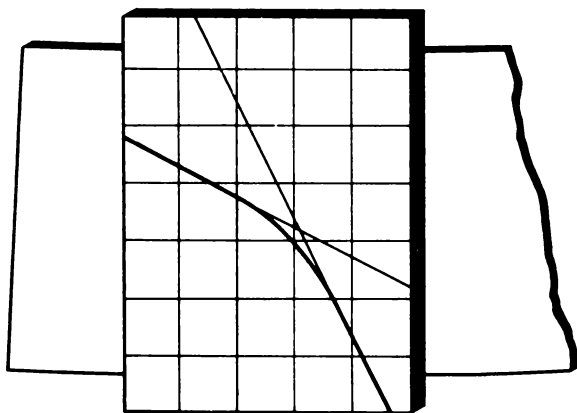


PROCEEDINGS
of the
NORTH DAKOTA
ACADEMY OF SCIENCE

ABSTRACTS



67TH ANNUAL MEETING
APRIL 25 and 26, 1975

Valley City State College
Valley City, North Dakota

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ABSTRACTS

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EDITOR'S NOTE

The abstracts are arranged alphabetically by author or first author if more than one author are involved. An author index of all authors is on pages 35 and 36.

SYNTHESIS AND REACTIONS OF PALLADIUM AND COBALT COMPLEXES CONTAINING A METAL-CARBON BOND. Bruce B. Anderson and Kenneth Klambunde. Dept. of Chemistry, Arts and Sciences, Univ. N.Dak., Grand Forks, N. Dak. 58202

Palladium atoms cocondensed with bromopentafluoro benzene (C_6F_5Br) oxidatively insert into the carbon-bromine bond to form $[C_6F_5PdBr]_n$. This intermediate can be prepared under mild conditions and is stable in acetone solutions at room temperature in air. $[C_6F_5PdBr]_n$ has been isolated as a moderately stable brown powder. $[C_6F_5PdBr]_n$ in acetone reacts with a variety of ligands to form stable, isolable complexes, $C_6F_5PdL_2Br$. ($L=PEt_3$, SPh_2 , SMe_2 , SEt_2 , NH_3 , NH_2Et , $NHEt_2$, NMe_3 , C_6H_5N ; $L_2=2,2'$ -bipyridine, 1,5-cyclooctadiene, norbornadiene). Cobalt atoms cocondensed with C_6F_5Br yield $[C_6F_5CoBr]_n$ which is less stable than the palladium intermediate. Data from trapping experiments with PEt_3 indicate the synthesis of $C_6F_5CoBr(PEt_3)_2$. We are currently investigating the reactions of other transition metals with similar RX compounds (eg. CF_3Br). These RMX compounds are highly coordinatively unsaturated and thus quite interesting in terms of catalysis species. (Supported by NSF-42375.)

PRELIMINARY GEOLOGIC AND HYDROLOGIC INVESTIGATIONS FOR PROPOSED COAL-GASIFICATION PLANTS IN NORTH DAKOTA. B. M. Arndt, L. Clayton, S. R. Moran. Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dak., 58202 and J. Cherry. Dept. of Earth Sciences, University of Waterloo, Waterloo, Ontario, Canada.

An initial step in assessing environmental impact of coal gasification is to establish the baseline conditions prior to development. Preliminary geologic and subsurface hydrologic studies were made at two proposed mine sites in western North Dakota. These studies were designed to (1) summarize the structural, stratigraphic, and lithologic information in each area, (2) summarize what is known about the groundwater-flow system in each area, (3) make a preliminary assessment of the impact of mining, and (4) outline procedures for detailed assessment of the impact of mining.

The general aspects of the geologic setting of the proposed sites can be determined using existing data. The detailed aspects of the geologic setting necessary to assess the environmental impact of mining requires additional data. The groundwater-flow systems at both sites include local, intermediate, and regional sub-systems. The removal of the lignite and disturbance of the overburden materials will alter permeability characteristics of the sediments, may change groundwater-flow gradients, and may destroy aquifers that are of local significance.

Proposed future studies include detailed stratigraphic analyses, groundwater flow-system monitoring through the use of an extensive piezometer network, and computer simulation of groundwater-flow patterns.

NORTH DAKOTA FLEAS. VI. SEASONAL VARIATION OF FLEAS ON RICHARDSON'S GROUND SQUIRRELS. Larry G. Baesler. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

Seasonal fluctuations in numbers of adult fleas parasitizing Richardson's ground squirrels, Citellus richardsonii (Sabine) were determined from April 10 to September 24, 1974.

One hundred ninety-six ground squirrels collected in Grand Forks County with rifles and kill traps yielded 607 fleas. These included 456 Opisocrostis bruneri, 130 Neopsylla inopina, and 21 Rectofrontia fraterna.

Collection data were divided into 12 2-week periods and flea indices were calculated for each. O. bruneri was present throughout the entire collecting period, while N. inopina disappeared in July and remained scarce until September. R. fraterna was too scarce to draw any conclusions concerning its population dynamics.

Precipitation totals for simultaneous periods suggest a direct relationship with the abundance of O. bruneri. Peaks in precipitation were followed 2-4 weeks later by peaks in the numbers of O. bruneri. This lag effect suggests that increased precipