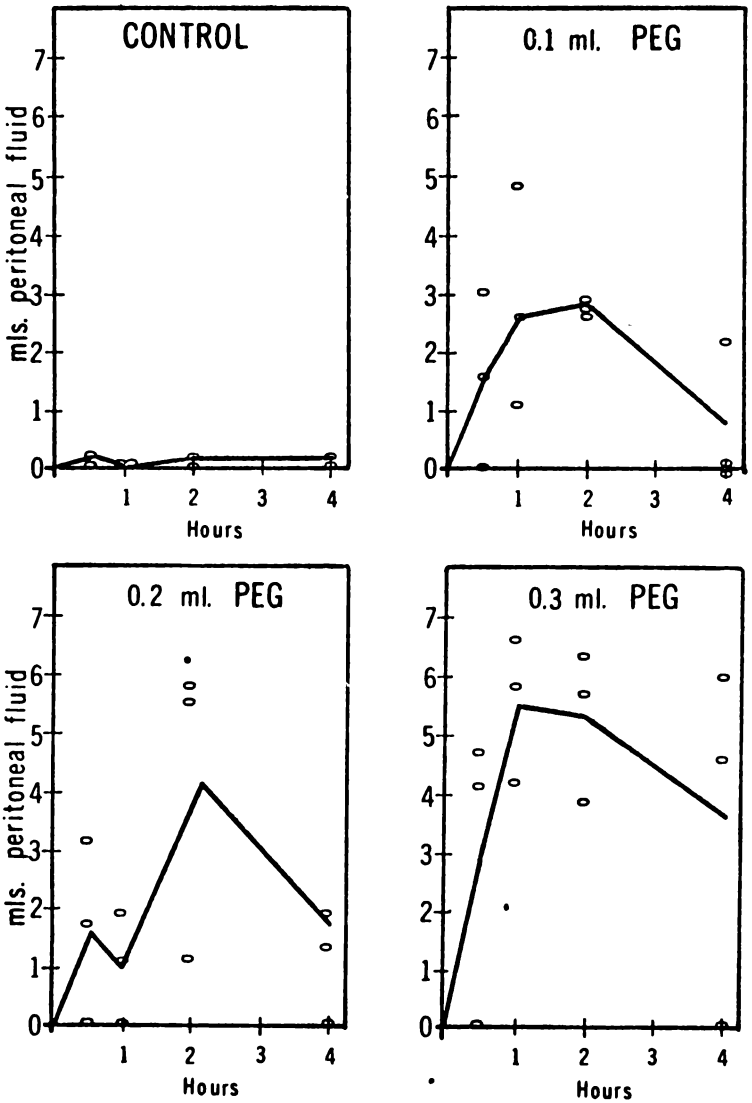

Figure 1. Milliliters of peritoneal fluid recovered from rats after IP injection of polyethylene glycol. o = observations, ——— = means.

tion of 0.3 ml PEG. This animal weighed 366 g and had a calculated blood plasma volume of 10.9 ml (3% body weight). Such an accumulation of peritoneal fluid, equal to 60% of the animal's plasma volume, could alter markedly the results of a study in body fluid move-

ment, volume or pressure. Although these results show that PEG caused this fluid accumulation, the mechanism of its action is not clear. Osmosis is not a problable mechanism since up to 600 times more fluid was recovered than would be necessary to osmotically balance the quantity of PEG injected. The moderate amounts of protein present in the peritoneal fluid also refute osmosis which would cause primarily an accumulation of water. Our results indicate that the mechanism of action of PEG in causing these large accumulations of lymph-like fluid is increased capillary permeability with associated vasodilation of peritoneal blood vessels. PEG-400 is readily absorbed into the vascular system from the peritoneal space and excreted by the kidneys (Smyth et al., 1950). A resultant fluid accumulation occurs which is maintained for a dose dependent time and the fluid is then reabsorbed into the vascular system. The greater the dose, the longer is the time required for reabsorption (Figure 1). The vascular system is the immediate fluid source since it is improbable that these large volumes could arise from extravascular tissue in immediate contact with the peritoneal space. One would expect that as the vascular system lost fluid to the peritoneal space, a concomitant increase should occur in the hematocrit. We are unable to show statistically significant differences in the hematocrits but changes in hematocrit corresponding with fluid changes were noted. It is plausible that the vascular fluid lost to the peritoneal space was rapidly replaced by tissue fluid from other areas of the body. Expected changes were observable in plasma and peritoneal fluid protein concentrations even though these differences were not statistically significant. In light of the fluid shifts we observed and variance in observations (Figure 1), caution should be exercised in the interpretation of experimental results where polyethylene glycol is used.

ACKNOWLEDGMENTS

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REFERENCES CITED

Carpenter, C. P., and C. B. Shaffer. 1952. A study of the polyethylene glycols as vehicles for intramuscular and subcutaneous injection. J. Amer. Pharm. Ass. 41:27.

Duncan. D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1.

Shaffer, C. B., and F. H .Critchfield. 1947. The toxicity of high molecular weight polythylene glycols; chronic oral and parenteral administration. J. Amer. Pharm. Ass. 36:157.

Smyth, H. F., C. P. Carpenter, and C. B. Shaffer. 1945. The subacute toxicity and irritation of polyethylene glycols of approximate molecular weights of 200, 300 and 400. J. Amer. Pharm. Ass. 34:172.

Smyth. H. F., C. P. Carpenter, and C. S. Weil. 1950. The toxicology of the polyethylene glycols. J. Amer. Ass. 39:349.

Smyth. H. F., C. P. Carpenter, and C. S. Weil. 1955. The chronic oral toxicities of the polyethylene glycols. J. Amer. Pharm. Ass. 44:27.

Tusing, T. W., J. R. Elsea, and A. B. Sauveur. 1954. The chronic dermal toxicities of a series of polyethylene glycols. J. Amer. Pharm. Ass. 43:489.

Union Carbide Chemicals Company. New York, N.Y. 1959. Carbowax, polyethylene glycols for pharmaceuticals and cosmetics.

PRESENT AND PAST MOLLUSKS OF THE FOREST RIVER, NORTH DAKOTA

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ABSTRACT

The Forest River in northeastern North Dakota was studied for living and fossil mollusks primarily during the summers of 1965 and 1966; additional data were gathered occasionally during 1967-1970. Twenty-two stations were sampled for living forms and fossil species were collected at cutbank exposures at four sites. Twenty-one species were found living in the river: 6 unionid bivalves (mussels), 5 pisidiid bivalves (fingernail clams) and 10 gastropods. Most of the mollusks occurred in the middle section of the river between Fordville and Minto. Unionids were found farthest downstream (few miles downstream from Minto), followed by pisidiids and gastropods. High chloride content (up to 1230 ppm) in the lower reaches of the river is perhaps the primary ecologic factor influencing the occurrence of mollusks. High turbidity, also in the lower reaches, may also be a limiting factor. The fossil fauna was very similar to that of today, lacking in only five species or less and possessing no different forms. This suggests a simlar regimen for the Forest River since the oldest sampled mollusks lived in late (?) Holocene time.

INTRODUCTION

Detailed studies of all mollusks of an entire river system are lacking in North Dakota except that of Norby (1967). One group, the unionids (mussels), has been studied rather extensively in the rivers of the eastern part of the state (Cvancara and Harrison, 1966; Cvancara, Heetderks and Iljana, 1967; Cvancara, 1970; and Cvancara and Erickson, 1968). This paper presents all known aquatic mollusks of the Forest River and evaluates the primary ecological factors influencing their occurrence, and compares the fossil fauna with that of today.

MATERIALS AND METHODS

Most of the field work was done during the summers of 1965 and 1966; additional collecting or measurement of water chemical factors was accomplished periodically during 1967-1970. Twenty-two stations

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for living mollusks were established on the river, most of them during 1966. Unionids were collected by hand-picking with the aid of a Turtox Fishscope, an aluminum alloy cylinder (0.61 m long by 0.15 m in diameter) fitted with a glass plate at one end. Other mollusks were collected by passing a food strainer (with wire screen) through the sediment and aquatic vegetation. Chemical factors and turbidity were measured in the field with a Hach Chemical Company portable chemical kit (Model DR-E); pH was measured with a Taylor pH Slide Comparator, Model T-1. Specific conductance was measured with a Beckman Solu Bridge, Model RB3-338.

For the fossil mollusks, each recognized lithologic unit was channel sampled at a measured section; the vertical channel was 9-15 cm wide and 3-4 cm deep, depending on the lithology. Units containing unionids were also grab sampled for these organisms. Volumes of lithologic samples were measured in a graduated cylinder, and the sediment was dry sieved through 4, 24 and 115 mesh sieves; only fractions retained on these sieves were picked for fossils.

RESULTS

Geologic setting.—The Forest River originates in northeastern Nelson and western Walsh Counties, northeastern North Dakota; its three branches converge near the town of Fordville (Figure 1). Flowing eastward and northeastward, the river joins the Red River 49 km north of Grand Forks.

Throughout its extent, the river is cut into glacier-related sediments of late Quaternary age. West of a line passing near stations 1, 5 and 7 (Figure 1), the three branches flow across the Drift Plains district (Hansen and Kume, 1970, p. 7) where the shallow valleys are cut into boulder-clay (till; Bluemle, 1971). East of this line the river enters the low sloping Agassiz Lake Plain district and, to about station 13, is incised largely into sand and gravel (of the Elk Valley delta-outwash plain; Hansen and Kume, 1970). Here, the valley may be up to 30 m deep but is usually about 14 m. From station 13 to station 16. the river valley is incised to about 12 m into boulder-clay (waterworn till) and sand and gravel (fluvial or nearshore deposits). Downstream from station 16 to the mouth, the river is cut through mostly clay and silt (deposited in glacial Lake Agassiz) to about 4.5 m.

Stream characteristics.—The Forest River drains a basin of about 2200 km^2 (850 mi^2) and has an average gradient of 0.72 m/km (3.8 ft/mi). Upstream from about station 19, the gradient is higher, about 1.2 m/km (6.4 ft/mi) (Harrison, 1965, p. 19-20), and corresponds to areas of coarser bottom sediment, riffles and pools. Below station 19 the bottom sediment changes from sand to mud (Table 2), and riffles and pools are absent. Channel sediment generally reflects the surficial geology except between stations 16 and 19, where the river cuts through clay and silt but the bottom sediment is sand and muddy sand. Turbidity generally increases downstream as the bottom sediment becomes finer (Figure 2).

Discharge data are given for two gaging stations on the river (Figure 1). The Forest is but a small tributary of the Red River; mean discharges at Minto and Drayton (on the Red River 33 air km

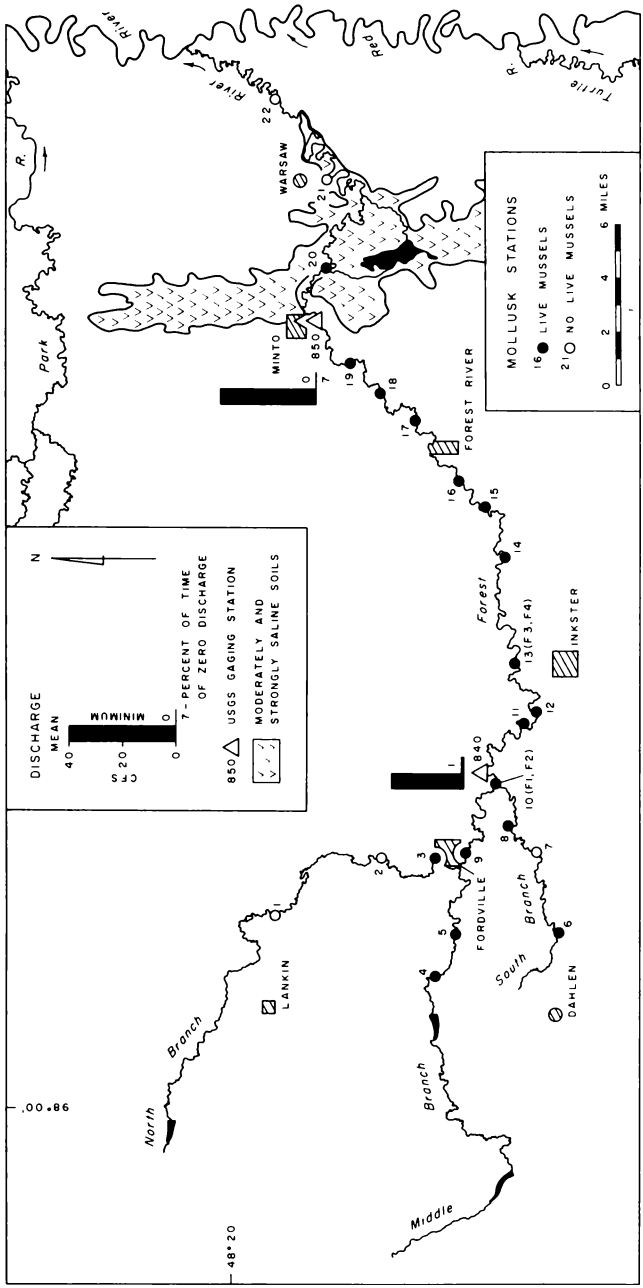

Figure 1. Location map showing mollusk stations and discharge values for two gaging stations on the Forest river. The numbers F1-F4 indicate fossil sites. Discharge values are for the 10-year span from October 1, 1955 to September 30, 1965 (U.S. Geological Survey, 1961-1966 and 1964). The area of saline soils was taken from a soil map of U.S. Dep. of Agriculture, Soil Conservation Service (1967).

Table 1. Specific conductance and several chemical factors for five stations on the Forest River*

Station	Specific Conductance (μmhos/cm)	pH		Total Phosphates (ppm)		Total Nitrites (ppm)		Total Nitrates (ppm)	Iron (ppm)
	1968	1965	1966	1965	1966	1965	1966	1965	1966
9	660	7.9	8.4	0.64	0.19	0.017	0.002	1.6	0.07
13	660	8.1	8.5	0.95	0.36	0.031	0.002	2.8	0.08
19	725	8.1	8.4	0.28	0.15	0.010	0.001	1.1	0.23
20	900	8.4	8.4	0.11	0.23	0.014	0.001	—	0.23
22	4600	8.0	8.3	0.32	0.28	0.007	0.000	—	0.34

*Specific conductance was measured on October 24, 1968; other factors were measured on July 15, 1965 and August 20, 1966.

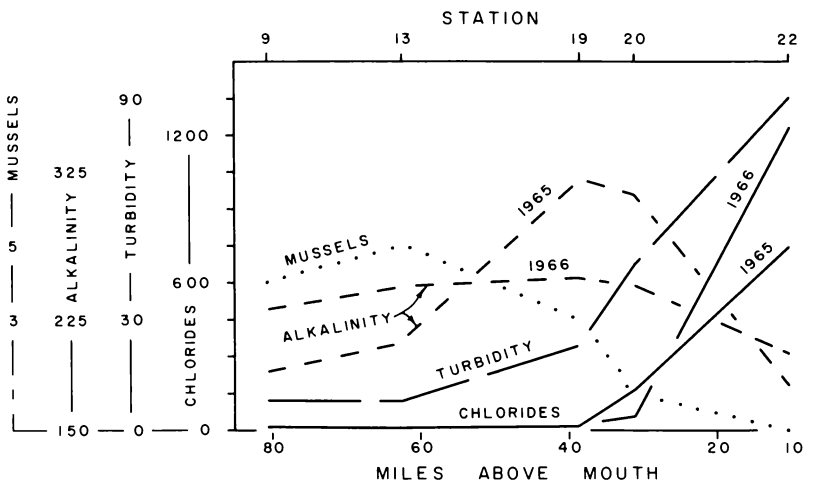

Figure 2. Graph showing the variation of unionid species (mussels), total alkalinity (ppm), total chlorides (ppm) and turbidity (Jackson turbidity units) with station on the Forest River. Total alkalinity and total chlorides were measured at the five stations on July 15, 1965 and August 20, 1966; turbidity was measured on August 20, 1966.

north-northeast of Minto) are about 1 m^3s (36 ft^3s) and 80 m^3s (2837 ft^3s), respectively. Flow is intermittent in the uppermost reaches as well as at Minto where no flow occurs 7% of the time.

Various chemical factors of the river water are given in Table 1 and Figure 2. Specific conductance (and hence total dissolved solids) increases markedly downstream, probably largely because of increasing total chlorides (highest value measured, 1230 ppm). Total alkalinity seems to have an inverse relationship with total chlorides, i.e., alkalinity is high where chlorides are low.

Living mollusks.—Twenty-one species of mollusks were found to

inhabit the Forest River: 6 unionid bivalves, 5 pisidiid bivalves (fingernail clams), and 10 gastropods (Table 2). Most of the mollusks occurred in the middle section of the river between Fordville and Minto. Unionids were found farthest downstream, followed by pisidiids and gastropods. No live mollusks were observed in the lowest reaches of the river (few miles below Minto), an area associated with high total chlorides of the water, saline soils and a saline lake (Figure 1).

Unionids were collected in greatest concentrations in the upper section of the main river, between Fordville and near Forest River. *Anodonta grandis* occurred most frequently and *Strophitus rugosus* least frequently (collected alive at only two stations). *A. grandis* also persisted farthest downstream (station 20), and it and *Anodontoides ferussacianus* appeared to persist farthest upstream in the intermittent sections of the river. Of the two species of *Lasmigona*, *L. complanata* was always found in greater numbers. Unionids occurred on all types of bottom but rarely between boulders and cobbles. The raccoon seemed to be the primary predator; shells broken posteriorly and with teeth and claw marks, and feeding sites were observed at several stations. *Pisidium compressum and Sphaerium striatinum* were the most frequently found pisidiids, whereas *P. casertanum* was collected at only one locality. *P. compressum* occurred alive farthest upstream and *S. striatinum* and *S. simile* were collected farthest upstream. Pisidiids generally occurred adjacent the banks where the current was slower and the bottom sediment finer, usually very fine to fine muddy sand.

The most frequently found gastropod was *Physa gyrina,* followed by *Amnicola integra;* although at fewer stations, *A. integra* usually occurred in greater numbers. (The two species of *Physa* were identified following the characterizations of Clampitt, 1970.) Two species more characteristic of stagnant water, *Stagnicola palustris* and *Gyraulus parvus,* were rare. At station 12, *S. palustris* occurred in nearly stagnant water where a spring entered the river. Of the two species of *Helisoma, H. anceps* was more frequently observed. Branchiate (operculate) snails (*Probythinella lacustris* and *A. integra*) occurred in habitats very similar to those of the pisidiids. Other snails were generally found crawling on various types of bottom sediment or attached to aquatic vegetation. The limpet *Ferrissia rivularis* was found commonly on unionid shells.

Fossil mollusks.—Fossil mollusks were discovered at essentially three sites (Table 3). All sites are at cutbank exposures along the present course of the Forest River. Measured sections displayed considerable lithologic variation, precluding correlation of units between sites. All sections were bottomed by pebble gravel (base not exposed) at or just above river level, and capped by fine sand and mud. Fossils were generally more frequent in the coarser sand and gravelly sand, both of which were commonly cross-stratified.

Seventeen species of aquatic mollusks, 4 unionids, 4 pisidiids and 8 gastropods, were found as fossils. Also, *Pisidium casertanum* may be represented among "*Pisidium* sp." (Table 3) which includes specimens unidentifiable to species with certainty, as does "*Sphaerium*

Table 2. Distribution and relative abundance of live mollusk species and predominant bottom sediment at each station sampled on the Forest River

MOLLUSK SPECIES	STATION							
	1 *(A359)	2 (A358)	3 (A172)	4 (A177a)	5 (A357)	6 (A356)	7 (A176)	8 (A171)
Unionid bivalves								
1. *Anodonta grandis* Say		**R	22.0	U	R	R	U	1.0
2. *Anodontoides ferussacianus* (Lea)			R	2.0	4.0	1.5	R	23.0
3. *Strophitus rugosus* (Swainson)								R
4. *Lasmigona complanata* (Barnes)								2.0
5. *L. compressa* (Lea)			R					1.0
6. *Lampsilis radiata luteola* (Lamarck)			42.0					
Pisidiid bivalves								
7. *Pisidium casertanum* (Poli)								
8. *P. compressum Prime*		U	A	U	A	C		U
9. *Sphaerium lacustre* (Muller)				U	U	U		
10. *S. simile* (Say)		U	U			U		
11. *S. striatinum* (Lamarck)			A					C
Gastropods								
12. *Probythinella lacustris* (Baker)								
13. *Amnicola integra* Say			A					
14. *Physa gyrina* (Say)	U		U		U	U		U
15. *Physa integra* Haldeman								
16. *Fossaria obrussa* (Say)								
17. *Stagnicola palustris* (Muller)					U			
18. *Gyraulus parvus* (Say)			R					U
19. *Helisoma anceps* (Menke)			U					C
20. *H. trivolvis* (Say)					U			
21. *Ferrissia rivularis* (Say)						U		
PREDOMINANT BOTTOM SEDIMENT	***GS	GS	SG	SG	SG	SG	GS	GS

*Accession numbers of the Department of Geology, University of North Dakota.

**Letter symbols for estimated relative abundance are: A = abundant, C = uncommon, U = uncommon, and R = rare; where underlined a species presence was evidenced only by empty shells. Numerical values for the unionids are the individuals collected per hour by two persons.

***GS = gravelly sand, SG = sandy gravel, S = sand, GMS = gravelly muddy sand, MS = muddy sand, SM = sandy mud, and M = mud, which is a mixture of silt and clay.

(Table 2, continued)

	STATION													
	9 (A43)	10 (A174)	11 (A448)	12 (A449)	13 (A42)	14 (A175)	15 (A154)	16 (A169)	17 (A173)	18 (A170)	19 (A44)	20 (A45)	21	22
Unionid bivalves														
1.	186	23.0	A	12.0	2.0	2.0	16.5	4.0	14.0	13.6	18.0	1.3		
2.	20.0	22.0	C	33.0	5.0	6.0	6.5	4.0	2.0	1.6	1.3			
3.				R			0.5	1.0						
4.	17.0	9.0	A	22.0	2.5	13.0	14.5	26.0	13.0	2.4	0.7			
5.		3.0	U	1.5	1.0	1.0	1.5	1.0	1.0	0.8				
6.	13.0	10.0	R	7.0	3.0	14.0	3.0	14.0	1.0	0.8				
Pisidiid bivalves														
7.						U								
8.	U	R	C	U		A		U						
9.	U								R					
10.		R						U		U				
11.	C	U		C	C	A		A	U	C				
Gastropods														
12.			U			C		C						
13.			A	U	A	A		C						
14.			A	C	U	U		U						
15.			A	C										
16.			C	C										
17.				R										
18.			R	R										
19.				U	C									
20.				U										
21.			C	C										
PREDOMINANT BOTTOM SEDIMENT	GS	GS	GS	GS	GS	SG	GS	S	S	MS	MS	SM	M	M

Table 3. Occurrence of fossil mollusks at four sites on the Forest River
(Figure 1 shows location of fossil sites and Table 2 explains symbols for sediment type)

SPECIES	FOSSIL SITE, UNIT AND SAMPLE SIZE*										
	F1	(A179)**		F2	F3	(A180)		F4	(A456)		
Unionid bivalves	unit 1 1000cc	unit 2 1300cc	unit 3 1700cc	(A178) 2000cc	unit 1 1100cc	unit 2 1100cc	unit 3 1400cc	units 1-3 1500cc	unit 4 1700cc	unit 5 2200cc	unit 6 3000cc
1. *Anodonta grandis* Say	—	—	—	X	X	X	X	—	—	—	—
2. *Anodontoides ferussacianus* (Lea)	—	—	—	—	X	X	—	—	—	—	X
3. *Lasmigona compressa* (Lea)	—	—	—	—	X	—	X	—	—	—	—
4. *Lampsilis radiata luteola* (Lamarck)	—	—	—	X?	X	—	—	—	—	—	—
Pisidiid bivalves	—	—	—	—	—	—		—	—	—	—
5. *Pisidium compressum* Prime	—	—	—	—	—	—	65½	—	—	—	—
6. *Pisidium* sp.	4	1	—	3	57	6	15	—	½	—	—
7. *Sphaerium lacustre* (Muller)	—	—	—	1?	1½	—	—	—	—	—	—
8. *Sphaerium simile* (Say)	—	—	—	20½	41½	—	1	—	—	—	4½
9. *Sphaerium striatinum* (Lamarck)	6	3½	2	3½	255	—	36½	—	—	½	3
10. *Sphaerium* sp.	1½	½	—	5	21	1½	2	—	1½	—	—
Gastropods											
11. *Probythinella lacustris* (Baker)	—	2	—	1	226	3	24	—	—	—	6
12. *Amnicola integra* Say	2	3	—	4	1022	20	164	—	—	1	16
13. *Physa gyrina* (Say)	—	—	—	1	—	2	—	—	—	—	—
14. *P. integra* Haldeman	—	1	1	3	70	9	36	—	—	—	—
15. *Fossaria obrussa* (Say)	—	—	—	1	15	8	12	—	—	—	—
16. *Gyraulus parvus* (Say)	—	2	—	2	74	12	39	—	—	—	2
17. *Helisoma anceps* (Menke)	—	1	—	1	46	5	15	—	—	—	3
18. *Helisoma trivolvis* (Say)	—	—	—	—	2	—	—	—	—	—	—
19. *Ferrissia rivularis* (Say)	—	1	—	1	46	26	27	—	—	—	4
Various terrestrial species	X	X	X	X	X	X	X	—	—	X	X
PREDOMINANT SEDIMENT TYPE	SG	MS	S	S	GMS	S	GS	GS,MS,S	S	S	GS
THICKNESS OF UNIT (M)	1.1	1.0	1.1	2.3	0.9	0.8	0.6	0.8	0.2	1.1	0.1

*Sites F3 and F4 are at the same locality but collections were made two years apart. For each sample are given numbers of individual specimens except for mussels. Units with lower numbers are relatively older.
**Accession number of Department of Geology, University of North Dakota.

sp." Gastropod shells were generally more numerous than those of bivalves, and the two branchiates, *Amnicola integra* and *Probythinella lacustris*, were most frequently represented. Of the pulmonates, shells of *Gyraulus parvus*, *Physa integra* and *Ferrissia rivularis* were most numerous. Terrestrial gastropods were evidenced by several unidentified species. Shells of pisidiids were more numerous than those of the unionids, which were sparse.

DISCUSSION

Living mollusks and ecologic factors.—Perhaps the primary ecologic factor influencing the occurrence of mollusks in the Forest River is high chloride content. Species decrease in number downstream apparently to zero as total chlorides increase (Table 2 and Figure 2). This relationship was also observed for the unionids in the Turtle River, the next Red River tributary to the south (Cvancara and Harrison, 1966). Only three tributaries (also the Park River, north of the Forest) in the Red River Valley of North Dakota and Minnesota appear to contain high total chlorides in their lower reaches (Cvancara, 1970, p. 84). Perhaps high turbidity in the lower reaches is another determinant factor (Figure 2); it generally increases downstream in the tributaries of the Red River in the upper Valley (Cvancara, 1970, p. 80).

Other "natural" ecologic factors may also be limiting to mollusks in the river; however, the relationships may only be revealed if each species is analyzed in detail. Man's building of dams (and, e.g., encouragement of stagnant water species) and channel dredging and straightening (destruction of habitat) has occurred largely in the upper and lower reaches of the Forest where mollusks are relatively sparse.

Comparison of fossil and present faunas.—The fossil fauna contained all but five or less of the species presently living in the Forest River; no fossil forms differed from those in the river now. Most conspicuously absent from the fossil fauna were *Strophitus rugosus* and *Lasmigona complanata*. *S. rugosus* is rare in the river today (Table 2) and was probably rare (or absent) in the past. It occurred as fossil in previous sediments of the Turtle River (Cvancara and Harrison, 1966). *L. complanata* was also absent as a fossil in the Turtle River and may be a later introduction into these two rivers, although it is now one of the four most common unionids in the Red River Valley (Cvancara, 1970, p. 76). *Pisidium casertanum* may well be present in the fossil assemblages but worn shells preclude establishing the former existence of this pisidiid in the river. *Stagnicola palustris* is generally rare in the river today and no shells of the species were found in the fossil assemblages. Similarly, *Helisoma trivolvis* is generally uncommon today and occurred only rarely as a fossil (Table 3).

The fossil assemblages represent the mollusks living in the river in the recent past but alterations have occurred. Considerable current sorting has probably biased the assemblages toward species of smaller shell size. Shells of larger species may have been commonly fragmented beyond recognition. Those of the large gastropods *Stagnicola palustris* and *Helisoma trivolvis*, however, may be absent or rare in

the assemblages because these species are characteristically stagnant water types and may have been uncommon or rare in the river earlier.

The generally close similarity of the fossil and living faunas suggests that the regimen of the Forest River has remained much the same since the recent past. Rates of sedimentation, however, may have been different.

Age of the fossil mollusks.—No definite age can be assigned presently to the fossil molluscan fauna. It has to be younger than about 9,000 years, the time when glacial Lake Agassiz was drained from the upper Valley (Elson, 1967, Table 6 and Fig. 6). Alluvial sediments along the western margin of the valley postdate Lake Agassiz and *might* be middle Holocene. The Forest River and other streams are incised into these alluvial sediments and so post-date them. Fossil mollusks in Forest River sediments may, therefore, be late Holocene and lived about 4,500 years ago or less (Dr. Lee Clayton, University of North Dakota; personal communication, February 22, 1971). At fossil site F4 an elk antler was found at the base of unit 5. The antler suggests that unit 5 was deposited during or prior to the late 1880s when the last native elk lived in North Dakota (Bailey, 1926, p. 34-35) and unit 6 may have been deposited since that time.

ACKNOWLEDGMENTS

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REFERENCES CITED

Bailey, Vernon. 1926. A biological survey of North Dakota. I. Physiography and life zones. II. The Mammals. U. S. Government Printing Office, Washington. 226 p. (U. S. Dep. Agriculture, Bur. Biol. Surv., North American fauna, No. 49).

Bluemle, J. P. 1971. Geology of Nelson and Walsh Counties. N. Dak. Geol. Survey Bull. (In preparation).

Clampitt, P. T. Comparative ecology of the snails *Physa gyrina* and *Physa integra* (Basommatophora: Physidae). Malacologia 10(1): 113-151.

Cvancara, A. M. 1970. Mussels (Unionidae) of the Red River Valley in North Dakota and Minnesota, U.S.A. Malacologia 10(1): 57-92.

————, and J. M. Erickson. 1968. Mussel distribution and ecology, Forest River, North Dakota. N. Dak. Acad. Sci., Proc. (Abstr.) 22:17.

————, and Harrison, S. S. 1966. Distribution and ecology of mussels in the Turtle River, North Dakota. N. Dak. Acad. Sci., Proc. 19: 128-146.

————. R. C. Heetderks, and F. J. Iljana. 1967. Local distribution of mussels. Turtle River, North Dakota. N. Dak. Acad. Sci., Proc. 20: 149-155.

Elson, J. A. 1967. Geology of glacial Lake Agassiz, p. 37-95. *In* W. J. Mayer-Oakes (ed.) Life, land and water. Proc. 1966 Conf. Environ. Studies Glacial Lake Agassiz Region. Univ. Manitoba Press, Winnipeg.

Hansen, D. E., and J. Kume. 1970. Geology and ground water resources of Grand Forks County. Part I. Geology. N. Dak. Geol. Survey Bull. 53. 76 p.

Harrison, S. S. 1965. Relationship of the Turtle, Forest, and Park Rivers to the history of glacial Lake Agassiz. Master's thesis. Univ. N. Dak. 50 p.

Norby, R. D. 1967. Past and present molluscan fauna, Sheyenne River, North Dakota. Senior thesis. Univ. N. Dak. 76 p.

U. S. Dep. Agriculture, Soil Conservation Service. 1967. General soil map, Walsh Co., N. Dak. USDA, Washington, D. C. (unpublished; revised March 7, 1967).

U. S. Geological Survey. 1961-1966. Surface water records of North Dakota and South Dakota. U. S. Government Printing Office, Washington, D.C. 236 p., 254 p., 253 p., 258 p., 156 p., 154 p.

————. 1964. Compilation of records of the United States, October 1950 to September 1960. Part 5. Hudson Bay and Upper Mississippi River basins. U. S. Geol. Survey Water-Supply Paper 1728. 576 p.

THE GEOGRAPHIC DISTRIBUTION OF SKUNK RABIES IN NORTH DAKOTA

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ABSTRACT

Records of the North Dakota Veterinary Diagnostic Laboratory and the State Public Health Department for 1957-1970 were reviewed to determine the reported incidence of rabies in striped skunks *(Mephitis mephitis)* in the state. Inhabitants east of the Missouri Coteau submitted significantly more striped skunks, both per unit area and per 1,000 human rural population; inhabitants from the southeastern part of the state submitted a disproportionately large number of skunks for rabies diagnosis, but there was no significant difference in rabies incidence in that area, or in any other area of the state examined. There was no statistical correlation between the number of submitted skunks and the percent diagnosed positive, but significant annual differences in rabies incidence among submitted specimens were disclosed. Factors that may influence the reported incidence of the disease are land use, human rural population density, soil type, and the proximity to specimen collection centers.

INTRODUCTION

This paper examines the geographic distribution of rabies in the

striped skunk *(Mephitis mephitis)* in North Dakota during 1957-1970 and discusses factors affecting the distribution.

The first report of rabies in the state appeared in the Fourth Annual Report of the State Public Health Laboratories (1911); this report involved rabid dogs in the Lidgerwood and Carrington areas. Based on reports submitted by the North Dakota Department of Health in Bismarck to the U. S. Center for Disease Control (CDC), Atlanta, Georgia, all but four counties in North Dakota have reported skunk rabies since 1957. Gustafson and Koons (1960) and Scholtens and Tierkel (1963) referred to a rabies epidemic in the early 1950's in North Dakota. This epidemic apparently resulted from the northward expansion of a focus of sylvatic rabies in Iowa. Schoening (1956) stated that rabies in wildlife is related to the density of the susceptible host. Parker (1961) reported a dramatic increase in skunk rabies following a decrease in pelt value in the early 1950's. He postulated that the factor responsible for the change in the rabies incidence was an increase in the skunk population.

MATERIALS AND METHODS

We utilized only the records of striped skunks submitted for rabies diagnosis to the North Dakota State Veterinary Diagnostic Laboratory (Fargo) and the Public Health Department (Grand Forks) since 1957. By limiting the number of laboratories involved, we did not materially reduce the available sample of skunks. Geographic distribution of skunk rabies and calculated incidence rates presented in this paper were totally dependent on these data. Skunks submitted for diagnosis by personnel or institutions monitoring rabies for research projects were not considered. CDC records, which included only animals diagnosed positive, served as checks on the completeness of our records.

RESULTS AND DISCUSSION

North Dakota is divided into three principal physiographic regions, the Missouri Plateau, the Drift Prairie and the Red River Valley (Omodt et al., 1968). The Missouri Coteau forms the northeast edge of the Missouri Plateau and is composed of dead-ice moraine (Clayton, 1967). These regions have characteristic soils which largely determine the natural vegetation and agricultural practices (Figure 1).

Our field observations indicate that in areas where small grains dominate, skunks are more abundant; where livestock assumes primary importance, there are fewer striped skunks. These generalizations regarding skunk densities are modified by land use practices; with mixed farming there is a more discontinuous pattern of land use. This interspersion of habitats may lead to higher skunk densities by virtue of an edge effect (Leopold, 1933). The above field observations were confirmed by reports of district field agents of the Division of Wildlife Services (Rew Hanson, personal communication).

When tested by Chi square, significantly more skunks per square mile were submitted by the human rural population from the region east of the Coteau than from the west ($p<0.05$). This may be due to higher skunk densities east of the Coteau and/or greater human population density in the region east of the Coteau increasing skunk-human interaction.

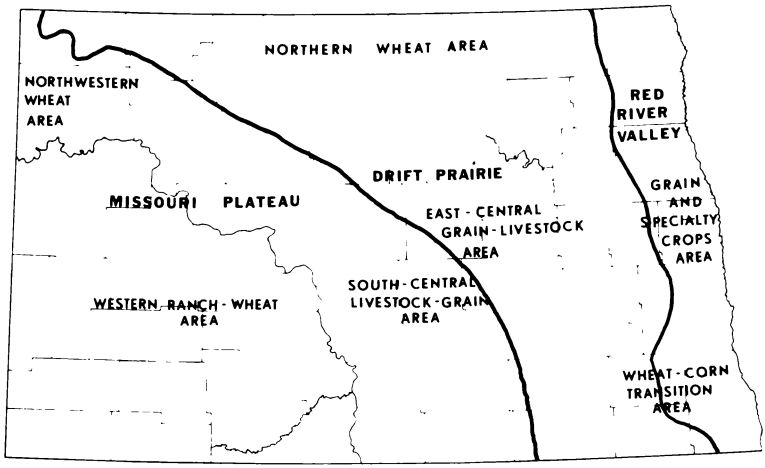

Figure 1. Physiographic and agricultural regions of North Dakota.

Skunk submissions were tested on a per capita of the rural population. The rural population was considered to include all persons living in communities with less than 1,000 inhabitants because we felt that people of larger centers were not as likely to be exposed to skunks. Human rural population density was greater in more intensively cultivated areas east of the Coteau than in grazing areas to the west. A significant difference was found when tested by Chi square ($p<0.05$) in the numbers of skunks submitted for rabies diagnosis from the region east of the Missouri Coteau as compared with those in the western areas (Figure 2). Significantly more skunks per unit rural population were submitted from the eastern area (3.29 per thousand rural population). West of the Coteau only 1.87 skunks per thousand rural population were submitted.

A disproportionate number of striped skunks were submitted for rabies diagnosis from the southeastern counties of Barnes, Cass, Richland, Ransom, Lamoure, Sargent and Dickey. Thirty-two percent of the North Dakota rural population exists east of the Coteau in these counties, and these people were responsible for 52% of the skunks submitted for rabies diagnosis during the 1957-1970 period. A Chi square test indicated that this difference was significant ($p<0.05$). We felt that this could be the result of the proximity of the North Dakota Veterinary Diagnostic Laboratory and/or the people of the region being particularly aware of rabies. Arthur Gustafson (Assistant Director, Division of Laboratories, North Dakota Department of Health, personal communication), believes that the people in the southeastern area are particularly alert to the problem because North Dakota's first rabid skunk came from Lisbon, Ransom County, in 1951, and the public health officers probably brought this to the attention of the inhabitants of this area.

Because of the disproportionate number of skunks submitted from

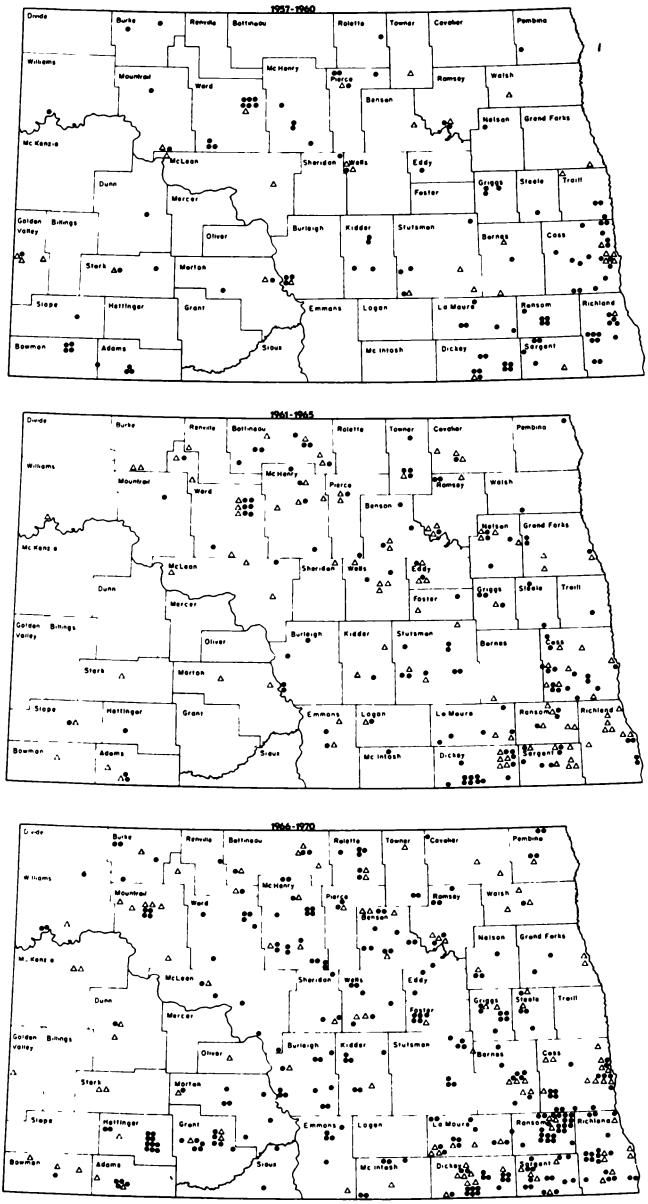

Figure 2. Geographic distribution of striped skunks submitted for rabies diagnosis, 1957-1970.

the southeast, the differences were tested in rabies incidence among the submitted sample. There was no significant difference, when tested by Chi square, in the rabies incidence rate among striped skunks submitted from the southeastern area versus the rest of the state, nor between the southeastern area and the remainder of the area east of the Coteau.

Over the 14-year period (1957-1970) the annual rabies incidence rate among skunks submitted for diagnosis varied between 88% (1957) and 38.33% (1963). The mean percent positive was 67.4% (Table 1). This indicated effective selection for rabid animals by persons submitting skunks for diagnosis. If these people were selecting rabid animals, as our data imply, then one might expect a higher percentage of rabid diagnoses in those years with increased submission rates; however, over the 14-year period there was no significant statistical correlation between the number of submitted skunks and the percent diagnosed positive statewide, either from the southeastern area ($r = -0.26$) or from the area east of the Coteau ($r = -0.06$).

The statewide skunk rabies incidence rates were tested for significance of annual differences using a G-test (Sokol and Rohlf, 1969) (Table 2). Such differences are highly dependent on the size of the sample submitted for diagnosis. We can document the fact that the measured rabies incidence rate varies between years.

Table 1. Numbers and rabies incidence in North Dakota striped skunks submitted for rabies diagnosis to the Grand Forks Public Health Laboratory and North Dakota State Veterinary Diagnostic Laboratory during 1957-1970

YEAR	TOTAL	POSITIVE	% POSITIVE
1957	25	22	88.0
1958	59	46	78.0
1959	49	36	73.5
1960	16	13	81.2
1961	22	16	72.7
1962	49	32	65.3
1963	60	23	38.3
1964	52	29	56.9
1965	49	24	49.0
1966	81	50	61.7
1967	110	81	73.6
1968	96	78	81.2
1969	54	37	68.5
1970	74	41	55.4
TOTAL	796.0	528.0	
MEAN	56.9	37.7	67.4

Table 2. Vertical lines link yearly incidence rates not significantly different ($p<0.05$) when tested by the G-test (Sokol and Rohlf, 1969)

1957	0.880
1960	0.812
1968	0.812
1958	0.780
1967	0.736
1959	0.735
1961	0.727
1969	0.685
1962	0.653
1966	0.617
1964	0.569
1970	0.554
1965	0.490
1963	0.363

A major question we must explore is what relationship the data available for establishing rabies incidence rates have to actual rabies incidence in the population. A number of biases are inherent; for example, sample size was not constant, varying between 16-110 during 1957-1970. Perhaps more important, the data were heavily weighted to animals that acted strangely in the vicinity of human habitations and livestock. Bias was also perhaps introduced by the local history of rabies. For example, if there was a recent human exposure in an area, more animals were submitted for diagnosis.

If foci of rabies incidence exist in the state, the current method of data collection is inadequate to detect these foci, and it offers no definite proof of the existence of such centers. To measure the true rabies incidence rate we must have unbiased samples of skunks for rabies diagnosis and estimates of skunk population densities. Regional field workers (conservation officers, USDA biologists) could be required to collect random samples of skunks within the state. Techniques have been developed at North Dakota State University for working with live skunks and could be put to good use for monitoring rabies. Jacobson (1969) developed a night shining technique to census and sample skunk populations. Burkel, Andrews and Meslow (1970) tested the feasibility of using road-killed skunks for

monitoring rabies. They found that rabies diagnosis was sometimes difficult, and there was reason to suspect that the sample did not represent the true incidence in the population.

Since skunk rabies is becoming an imminent problem in North Dakota, and because there are better means available for sampling skunks than voluntary contributions by the general public, a program should be instituted to effectively determine the actual rabies incidence in the state.

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REFERENCES

Burkel, M. D., M. F. Andrews, and E. C. Meslow. 1970. Rabies detection in road-killed skunks. J. Wildl. Dis. 6(4):496-499.

Clayton, L. 1967. Stagnant-glacier features of the Missouri Coteau in North Dakota, p. 35-46. *In* L. Clayto,n and T. F. Freers [eds.] Glacial geology of the Missouri Coteau and adjacent areas. N. Dak. Geol. Surv. Misc. Pub. 30. 170 p.

Fourth Annual Report of the State Public Health Laboratories. 1911. Univ. of North Dakota, Grand Forks.

Jacobson, J. O. 1969. Application of a nighttime roadside census to striped skunk population studies. M.S. Thesis. N. Dak. State Univ. 83 p.

Gustafson, A. A., and M. E. Koons. 1960. Rabies in North Dakota. Lancet 80 (7):320-323.

Leopold, A. 1933. Game management. Charles Scriber's Sons, New York. 481 p.

Omodt, H. W., G. A. Johnsgard, D. D. Patterson, and O. P. Olson. 1968. The major soils of North Dakota. N. Dak. Exp. Sta. Bull. 472. 60 p.

Parker, R. L. 1961. Rabies in skunks in the north-central states. Proc. U.S. Livestock Sanit. Assoc. 65:273-280.

Schoening, H. W. 1956. Rabies, p. 195-207. *In* Yearbook of agriculture, 1956, animal diseases. U. S. Government Printing Office, Washington, D.C. 591 p.

Scholtens, R. G., and E. S. Tierkel. 1963. Incidence of animal rabies in the United States. J. Amer. Vet. Med. Assoc. 143(1):52-56.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman Co., San Francisco. 776 p.

THE AGE AND RATE OF GROWTH OF WALLEYE *(STIZOSTEDION VITREUM)* AND SAUGER *(STIZOSTEDION CANADENSE)* IN LAKE SAKAKAWEA, NORTH DAKOTA, 1968-1969

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ABSTRACT

The age and growth rate of walleye, *Stizostedion vitreum* (Mitchill), and sauger, *S. canadense* (Smith), in Lake Sakakawea, North Dakota, were investigated during the summers of 1968 and 1969. A total of 236 walleye and 447 sauger were captured during the study. Scales were taken, and annual growth was back calculated. The maximum age of walleye was 10 years; that of saugers was 7 years. The overall sex ratio for walleye was 1.54 females per male whereas sauger had a ratio of 0.87 females per male. The overall coefficient of condition was 0.86 for walleye, and 0.73 for sauger.

Walleye from Lake Sakakawea have good linear growth when compared with walleye from waters in the north-central United States but not as good as walleye from Norris Reservoir, Tennessee. Sauger from Lake Sakakawea had somewhat slower growth than those from Lake of the Woods, Lake Winnipeg and Lake Erie. Good growth rates were attributed to an abundance of small goldeye, *Hiodon alosoides* (Rafinesque), and yellow perch, *Perca flavescens* (Mitchill).

INTRODUCTION

Walleye, *Stizostedion vitreum* (Mitchill), and sauger, *S. canadense* (Smith), are important game fishes of lakes and reservoirs of the northern United States and central Canada. The walleye population in Lake Sakakawea had not been studied prior to our research, whereas sauger had been studied by Carufel (1963). Annual test netting records of the North Dakota Game and Fish Department reveal that the numbers of walleye and sauger taken from the reservoir have steadily increased since the dam was closed in 1953. This increase has been attributed to formation of new spawning areas. The purpose of this study is to present the age, growth and condition factors of walleye and sauger found in Lake Sakakawea.

DESCRIPTION OF THE AREA

Lake Sakakawea, the largest of the main stem Missouri River reservoirs, encompasses 326,000 acres when full and has a maximum storage capacity of 24,500,000 acre-feet (Neel, 1963). This reservoir is 200 miles long, has 1600 miles of shoreline, an average width of 3 miles and a maximum depth of 180 feet (Hill, 1967). The principal source is the Missouri River, although five tributaries, the Little Missouri River, Shell Creek, White Earth River, Tobacco Garden Creek, and the Little Muddy River flow into the impoundment

(Figure 1). The Little Missouri River, the largest tributary, has a length of 1,506 miles and 9,500 square miles of drainage.

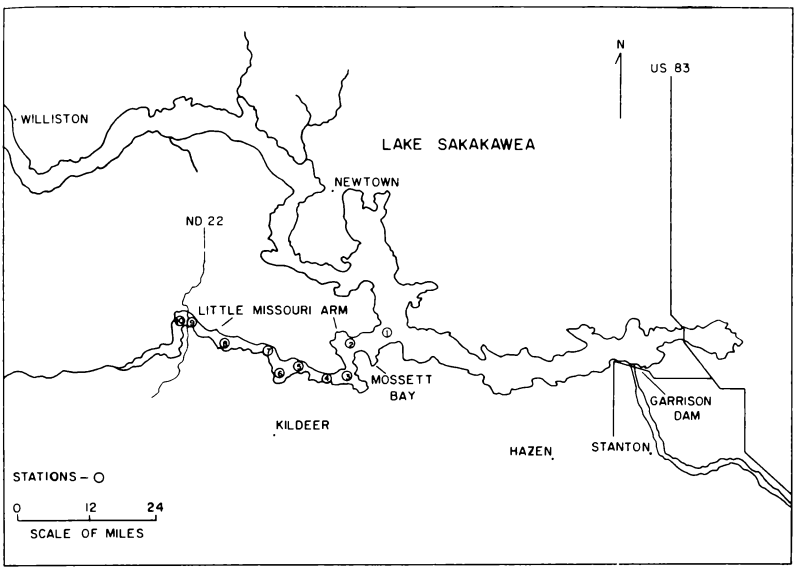

Figure 1. Lake Sakakawea showing collecting stations.

The Little Missouri arm of the reservoir is located in a glaciated plateau characterized by rough, local badlands. The area is semiarid, with an average annual precipitation of 16 inches which includes approximately 30 inches of snow. The majority of the rainfall occurs between April and October. The summer temperature may reach a high of 110 F and low winter temperatures may range from -20 to -60 F (Neel, Nicholson, and Hirsch, 1963). The ice usually breaks up by 15 April on the main reservoir (Hieb, 1968).

The reservoir elevations usually fluctuate from 10-16 feet annually with rises in water level occurring each year in May and June. During the winter, the reservoir is drawn down to accommodate the spring flood waters (Carufel, 1963).

MATERIALS AND METHODS

Ten stations were established on the reservoir and sampled at weekly or biweekly intervals (Figure 1). These were sampled between 25 April and 1 September 1968 and between 15 April and 28 September 1969.

Three types of experimental gill nets were used: 1) a 36-ft wide, 50-ft deep, vertical gill net with 6 panels, each 6 ft wide and 50 ft deep of ½, 1, 1¼, 1¾ and 2-inch bar mesh, respectively; 2) a 36-ft

wide, 25-ft deep, vertical gill net with 6 panels each 6 ft wide and 25 ft deep of ¾, 1, 1¼, 1½, 2 and 2½ bar mesh, respectively, and 3) a 125-ft × 6-ft experimental gill net with five, 25-ft × 6-ft panels of ¾, 1, 1¼, 1½ and 1¾ inch mesh, respectively.

The nets were fished singly or in combination, one above the other, according to the depth of water so that the nets extended from the surface to the bottom at each location. Nets were set for 24-hour periods in order to sample all major activity cycles of the fish as recommended by Carlander (1953).

Two hundred thirty six walleye and 447 sauger were captured during the study. Walleye and sauger were sexed by inspection of gonads, measured to the nearest millimeter (total length) and weighed to the nearest gram. Essentially all fish were sexed in 1968; however, in 1969, time did not permit examination of all the fish and unsexed fish were recorded as undetermined. Scales were taken from the left side beneath the lateral line directly below the first dorsal fin (Lagler, 1952). Scale impressions were made on cellulose acetate slides with a roller press following the technique described by Smtih (1954). Scale impressions were projected and magnified at 30× by means of a Bausch and Lomb microprojector. Paper strips were superimposed on these images, and the anterior scale length and point of interception of the annuli were marked.

A straight line relationship between body length and scale length for each species was calculated by the method of least squares. Body-scale, and age and growth computations were made on all fish but were not calculated for each sex. Since equations for the body-scale relationship were $Y = 20.33 + 0.37$ and $Y = 0.73 + 0.35$ for walleye and sauger, respectively, a correction factor of 20 mm was used in computation of lengths from walleye scale data; a correction factor was not considered necessary for sauger.

The average annual growth increments were calculated by the use of a direct proportion nomograph (Carlander and Smith, 1944).

The coefficient of condition was calculated for all walleye and sauger which were aged by the formula $K = 100{,}000\ W/L^3$ (Bennett, 1962).

The equation for the length-weight regression, expressed in logarithmic form, was calculated by the method of least squares. Regressions were computed for both species for each year; sexes were not separated.

RESULTS

Average total lengths at each annulus were computed for the 236 walleye and 447 sauger captured in 1968 and 1969. Most of the walleye captured were in age classes II-VI (Tables 1 and 2). Only 16 walleye of age class VII or older and six walleye of age class I were captured out of the 236 walleye taken. Seven age groups were established for sauger collected in 1968 and 1969. Age group II was most numerous in both years and the frequency of occurrence of the older age groups diminished as they grew older (Tables 3 and 4). The most rapid growth was obtained in both walleye and sauger during

their first year of life. Growth rates were reduced during the second year and continued to diminish in subsequent years.

The average coefficient of condition for all walleye collected during 1968 and 1969 was 0.86. The 1968 average was 0.88; the 1969 average was slightly less, 0.85. The average coefficient of condition for all sauger taken during 1968 and 1969 was 0.73. The 1968 average was 0.74; the 1969 average was 0.72.

Table 1. Average total length (mm) at each annulus calculated for 84 walleye, Lake Sakakawea; 1968

Year class	No. fish	Length at capture	Annulus I	II	III	IV	V	VI	VII	VIII	IX	X
1967	1	229	170									
1966	13	313	154	246								
1965	12	362	133	239	309							
1964	16	436	148	241	314	378						
1963	22	498	152	246	323	390	454					
1962	13	578	148	245	327	399	471	535				
1961	4	541	159	251	328	375	448	503	540			
1960	2	582	131	203	284	352	401	468	533	573		
1958	1	745	160	256	320	400	456	528	484	656	696	736
Average calculated length			149	243	318	386	456	521	544	601	696	736
Average annual increment			149	94	75	68	70	65	23	57	95	40

Table 2. Average total length (mm) at each annulus calculated for 152 walleye, Lake Sakakawea; 1969

Year class	No. fish	Length at capture	Annulus I	II	III	IV	V	VI	VII	VIII	IX
1969	5	234	163								
1967	34	300	151	243							
1966	47	347	148	241	311						
1965	26	427	148	249	323	381					
1964	21	499	159	249	327	396	452				
1963	10	529	147	238	320	386	443	494			
1962	6	652	144	228	308	396	469	514	571		
1961	2	670	138	210	285	365	413	468	535	600	
1960	1	580	135	205	290	355	390	435	490	515	550
Average calculated length			147	240	314	384	440	497	554	572	550
Average annual increment			147	93	74	70	56	57	57	18	22

Table 3. Average total length (mm) at each annulus calculated for 264 sauger, Lake Sakakawea; 1968

Year class	No. fish	Length at capture	Annulus I	II	III	IV	V	VI	VII
1967	15	221	159						
1966	82	292	121	228					
1965	75	334	119	220	297				
1964	47	420	135	227	308	378			
1963	31	472	132	227	303	373	433		
1962	13	487	140	200	274	336	391	441	
1961	1	511	120	175	265	330	405	465	490
Average calculated length			128	224	300	372	428	460	490
Average annual increment			128	96	76	72	56	32	30

Table 4. Average total length (mm) at each annulus calculated for 183 sauger, Lake Sakakawea; 1969

Year class	No. fish	Length at capture	Annulus I	II	III	IV	V	VI	VII
1968	3	262	168						
1967	58	282	132	231					
1966	57	338	126	224	296				
1965	34	387	127	213	292	347			
1964	12	457	126	220	293	363	418		
1963	16	516	148	237	313	379	431	480	
1962	3	530	138	217	268	342	403	475	487
Average calculated length			131	225	296	357	423	475	487
Average annual increment			131	94	71	61	66	52	12

Table 5. Average length and sex ratios of walleye and sauger, Lake Sakakawea; 1968-69

	No. males	Males (mm)	No. females	Females (mm)	undetermined	Ratio of males to females
Walleye						
1968	32	385	50	509	2	1.00 : 1.56
1969	37	358	56	433	59	1.00 : 1.51
Total	69		106		61	1.00 : 1.54
Sauger						
1968	131	329	116	395	17	1.00 : 0.88
1969	25	333	21	388	137	1:00 : 0.84
Total	156		137		154	1.00 : 0.87

The overall sex ratio of walleye during 1968-1969 was 1.54 females per male. The yearly sex ratios were 1.56 and 1.51 females per male for 1968 and 1969 catches, respectively (Table 5). The sex ratio of sauger during 1968-1969 was 0.87 females per male. The ratio was 0.88 females per male during 1968, and 0.84 females per male during 1969 (Table 5).

The equation for the line of best fit for the length-weight regression of walleye in 1968 was found to be Log W = -5.60896 + 3.20910 Log L; in 1969 it was described by the equation Log W = -5.80964 + 3.20447 Log L (Figure 2). The equation for the length-weight relationship for sauger in 1968 was Log W = -5.41004 + 3.10705 Log L; the line of best fit for the 1969 data was Log W = -4.80952 + 3.00156 Log L (Figure 3). Walleye males and females captured in 1968 were, on the average, 27 mm and 76 mm longer, respectively, than those captured in 1969 (Table 5). Females averaged 124 mm and 75 mm longer than males in 1968 and 1969, respectively. Average length of sauger varied little from 1968 to 1969 (Table 5). However, males were smaller than the females in both years.

DISCUSSION

Ten age groups were found in the 236 walleye sampled; however, only four were in age groups VIII, one in age group IX and one in age group X (Tables 1, 2). Hill (1967) reported only eight age groups in the 76 walleye taken in test netting; however, the sample may have been too small to have included all age groups present. Walleye may live as long as 18 years (Carlander, 1950a) but fish older than eight years are relatively rare and are therefore difficult to sample adequately for comparative purposes. For example, Carlander (1945), in extensive sampling in Lake of the Woods, Minnesota, found that only 23 (0.8%) of 2,898 walleye were older than age class VIII. Walleye from Lake Sakakawea apparently have good linear growth rates when compared with walleye from representative lakes and reservoirs in the north-central United States (Table 6). However, suitable southern waters such as Norris Reservoir, Tennessee apparently support greater growth rates than those found farther north.

Seven age groups were identified in the 447 sauger collected in this study. Carufel (1963) found six age groups in the 318 sauger and Hill (1967) found only five age groups in the 83 sauger he sampled from the Lake Sakakawea reservoir. The latter sample may have been too small to adequately represent older age groups. Carlander (1950b) reported group XIII, but sauger older than age group V would be rarely included in a small sample. Although statistical tests were not made, it is obvious that the estimates of annual growth increments presented for sauger should be more accurate for age groups I-V than for the older groups. The average lengths of the first five age groups compared favorably with those of Carufel (1963); however, Hill (1967) reported greater lengths for each group (Table 7). Sauger from Lake Sakakawea apparently have somewhat slower growth than those from Oahe, Fort Randall and Lewis and Clark Reservoirs on the Missouri River and Norris Reservoir in Tennessee, but more rapid growth than those from Lake of the Woods, Lake Winnipeg and Lake Erie (Table 7).

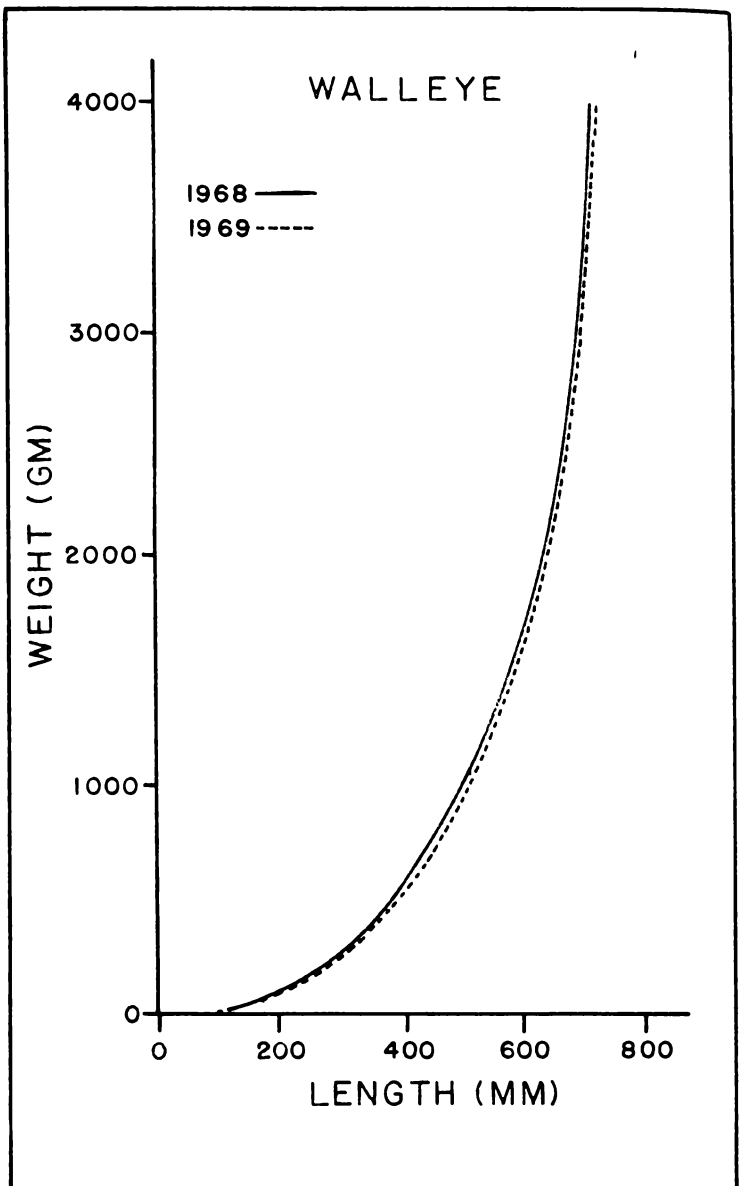

Figure 2. The length/weight regressions for walleye during 1968 and 1969, respectively, are Log W = -5.60896 + 3.20910 Log L and Log W = -5.80964 + 3.20447 Log L.

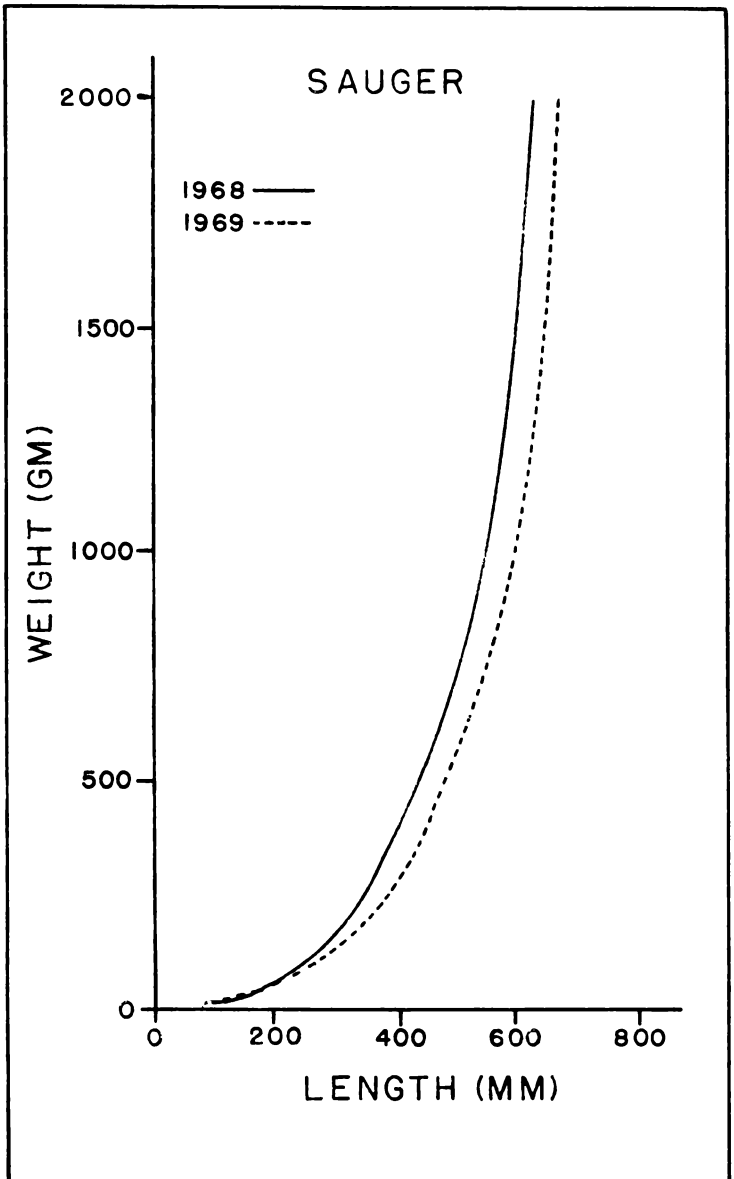

Figure 3. The length/weight regressions for sauger during 1968 and 1969, respectively, are Log W + -5.41004 + 3.10705 Log L and Log W = 4.80952 + 3.00156 Log L.

Table 6. Comparison of average calculated total lengths (inches) of Lake Sakakawea walleye with those from other areas

Area	No. of fish	Annulus I	II	III	IV	V	VI	VII	VIII	IX	X
Lake Sakakawea											
Present study[a]											
1968	84	5-9	9.6	12.5	15.2	18.0	20.5	21.4	23.7	27.4	29.0
1969	152	5.9	9.6	12.5	15.2	17.6	19.6	22.1	22.5	21.6	——
Hill[a]											
1967	76	5.5	10.2	14.3	17.9	20.5	23.0	25.5	27.5	——	——
Lake of the Woods[b]	2898	6.4	9.3	11.5	13.5	14.9	16.7	18.3	19.9	21.6	——
Minnesota Lakes[b]	6599	4.6	8.6	12.0	15.0	18.1	20.5	22.9	25.2	26.7	——
Trout Lake, Wisconsin[b]	427	5.3	9.7	13.7	16.6	19.0	20.7	21.7	22.3	23.1	——
Iowa lakes[b]	216	5.0	9.2	12.4	15.0	17.1	18.6	19.9	21.5	23.2	——
Norris Reservoir,[b] Tennessee	96	8.3	16.0	20.5	——	——	——	——	——	——	——
Clear Lake, Iowa[b]	319	5.9	10.9	14.5	17.2	19.3	21.4	23.6	27.0	27.0	——

[a]Lengths at each annulus converted to inches for comparison purposes.
[b]Compiled by Cleary (1949).

Table 7. Comparison of average calculated total lengths (inches) of Lake Sakakawea sauger with those from other areas

Area	No. of fish	Annulus I	II	III	IV	V	VI	VII	VIII	IX	X
Lake Sakakawea											
Present study[a]											
1968	264	5.0	8.8	11.8	14.6	16.8	18.1	19.3	—	—	—
1969	184	5.1	8.8	11.6	14.0	16.7	18.7	19.2	—	—	—
Hill[a]											
1967	83	5.7	9.8	13.2	16.3	18.5	—	—	—	—	—
Carufel[a]											
1963	318	4.9	8.7	12.2	15.2	18.2	23.1	—	—	—	—
Lewis and Clark Lake[b] 1962	479	6.3	12.3	16.3	19.0	20.5	21.2	—	—	—	—
Fort Randall Reservoir[b]											
1955, 1956, 1957											
1961	488	5.6	12.4	16.4	19.1	21.0	22.7	—	—	—	—
Oahe Reservoir[b]											
1961	357	6.2	11.2	14.7	16.9	18.7	19.1	—	—	—	—
Norris Reservoir[b]											
1957	3393	8.4	13.3	15.6	17.2	18.6	19.6	20.3	—	—	—
Lake of the Woods[b]	883	5.0	7.3	9.3	10.9	12.3	13.3	14.3	14.1	15.2	15.1
Lake Erie[b]	905	3.9	7.0	10.4	12.2	13.6	15.8	—	—	—	—
Lake Winnipeg[b]	1039	—	—	10.0	12.1	13.3	14.3	15.0	15.8	16.5	18.0

[a]Lengths at each annulus converted to inches for comparison purposes.
[b]Compiled by Vanicek (1964).

It is difficult to compare the sex ratio of 1.54 females per male for Lake Sakakawea walleye with sex ratios for this species found from other bodies of water. These fish were sampled after the spawning season and throughout the summer. Mraz (1968) found 0.37 females per male in samples taken during the spawning run and that males usually mature at least one year before the females. Therefore, males were found on the spawning grounds in numbers greater than the true sex ratio of the population.

A sex ratio of 0.88 females per male for sauger in Lake Sakakawea is in close agreement with the ratio of 0.89 females per male in Lake Winnebago, Wisconsin (Priegel, 1969). However, sex ratios reported for sauger from other waters are highly variable. Ratios of 1.23 and 1.96 females per male have been found in Norris Reservoir, Tennessee (Hassler, 1957) and Lake of the Woods, Minnesota (Carlander, 1950b).

Generally it is recognized that the n value or the slope of the length-weight regression for an average population of fish should be approximately 3.0. Deviations above or below this value reflect changes in relative plumpness in relation to length. The n value for both walleye and sauger in Lake Sakakawea is above 3.0, suggesting that these fish become relatively more plump as they increase in length.

The good growth and condition of walleye and sauger, especially of the larger sizes, can be attributed to an abundance of food. Yellow perch, *Perca flavescens* (Mitchill) and goldeye, *Hiodon alosoides* (Rafinesque) are abundant in the reservoir; both species are stunted (Hieb, 1968; Wahtola et al. 1970). The yellow perch are particularly small and therefore may be vulnerable to predation by larger walleye and sauger over their entire size range.

The increasing number of walleye and sauger taken in annual test netting catches of the North Dakota Game and Fish Department can probably be attributed to improved spawning habitat for these species. As the reservoir matures, yearly fluctuations in water level with resultant beach erosion continually produce new areas of sandy substrate that are excellent spawning grounds.

ACKNOWLEDGMENTS

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REFERENCES CITED

Bennett, G. W. 1962. Management of artificial lakes and ponds. Reinhold Publishing Corp., New York.

Carlander, K. D. 1945. Age, growth, sexual maturity, and population fluctuations of the yellow pikeperch, *Stizostedion vitreum vitreum* (Mitchill), with reference to the commercial fisheries, Lake of the Woods, Minnesota. Trans. Amer. Fish. Soc., 73:90-109.

Carlander, K. D. 1950a. Handbook of freshwater fishery biology. Wm. C. Brown Co., Dubuque, Iowa.

———. 1950b. Growth rate studies of sauger, *Stizostedion canadense canadense* (Smith) and yellow perch, *Perca flavescens* (Mitchill), from Lake of the Woods, Minnesota. Trans. Amer. Fish. Soc. 79:30-42.

———. 1953. Use of gill nets in studying fish populations, Clear Lake, Iowa. Iowa Acad. Sci. Proc., 60:621-625.

———, and L. L. Smith. 1944. Some uses of nomographs in fish growth studies. Copeia, 1944:157-162.

Carufel, L. H. 1963. Life history of saugers in Garrison Reservoir. J. Wildl. Mgmt. 27:450-456.

Cleary, R. E. 1949. Life history and management of the yellow pike-perch, *Stizostedion v. vitreum* (Mitchill), of Clear Lake, Iowa. Iowa State College J. Sci. 23:195-208.

Hassler, W. W. 1957. Age and growth of the sauger, *Stizostedion canadense canadense* (Smith), in Norris Reservoir, Tennessee. J. Tennessee Acad. Sci. 32(1):55-76.

Hieb, R. N. 1968. Observations of the life history of the goldeye *Hiodon alosoides* (Rafinesque) in Moccasin Bay on the Little Missouri arm of Garrison Reservoir, North Dakota. Thesis, Univ. of North Dakota 45 p.

Hill, W. J. 1967. Test netting survey of Garrison Reservoir. N. Dak. State Game and Fish Dep., Dingell-Johnson Project F-2-R-14 (Mimeographed).

Lagler, K. F. 1952. Freshwater Fishery Biology. Wm. C. Brown Co., Dubuque, Iowa.

Mraz, D. 1968. Recruitment, growth, exploitation and management of walleyes in a southeastern Wisconsin lake. Tech. Bull. No. 40, Dep. of Natur. Resources, Madison, Wisconsin.

Neel, J. K. 1963. Impact of reservoirs, p. 575-593. *In* D. G. Frey [ed.] Limnology in North America. Univ. Wisconsin Press, Madison.

———, H. P. Nicholson and A. Hirsch. 1963. Main stem reservoir effects on water quality in the central Missouri River. U. S. Dep. Health Educ. and Welfare, Pub. Health Service, Region VI Water Supply and Pollution Control. Kansas City, Missouri.

Priegel, G. R. 1969. The Lake Winnebago sauger age, growth, reproduction, food habits and early life history. Tech. Bull. No. 43, Dep. of Natur. Resources, Madison, Wisconsin.

Smith, S. 1954. Method of producing plastic impressions of fish scales without using heat. Progr. Fish-Cult. 16:75-78.

Vanicek, D. C. 1964. Age and growth of sauger, *Stizostedion canadense* (Smith), in Lewis and Clark Lake. Iowa State College, J. Sci. 38:481-502.

Wahtola, C. H., Jr., B. L. Evenhuis, and J. B. Owen. 1970. The age and growth of the yellow perch *Perca flavescens* (Mitchill) in the Little Missouri Arm of Lake Sakakawea, North Dakota, 1968. N. Dak. Acad. Sci. Proc., 24: 39-44.

MERCURY LEVELS IN TISSUES OF DUCKS COLLECTED IN SOUTH-CENTRAL NORTH DAKOTA

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ABSTRACT

Neutron activation analysis was used to determine total mercury content in liver, kidney and breast muscle tissues of pintails *(Anas acuta)* that fed on uplands as well as in wetlands in south-central North Dakota and shovelers *(Spatula clypeata)* that fed exclusively in wetlands. Tissues were obtained in 1969 and 1970 from adult birds during the spring and summer months and from juveniles collected during August of 1970 and others reared under penned conditions. A sample of food fed to the pen-reared birds was also examined for mercury.

Adult birds of both species contained individuals with concentrations of over 500 ppb mercury in their tissues. Individual adult pintails contained higher maximum levels of mercury in liver tissues than did shovelers; however, a greater percentage (83%) of adult shoveler liver tissues exceeded 500 ppb than did those of pintails (32%). Mercury levels in wild juveniles were lower than in adult birds but higher at the 0.05 probability level than in pen-reared birds of similar age. Levels in the liver and kidney tissues were of a similar magnitude, but breast muscle tissues were generally lower by a factor of 2 to 4 times.

INTRODUCTION

Relatively high levels of mercury have been reported in tissues of fish and birds examined in Japan (Takeuchi, 1971), northern Europe (Johnels and Westermark, 1969, Borg et al., 1969), and North America (Fimreite et al., 1970 and Wobeser et al., 1970). Maxima have often been reported in organisms at or near the top of the food chain, or in seed-eating species having direct access to treated grains.

Investigations of the ecology of shovelers and pintails have demonstrated different feeding niches for the breeding birds of each species. Shovelers feed exclusively in wetlands where they consume primarily aquatic invertebrates, whereas pintails feed in both upland and wetland habitats, and as a result, are exposed to two potential sources of mercury. Our information on the feeding ecology of the two species and the current concern over mercury concentrations in game birds led us to investigate mercury levels in the tissues of these two species.

MATERIALS AND METHODS

Tissues of 56 adult and juvenile pintails and shovelers collected during the spring and summer of 1969 and 1970 in Barnes and Stutsman Counties in North Dakota, and 6 pen-reared juveniles (3 of each of these species) were analyzed for total mercury. Liver and breast muscle tissues were analyzed for 8 adult pintails collected during the spring and early summer of 1969 and 1970 and 8 adult

shovelers collected during the spring of 1970. Liver tissues alone were also analyzed for an additional 22 adult pintail hens collected during the breeding season in 1969 and 1970. Liver and breast muscle tissues were also analyzed for four adults and eight juveniles (five wild and three pen-reared) of each species collected during August 1970. In addition, kidney tissues were included in the analysis for the first time. The pen-reared pintails were hatched from eggs produced by a pintail flock at the Northern Prairie Wildlife Research Center. The pen-reared shovelers were hatched from eggs collected in the wild. A sample of a prepared diet fed to the pen-reared birds was also analyzed for mercury.

Birds collected in the field were cooled immediately and brought into the lab where they were placed in polyethylene bags and frozen. The tissue samples were placed in chemically clean vials, freeze-dried in a VirTis freeze dryer, sealed in 1.5-ml vials and shipped to Gulf General Atomic Incorporated, San Diego, California, where total mercury was determined by the neutron activation technique as described in their report:

> "Weighed portions of each sample were sealed in quartz vials and irradiated for three days at a flux of 10^{12} thermal neutrons per cm^2 per sec together with a mercury comparator standard. The irradiated samples were digested, in the presence of mercury carrier, in a mixture of HNO_3 and H_2SO_4 under reflux conditions. Addition of $HClO_4$ and glycine (NH_2CH_2COOH) permitted distillation of $HgCl_2$. Mercury was electroplated from the distillate onto a gold foil cathode, and multichannel gamma ray spectrometry was used to identify and quantitate mercury."

The dry weight values were converted to wet weight values. Mercury concentrations presented in this paper are expressed as wet weight values unless otherwise stated in the text. The mercury levels presented must be considered to be minimal values because some mercury is lost during the freeze drying process. A reliable correction factor for this loss is not as yet available.

The methods and techniques used in gathering the information on foods consumed by adult and juvenile birds are similar to those described by Swanson and Bartonek (1970). Breeding and immature birds were collected while actively feeding, and the esophageal contents were removed immediately and preserved in 80% ethanol.

RESULTS AND DISCUSSION

Mercury levels in the three groups of birds (adult, wild juveniles and hand-reared juveniles) varied considerably (Tables 1 and 2). Certain adult birds of both species had mercury concentrations in their tissues that exceeded 500 ppb. The mercury levels in liver and kidney tissues of individual adult birds were of a similar magnitude; however, breast muscle tissues were generally 2 to 4 times lower. Results of a similar nature were reported by Wobeser et al. (1970) and Hannerz (1968) for fish, and Westoo (1969) and Borg et al. (1969) for birds.

Table 1. Total mercury content (ppb wet weight) of liver, kidney and breast muscle tissues from shovelers collected in south-central North Dakota during 1970

Collection period	Age group	Number	Liver[1]	Kidney[1]	Breast Muscle[1]
		Wild Birds			
April 29-May 6	Adult	8	1,012 (164-2,316)	.....[2]	250 (50-670)
August 7-August 22	Adult	4	1,038 (429-1,739)	717 (453-1,178)	389 (140-602)
August 6-August 11	Juvenile	5	382 (145-1,016)	267 (180- 456)[3]	153 (70-225)
		Pen-reared Birds			
August 18	Juvenile (42 days)	3	21 (18- 25)	24 (23- 26)	22 (20- 26)

[1]Mean and range expressed for each tissue
[2]Tissue not examined
[3]Data derived from four samples

Table 2. Total mercury content (ppb wet weight) of liver, kidney and breast muscle tissues from pintails collected in south-central North Dakota during 1969-1970

Collection period	Age group	Number	Liver[1]	Kidney[1]	Breast Muscle[1]
		Wild Birds			
April 18-July 8 (1969-70)[2]	Adult	8	299 (67- 889)	.....[3]	89 (11- 267)
July 23-August 11 (1970)	Adult	4	3,230 (140-9,512)	2,660 (144-9,146)	676 (29-2,141)
July 23-August 11 (1970)	Juvenile	5	123 (44- 190)	123 (49- 223)[4]	91 (26- 169)
		Pen-reared Birds			
August 19 (1970)	Juvenile (68 days)	3	32 (27- 36)	25 (23- 26)	18 (15- 21)

[1]Mean and range expressed for each tissue
[2]Four birds from each year
[3]Tissue not examined
[4]Data derived from four samples

Mercury concentrations in pintails were consistently low in April and early May, but varied widely later in the season. Mercury levels in shovelers fluctuated more than in pintails in April and early May, but less than in pintails in August; however, fewer shovelers were collected (Figure 1). Highest levels of mercury were found in liver tissues of adult pintails, but a greater percentage of liver and breast muscle tissues from adult shovelers (83 and 25%, respectively) exceeded 500 ppb than in adult pintails (32 and 8%, respectively). The high mean level of mercury found in adult pintails during the period

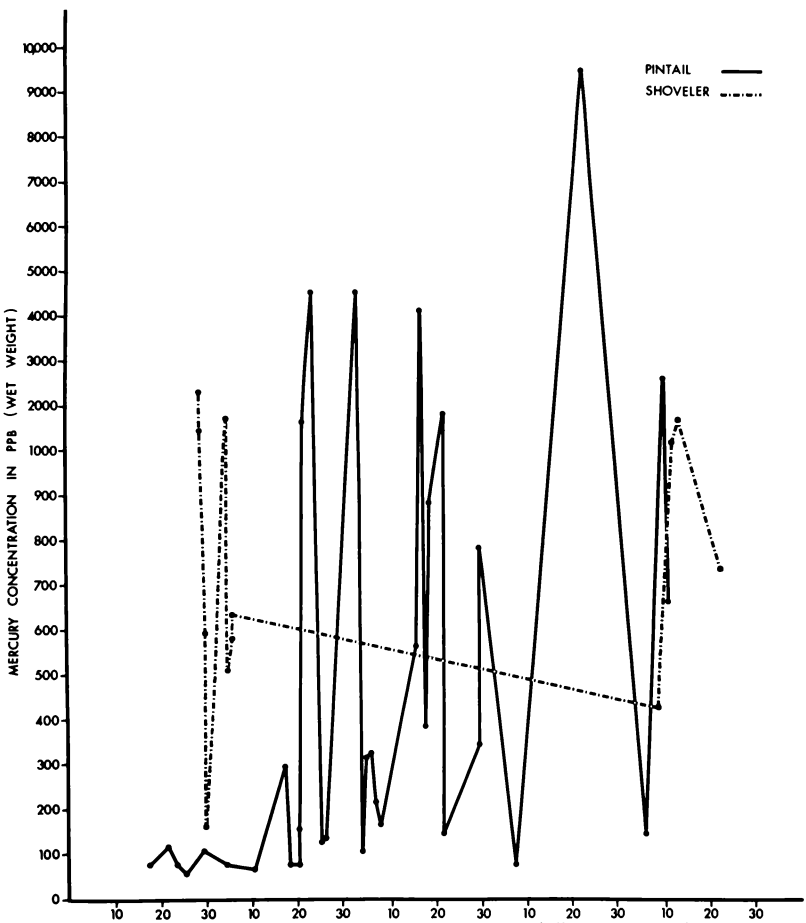

Figure 1. Total mercury content in liver tissues of 34 adult pintails and 12 adult shovelers collected in south-central North Dakota during 1969-70. Nine adult pintails were collected in 1969.

July 23 to August 11 (Table 2) may be attributed in part to a bird that contained 9.5 ppm in liver tissue, 9.2 ppm in kidney tissue and 2.1 ppm in breast muscle tissue.

Wild juveniles were consistently lower in mercury content than were adult birds from the same region. Only one liver sample from an immature shoveler exceeded 500 ppb. The mercury levels in wild juveniles, however, were significantly higher (0.05 probability level) than were pen-reared juveniles fed food that contained 39 ppb (dry weight) of mercury (Table 3). This suggests that considerably higher concentrations of mercury exist in foods eaten by wild juveniles. Westoo (1969) reported that domestic poultry examined in Sweden contained 13 ppb of mercury in meat and 30 ppb in liver tissues. The levels found in our control birds approached these levels.

Pintail ducklings of all age classes were found to consume 70% animal material, whereas shoveler ducklings and adults consume almost 100% animal material (Table 4). Therefore, adult shovelers and juveniles of both species apparently obtain the bulk of their mercury from aquatic invertebrates. Adult shovelers apparently ingest a more consistent dosage of mercury than adult pintails. Although pintails consume aquatic invertebrates, they feed primarily on seeds in both upland and wetland habitats. Pintails feeding in upland fields are exposed to treated grain that is spilled or subsequently exposed following spring planting. The esophagus of one pintail hen was filled with pink-colored wheat; this indicates that treated grain is being consumed in cultivated fields.

The status of our knowledge of mercury in the environment was reviewed by Wallace et al. (1971), U. S. Geological Survey (1970) and Adomaitis (1970). Mercury can conceivably enter wetlands from a number of sources. Jenne (1970) pointed out that mercury is continuously removed from the atmosphere and deposited on the earth's surface by dry fallout and rainfall. He further stated that the quantity of sediment that is transported by water is also an important factor in determining the movements of mercury. In a summary of the available information on mercury in the United States (U. S. Geological Survey, 1970) it is stated: "Because they serve as sediment traps and habitats for aquatic organisms, lakes and ponds are likely to serve as traps for mercury which enters them." Johnels and Westermark (1969) stated that the burning of various kinds of raw materials will release mercury to the atmosphere and suggested that chemical fertilizers (e.g. superphosphate) may contribute mercury. Fleischer (1970) pointed out that it has been implied that the widespread use of organic mercury compounds as seed fungicides has increased the content in cultivated soils; he added, however, that data are not available on this subject to confirm this postulation.

The majority of prairie wetlands are closed basins for surface water, and once mercury enters the area in significant amounts, it can only be removed by passage into the ground water, by evaporation into the atmosphere, or by transportation by some biological means such as insect emergence. It is likely, therefore, that upon entering a prairie wetland, mercury will be retained for a considerable period of time.

Table 3. A comparison of the mercury content (ppb wet weight) in liver and breast muscle of wild and pen-reared juvenile pintails and shovelers

Statistic	Pintail				Shoveler			
	Liver		Breast Muscle		Liver		Breast Muscle	
	Pen	Wild	Pen	Wild	Pen	Wild	Pen	Wild
Number	3	5	3	5	3	5	3	5
Range	27-36	44-190	15-21	26-169	18-25	145-1,016	20-26	70-225
Mean	32	123	18	91	21	382	22	153
Probability values[1]	0.036[2]		0.036		0.036		0.036	

[1]The "U" statistic (Mann-Whitney "U" Test, Siegel, 1956)
[2]Significant at 0.05 level

Table 4. Proportion of animal and plant foods consumed by shovelers in south-central North Dakota and pintails in southern Alberta

Food item	Breeding shovelers[1] No. (40)	Juvenile shovelers[1] No. (10)	Juvenile pintails[2] No. (144)
Mollusca	16	13	15
Crustacea	75	49	6
Insecta	7	38	48
Miscellaneous	2	T	1
Total plant	T[3]	T	30
Total animal	100	100	70

[1]Expressed as percent by volume
[2]Expressed as percent by dry weight (Sugden, 1969)
[3]Less than 1%

The relationships between the use of mercury for seed treatments and mercury levels found in the tissues of seed-eating birds have been discussed by Westoo (1969), Fimreite et al. (1970), Johnels and Westermark (1969), Holt (1969), Ljunggren (1968), Borg et al. (1969), and others. There is little information concerning mercury levels in birds that consume aquatic invertebrates. Johnels and Westermark (1969) reported that mercury accumulation in fish-eating birds follows a different course than that in terrestrial birds. Although fish dominated the diet of the great-crested greben *(Podiceps cristatus)* invertebrates accounted for one-third of the foods consumed.

Hannerz (1968), while studying accumulations of mercury in aquatic invertebrates, stated that "the predacious insect larvae, e.g. those of dragon flies and *Sialis*, water beetles (especially Hydrophilidae), all have high concentration factors, while species living on detritus and decaying plant material mostly have lower factors. The chironomids were found to vary in this respect." Johnels and Westermark (1969) found that invertebrates below a Swedish mill that discharged mercury wastes had mercury levels as high as fish. In summary, the authors stated: "It is evident that the aquatic environment is more susceptible to mercury contamination in general than the terrestrial environment; a study of the feathers of the fish-eating bird specimens gives evidence of and shows influence of mercury contamination." It has been demonstrated that inorganic mercury can be biochemically methylated in the aquatic ecosystem and, as a result, gain entrance into the aquatic food chain (Jernelov, 1969 and 1970).

The tissues from pintail and shoveler ducklings reared in Stutsman and Barnes Counties in North Dakota contained significantly higher levels of mercury than did pen-reared ducklings. It is likely that the major source of the mercury found in duck tissues comes from the foods that they consume. Mercury levels in the water are generally low (Wershaw, 1970) in comparison to those found in aquatic invertebrates or fish (Hannerz, 1968). Rucker and Amend (1969) have demonstrated that fish can absorb significant amounts

of mercury through their external tissues; however, it is unlikely that this occurs in ducks.

There is a paucity of information concerning mercury levels in tissues of aquatic birds. Johnels and Westermark (1969) reported on the great-crested grebe in Sweden; Fimreite et al. (1970), on the mallard (*Anas platyrhynchos*) in Canada; Borg et al. (1969), on the mallard, shelduck (*Tadorna tadorna*) and mute swan (*Cygnus olor*) in Sweden; and Holt (1969), on the mallard, goldeneye (*Bucephala clangula*), whooper swan (*Cygnus cygnus*) and the Canada goose (*Branta canadensis*) in Norway. Mercury levels found in our studies in North Dakota were of a similar magnitude to those found in agricultural areas of northern Europe and Canada.

Fimreite et al. (1970) reported that eggs from hen pheasants with mercury levels of 3-13 ppm in their livers had significantly lower hatchability than did controls. Borg et al. (1969) reported similar results. Ljunggren (1968) presented evidence that mercury poisoning resulted in poor hatchability in wood pigeons (*Columba p. palumbus*). In our study, mercury levels in liver tissue exceeded 2 ppm in one shoveler hen and 9 ppm in one pintail hen. Therefore, this would suggest possible interference with reproduction. Kiwimae et al. (1969) reported that domestic chickens fed grain treated with different mercury compounds reached levels ranging from 2.65-4.80 ppm in liver tissue.

Because of the large geographic area covered by migrating birds, it is often difficult to determine with certainty the sources of toxic materials, particularly those like mercury that are retained in the bird for a considerable length of time (Borg et al. 1969). There is some indication that ducklings reared in North Dakota obtain mercury on the breeding grounds. The fact that wild juveniles contained significantly higher levels than pen-reared juveniles and that at least one juvenile shoveler contained liver tissue containing 1 ppm of mercury suggests that significant levels are being ingested. Hammond (1971) reported that most rocks and soils contain mercury concentrations averaging about 0.1 ppm. Borg et al. (1969) suggested that soil levels of 0.2 ppm or less might be considered as reasonably unaffected by human activities. Wallace et al. (1971) in discussing "background" levels stated, "A literature survey suggests that normal values for eggs and the flesh of birds and animals are generally less than 0.02 ppm." Edwards (1970) pointed out that very little reliable information exists on the newer organic fungicides' toxicities to fish and other aquatic organisms. Harriss et al. (1970) reported that concentrations of organomercurial fungicides as low as 0.1 ppb in water reduced photosynthesis and growth in phytoplankton organisms which are basic elements in many aquatic food chains. Considerably more research needs to be done in this area in order to protect the aquatic environment and the terrestrial organisms that feed on aquatic life. We need to understand the processes through which mercury enters the aquatic food chains and to identify which organisms contain the highest concentrations. A knowledge of the foods consumed by an individual species is indispensable for determining the probability of its exposure to mercury and other toxic materials in the environment.

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REFERENCES CITED

Adomaitis, V. 1970. Mercury and the environment. N. D. Outdoors 33(6):5-7.

Borg, K., H. Wanntorp, K. Erne and E. Hanko. 1969. Alkyl mercury poisoning in terrestrial Swedish wildlife. Viltrevy 6:301-379.

Edwards, C. A. 1970. Persistent pesticides in the environment. Chemical Rubber Co. Press, Cleveland, Ohio. 78 p.

Fimreite, N., R. W. Fyfe, and J. A. Keith. 1970. Mercury contamination of Canadian prairie seed eaters and their avian predators. Canadian Field-Natur. 84:269-276.

Fleischer, M. 1970. Summary of the literature on the inorganic geochemistry of mercury, p. 6-13. *In* Mercury in the environment. U. S. Geol. Surv. Prof. Paper 713. 67 p.

Hammond, A. L. 1971. Mercury in the environment: natural and human factors. Science 171:788-789.

Hannerz, L. 1968. Experimental investigations on the accumulation of mercury in water organisms. Rep. Inst. of Freshwater Res., Drottningholm, Sweden. 48:120-176.

Harriss, R. C., D. B. White, and R. B. MacFarlane. 1970. Mercury compounds reduce photosynthesis by plankton. Science 170:736-737.

Holt, G. 1969. Mercury residues in wild birds in Norway: 1965-67. Nordisk Veterinarmedicin 21:105-114.

Jenne, E. A. 1970. Atmospheric and fluvial transport of mercury, p. 40-45. *In* Mercury in the environment. U. S. Geol. Surv. Prof. Paper 713. 67 p.

Jernelov, A. 1969. Conversion of mercury compounds, chapter 4. *In* M. W. Miller and G. G. Berg [eds.] Chemical Fallout: current research on perisistent pesticides. Charles C. Thomas, Springfield, Ill. 531 p.

————. 1970. Release of methyl mercury from sediments with layers containing inorganic mercury at different depths. Limnol. Oceanogr. 15:958-960.

Johnels, A. G., and T. Westermark. 1969. Mercury contamination of the environment in Sweden, chapter 10. *In* M. W. Miller and G. G. Berg [eds.] Chemical Fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Ill. 531 p.

Kiwimae, A., A. Swensson, U. Ulfvarson, and G. Westoo. 1969. Methylmercury compounds in eggs from hens after oral administration of mercury compounds. J. Agr. Food Chem. 17:1014-1016.

Ljunggren, L. 1968. The influence of mercury poisoning on the reproduction and general health of wood pigeons. Viltrevy 5:423-434.

Rucker, R. R., and D. F. Amend. 1969. Absorption and retention of organic mercurials by rainbow trout and chinook and sockeye salmon. Progressive Fish-Cult. 31:197-201.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, Inc., New York. 312 p.

Sugden, L. G. 1969. Foods, food selection, and energy requirements of wild ducklings in southern Alberta. Ph.D. thesis, Utah State Univ. 171 p.

Swanson, G. A., and J. C. Bartonek. 1970. Bias associated with food analysis in gizzards of blue-winged teal. J. Wildl. Mgmt. 34:739-746.

Takeuchi, T. 1971. Biological reactions and pathological changes of human beings and animals under the condition of organic mercury contamination. Proc. Internat. Conf. on Environmental Mercury Contamination. Ann Arbor, Mich. (in press).

U. S. Geological Survey. 1970. Summary, p. 1-5. *In* Mercury in the environment. U. S. Geol. Surv. Prof. Paper 713. 67 p.

Wallace, R. A., W. Fulkerson, W. D. Shults, and W. S. Lyon. 1971. Mercury in the environment: The human element. Oak Ridge Nat. Lab. NSF-EP-1. 61 p.

Wershaw, R. L. 1970. Sources and behavior of mercury in surface waters, p. 29-31. *In* Mercury in the environment. U. S. Geol. Surv. Prof. Paper 713. 67 p.

Westoo, G. 1969. Methylmercury compounds in animal foods, chapter 5. *In* M. W. Miller and G. G. Berg [eds.] Chemical Fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Ill. 531 p.

Wobeser, G., N. O. Nelson, R. H. Dunlop, and F. M. Atton. 1970. Mercury concentrations in tissues in fish from the Saskatchewan River. J. Fish. Res. Board Canada 27:830-834.

EFFECTS OF NOISE ON HAMSTER BEHAVIOR PATTERN

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ABSTRACT

This study involves the effect of noise on the behavior pattern or activities of seven, individual, male hamsters. Humidity, room temperature, light hours, light intensity, noise level and noise interval were controlled in the laboratory. The hamsters were individually placed in control cages equipped with counters and recorders to measure revolutions of the exercise wheel, nest time, uninterrupted nest time and exits from the nest. Observations were made and data collected, for 7 days of quiet, "no noise" conditions followed by 7 days of an intermittent 95-db noise, introduced 4 sec out of each minute during the hours of 8 AM to 5 AM. Noise has an apparent

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detrimental effect on the hamster behavior pattern under the described conditions. The hamsters were more skittish,, spent more time in the nest, and were more active on an exercise wheel during the week of noise. Under two of six conditions, average revolutions on the exercise wheel and average low revolution count, the results under noise are statistically, significantly greater (t test; $P<0.005$ and $0.10<P>0.005$, respectively).

INTRODUCTION

Biologists, scientists, legislators, laymen, blue and white collar workers alike, teachers, business men and the clergy have suddenly become specialists concerned with our environment. In the chambers of our State Capitol senators and representatives passed legislation requesting manufacturers to lessen the noise output of our farm tractors. The legislators regard any noise above 85 db to be harmful and deafening to man.

Since our nation and the public suddenly shows concern and interest in "noise pollution" it seemed timely to conduct tests to determine the effect of noise on hamster behavior patterns. Specifically, the problem was to determine the effect of an intermittent noise of 95 db on the hamsters' activity habits. The effect was determined by observation of the hamsters and also by measured and recorded activities.

MATERIAL AND METHODS

Homemade cages, built and designed especially for the experiment, were of two types, holding cages and control cages. The holding cage was an individual cage with no measuring devices. The control cage had an exercise wheel equipped with two counters to record the revolutions of the wheel. The second counter was used simply to check on the first counter. The control cage also had a nest box balanced on a platform. The platform was quarter-inch plywood measuring $4 \times 10\frac{1}{2}$ inches. The platform pivots or balances on a horizontal $\frac{3}{8}$-inch dowel which was off center, located 3 inches from one end of the plywood platform. Stoppers mounted on the cage hardware cloth permitted only a quarter-inch up and down movement of the platform at the nest end. Hangers for the recording pens extended from the platform through the hardware cloth to the outside of the cage.

The motor drive mechanism was salvaged from an old recording volt meter. A roll chart mounted on the back side of the cage and advanced by the motor drive was in position to record the up and down position of the ink pen suspended from the platform.

A small metal box opposite the nest end of the platform was carefully loaded with lead shot to just lift the nest end of the platform to the upper stopper. When the hamster stepped on the platform or entered his cage, the platform dropped to the lower stopper and likewise the pen lowered the same distance.

Four control cages were mounted as one unit. The platform of each cage was at a slightly different elevation so that the four recording pens, one from each cage, did not overlap. As the roll chart advanced, approximately 2 ft each 24 hrs, the ink pens recorded the

nest box empty in the upper position and the hamster in the nest at the lower level. Vertical lines on the chart indicated the time the hamster left or entered his nest. The roll chart served as a biological clock recording the nest time, uninterrupted nest time, "in" or "out" times from the nest in any given time period and the frequency of hygiene errands.

The noise source was a Klaxton 120 v electric horn. It put out 120 db of noise which could be deafening to the hamsters. Rather than muffle the Klaxton noise, the sound from the electric horn was recorded on a tape recorder and the volume was adjusted for a noise level of 95 db (rated very loud, yet safe from deafening). For various reasons, an intermittent sound rather than a continuous noise was used. To accomplish this, a motorized switch, which turned the tape recorder on 4 sec out of each minute, was used.

The spare bedroom in my home served as a laboratory. The room was very quiet, and the shades were pulled. A time clock turned on a tree lamp to control the light hours and also the noise hours from 8 AM to 5 PM, the hours that the family was not normally at home. Room temperature and humidity were constant throughout the experiment. Regular feeding and observation times were maintained.

Seven male hamsters were used in the test, four on the first run. No sound was used during the first week. Readings from the revolution counters were recorded on the data sheets and the roll chart recorded the nest activities. Observations of animal health, response to my entering the room, ambition to escape at feeding time, etc. were recorded. After controlled quiet conditions for one week, the hamsters were exposed to intermittent noise for 7 days. Observations were again recorded. When this test was completed the first four hamsters were returned to the holding cages and a new set of three animals was used to repeat the experiment.

RESULTS AND DISCUSSION

Immediately after noise was introduced, the hamsters became frightened. They pulled down their ears, rolled up in a ball and tried to hide when the cage cover was opened for feeding or checking counters. They remained skittish during all 7 days that noise was applied. The hamsters gave up the excessively lively or frivolous action a day or so after they were returned to the holding cages. The hamsters used more water during the week of noise.

The results from six conditions are significant (Table 1). All seven animals spent more time in the nest during the week of noise. Nest time was the number of hours a hamster spent each day in his nest. He may or may not be asleep, but he is in his nest box causing the platform to be weighted down, placing the recording pen in the lower position. The seven hamsters averaged 15 hrs each day in their nest when there was no noise, but 16 hrs and 12 min when there was noise . This is to be expected because certainly the noise must have interrupted their sleep. The hamsters needed the extra 1 hr and 12 min to catch up on the lost sleep because of the noise.

Noise also shortened the uninterrupted nest time for the hamster during the week of noise. Uninterrupted nest time was the longest

Table 1. Response of seven hamsters involving noise and no noise conditions*

SITUATION	INDIVIDUAL HAMSTERS							
	Red I	Black 1	Green I	Blue I	Black II	Green II	Blue II	Total Avg.
Average nest time NO NOISE	14:10	15:05	12:30	15:55	14:10	13:40	14:55	15:00
Average nest time INTERRUPTED NOISE	16:25	16:00	17:10	17:10	15:30	14:45	16:10	16:12
Average uninterrupted nest time NO NOISE	3:34	4:18	5:25	5:26	4:40	4:24	5:17	4:46
Average uninterrupted nest time INTERRUPTED NOISE	2:06	2:46	4:39	2:46	3:51	3:43	5:03	3:33
Average exits NO NOISE	23:00	26:00	24:40	15.00	34.00	19.30	18.30	22.90
Average exits INTERRUPTED NOISE	37.20	43.00	29.60	19.50	50.00	27.00	29.00	33.60
Average revolutions exercise wheel NO NOISE	8,881	9,729	13,148	11,253	1,034	7,242	12,257	9,078
Average revolutions exercise wheel INTERRUPTED NOISE	11,826	10,367	14,563	11,577	1,699	9,214	12,939	10,312
High revolution count on wheel NO NOISE	12,875	10,152	14,872	14,311	1,960	10,447	17,562	11,739
High revolution count on wheel INTERRUPTED NOISE	16,429	14,706	18,374	16,657	2,134	14,943	14,018	-13,869
Low revolution count on wheel NO NOISE	5,334	7,561	6,006	7,483	284	5,401	8,017	5,726
Low revolution count on wheel INTERRUPTED NOISE	8,477	7,561	9,226	9,717	1,116	5,519	11,209	7,546

*All averages are calculated for a 24-hour day from a seven-day experiment.

recorded interval of nest time in a 24-hr day. The hamsters averaged 4 hr and 46 min of uninterrupted nest time when there was no sound and this was reduced to 3 hr and 33 min when noise was applied.

Noise also had a noticeable effect on the number of exits by the hamster from his cage, marking up more exits during the week of noise.

The hamsters started operating the exercise wheel much later in the evening during the week of noise, but once they started, they used the wheel almost continuously.

The seven hamsters averaged 9,078 revoluutions on the exercise wheel each day in a quiet laboratory but averaged 10,312 revolutions each 24-hr period during the week noise was introduced. This difference is statistically significant (t test; $P<0.005$). It was as if the hamsters were running away from something. They did not take rest periods or stop to gnaw at the wire on their cages. Hamsters are not consistent in the revolutions that they operate the wheel during a 24-hr period. Each animal had a low and high recording for the week. Of importance is that the hamster has a higher "high" reading and a higher "low" reading during the week of noise. The average low revolution count was significantly greater during the week of noise ($0.10<P<0.05$).

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ANALYSIS AND CONTROL OF FLOW NOISE AND SOUND REVERBERATIONS IN HYPERBARIC CHAMBERS

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ABSTRACT

Experiments with animals in hyperbaric chambers at pressures up to 600 psig indicated that they developed severe emotional stress and even death due to the noise in the chamber. It became apparent that this noise level should be analyzed and controlled if other physiological experiments were to yield satisfactory results. Analysis was carried out with an audio spectrometer and a graphic level recorder, which yielded a graph of the sound amplitude vs. frequency. Noise levels above 120 db (re. 0.0002 microbar) were recorded. This exceeds the point of discomfort and approaches the region of pain. Methods of noise control included: placing fiberglass and cotton wadding in the chamber's inlet and outlet pipe and placing a fiberglass-filled expansion chamber in the chamber outlet line. Though

these methods did not completely solve the problem, they did pinpoint the noise source to aid in achieving a forthcoming solution.

INTRODUCTION

Statement of the problem.—In recent years, acquisition of deep-sea knowledge has become an important phase of technology. Of primary interest are the long-term effects of the high-pressure deep-sea environment on mammals, particularly man. Due to the shortcomings of using humans to actually make the journey into the deep-sea environment, it has become more practical to simulate the environment in high-pressure chambers, and to observe the reactions of guinea pigs or similar animals as they are subjected to the experiences (such as compression and decompression) that such an environment has to offer.

Such a simulation is taking place at the University of North Dakota. Although a full-scale laboratory is under construction, it is not yet completed, and research is currently taking place in small pilot laboratories on the campus. One such laboratory is operated by Dr. Carl Zogg in the Department of Physiology and Pharmacology, Medical School. A schematic diagram of the apparatus in that laboratory is shown in Figure 1.

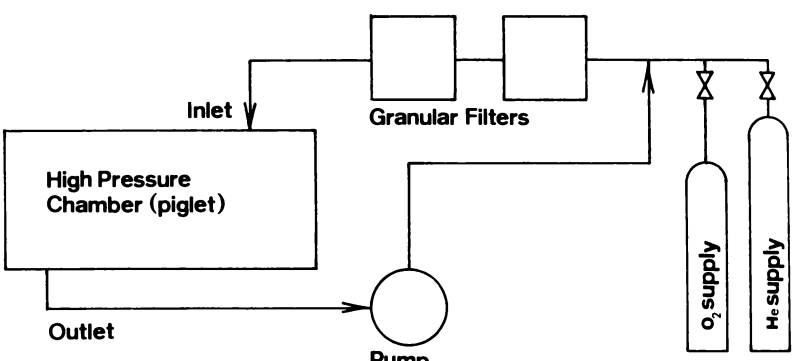

Figure 1. Schematic of high pressure life system.

The high pressure chamber is 17 inches in diameter and 39 inches long, and is called a piglet. It is pressurized from high-pressure storage tanks of helium and oxygen, with helium being the primary component. The gas is circulated through the system with a Bell & Gossett 1½ hp, 4-piston pump, with only one piston actually operating. Granular filters in the inlet line remove impurities from the gas. A small fan is used inside the piglet to prevent gas stagnation pockets from forming. All lines are ¾ inch, schedule 80, steel pipe and the entire system is designed to accomodate pressures up to 600 psig (guage pressure), simulating ocean depths of roughly 1000 feet.

In preliminary tests with animals in the piglet, it was observed

that the animals reacted adversely when the pump was operating, and noise in the chamber became a suspect as a probable cause of this reaction. Since the purpose of the facility was to test reaction to the deep-sea environment, and not to noise, it became apparent that, if the noise in the chamber was the cause of these observed reactions, this noise would have to be reduced if satisfactory results were to be obtained from other experiments.

Possible sources of high noise level.—A preliminary look at the system indicated several possible sources of high noise in the piglet. Since the main problem seemed to arise when the pump was running, it seemed reasonable to assume that the circulation pump was in some way the primary cause of the noise. This could be due to one or more of the following: noise of the gas flowing through the pipe and into the chamber, the actual pump noise being transmitted by the piping, the pump noise being transmitted through the gas stream, or the external pump noise being transmitted through the chamber walls with the piglet acting as a reverberation chamber to amplify the noise. The fan inside the chamber may also be a noise contributor and it was also investigated.

With these possible sources of extensive noise in mind, the problem became one of analyzing and isolating the actual source, determining the mode of transmission, and, if possible, finding a practical means to reduce the noise to the point where it would be insignificant with respect to the other tests being performed.

INSTRUMENTATION AND MEASUREMENT

Data needed and instruments used.—The first step in the analysis of this problem was to determine the actual sound level in the piglet, together with the frequency or frequencies at which the sound level, or levels, occurred. The primary instruments used to obtain this information were an audio frequency spectrometer, a graphic level recorder, and a piezoelectric microphone (Figure 2). This instrumentation provided a continuous scanning of the frequency spectrum from 25-20,000 Hz, and produced a graphical plot of sound amplitude vs. frequency.

The mechanics of carrying the signal from the microphone inside the high-pressure chamber (piglet) to the instruments outside was accomplished with the aid of a teflon-sealing, electrical throughput manufactured by Conax Corporation of Buffalo, New York. This device allowed the shielded coaxial microphone lead cable to pass across the pressure gradient with only a minimal amount of gas leakage from inside the chamber.

Actual measurement.—The microphone cable was inserted through the electrical throughput allowing approximately 20 inches of free cable inside the chamber. This permitted considerable movement inside the chamber to read sound levels at various locations within the piglet.

All sound measurements, taken during pressurization or under pressure, followed a consistent measurement sequence. The chamber was pressurized at 50-psi intervals from 0-600 psig. At each pressure interval, the ambient sound level was read and an audio spectrogram was made for three conditions: 1) "ambient" sound level in the

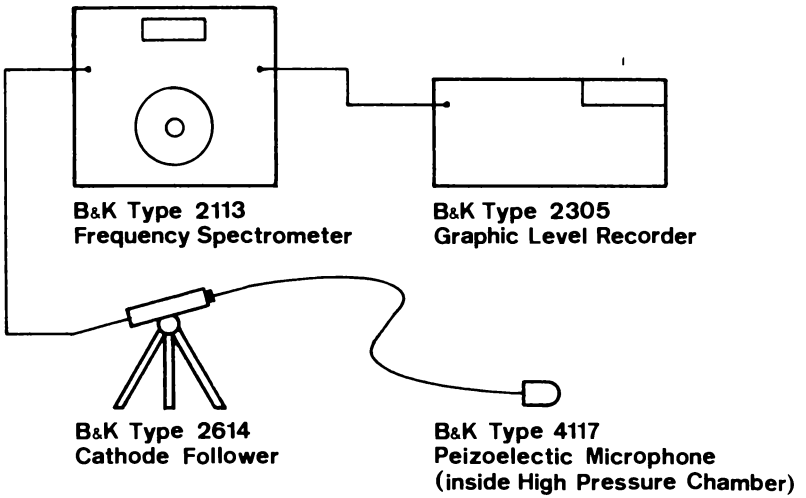

Figure 2. Instrumentation set-up used for sound measurements in the high-pressure chamber.

piglet, i.e. sound level without the fan or the pump running, 2) sound level with the fan running, and 3) sound level with both the fan and the pump running (normal operating conditions). Unless otherwise stated, the above-mentioned sequence of measurements pertains to all conditions discussed below.

The first sequence of measurements was run with the microphone directed towards the chamber's gas inlet. Following this, the microphone was placed near and directed towards the chamber's gas outlet and a measuring sequence was run.

Dr. Zogg believed that some success had been achieved in sound reduction in the high-pressure chamber when fiberglass and cotton wadding had been placed in the chamber's gas inlet and outlet, respectively. In an attempt to check the validity of this assumption, and to aid in localizing the sound, measuring sequences were run with wadding in position.

First, fiberglass wadding was placed in the gas inlet only, and a measuring sequence was run. (This was terminated after 250 psi when no change was noted over previous runs.) A complete sequence was then run with cotton wadding in the gas outlet only. Finally, a sequence was run with wadding in both the inlet and the outlet, respectively.

RESULTS AND CONCLUSIONS

Figure 3 is a summary of the results obtained from the sound measurements taken. Ambient sound conditions in the laboratory outside the piglet were roughly 65 db measured on the "linear" weighting network. The corresponding sound levels inside the closed chamber averaged only 38 db, or an average of 27 db lower than

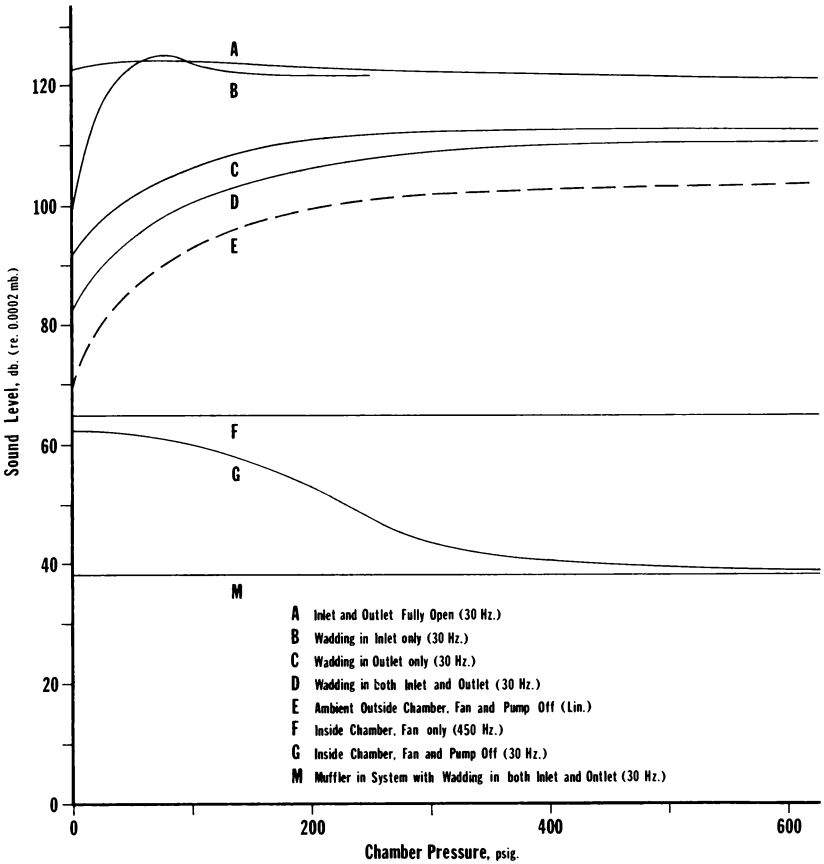

Figure 3. Maximum measured sound levels for varying chamber pressures, correlating the sound levels with their respective frequencies for different conditions. Fan and pump were both running except as noted.

outside conditions with the fan and pump off. The inside sound level with only the fan running varied from 60 db at 0 psig to only 40 db at 600 psig, and this occurred at 450 Hz. This attenuation can be attributed to the increasing density of the gas in the chamber as pressure increased.

With the fan and pump both running, and with the microphone located near the gas inlet inside the chamber with no wadding in either the chamber inlet or outlet, the sound was measured at levels exceeding 120 db at the frequency band of 30 Hz. Placing wadding in the inlet had little effect on the sound level. Merely relocating the microphone near the gas outlet with the fan and pump running

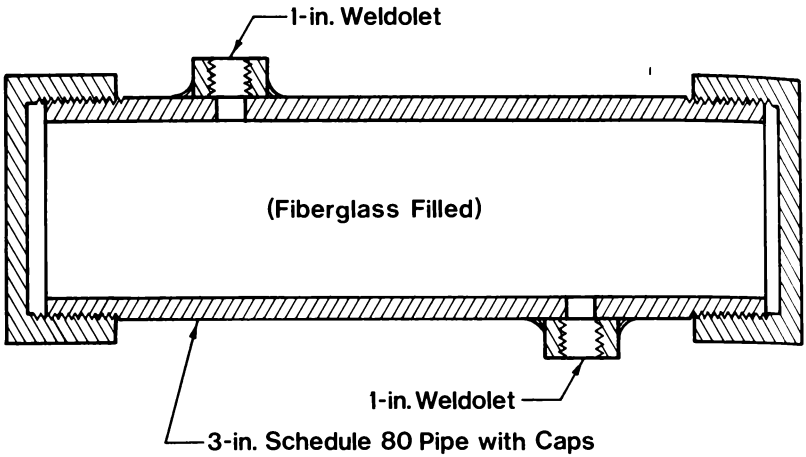

Figure 4. Experimental muffler.

indicated no noticeable change in the readings. However, when cotton wadding was placed in the gas outlet of the chamber the sound level dropped 14-18 db. It is also noted that with wadding in both the inlet and the outlet as well as with wadding in the outlet only, the sound level increased with increasing pressure. With wadding in the inlet only the sound decreased slightly with increased pressure. The maximum sound levels for the above conditions all occurred at the frequency band of 30 Hz.

Several conclusions were drawn from the analysis of the data:

1) Since sound levels as low as 70 db are known to cause measurable effects on living beings, the 120 db sound level recorded in the piglet is very likely the cause of the animals' observed distress.

2) Since the highest reading without the pump running was less than 70 db, the primary cause of the noise problem most likely stems from the gas circulation pump.

3) Since wadding in the chamber inlet had very little effect on the maximum sound level, the noise is not generated by the gas flow into the chamber.

4) Since wadding in the chamber outlet cut the sound level significantly, the noise must be from the pump pulse traveling against the flow through the gas stream in the outlet pipe. If the sound was being transmitted by the pipe itself, the cotton would not have had such a significant effect on the sound level. A rubber hose connection, which is in the line between the chamber outlet and the pump inlet, also seems to dispel any theory that the pipe is the transmission medium.

5) The granular filters in the inlet line are apparently absorbing the pump pulses which would otherwise enter the piglet's inlet connection.

6) Sound level inside the chamber without the fan or the pump

running is significantly below the ambient sound level in the room outside the chamber. Therefore, it appears that external sounds are not transmitted into the chamber in any significant amounts and do not materially contribute to high inside sound levels.

7) All of the peak sound levels occurred in the low frequency range (30-150 Hz). Therefore, if the low frequency sounds can be absorbed, the noise problem should be essentially solved.

ATTEMPTED SOLUTION USING MUFFLER

A simple expansion chamber type muffler filled with fiberglass was built and placed in the piglet's gas outlet line (Figure 4). It consists of a 3-inch, schedule 80, steel pipe, 16 inches long, and closed at both ends with threaded caps. Physical constraints limited the length of the pipe to 16 inches.

With this muffler placed in the system, a sound measuring sequence through the full pressure range was run (Figure 3). A sound level decrease of only about 10 db was achieved. This is in contrast to the 30-40 db attenuation which is required to provide the animals in the piglet with a compatible environment.

However, the fact that a rather significant attenuation was experienced with the muffler indicates that the previous conclusions are valid; i.e., the sound is coming from the pump and is being transmitted through the gas stream inside the pipe from the pump intake to the chamber outlet in the direction opposite to the flow of the gas stream. What is now needed is a more efficient muffler.

Lack of time caused by extended delays of instrumentation delivery by the suppliers prevented the design and building of another muffler. As a result, the task is not complete, and such a muffler is expected to be built and subsequently tested.

REFERENCES CITED

Beranek, L. 1960. Noise reduction. McGraw-Hill Book Co., Inc., New York.

Berland, T. 1970. Silencing invisible pollution. Today's Health 48: 57-58.

Broch, J. T. 1967. Acoustic noise measurements. Bruel and Kjaer.

Culliton, B. J. 1970. Noise: Polluting the environment. Science News 97: 132-133.

Peterson, A. P. G., and E. E. Gross, Jr. 1963. Handbook of noise measurement. General Radio Co., Concord, Mass.

Yerges, L. F. 1969. Sound, noise and vibration control. Van Nostrand Reinhold Co., New York.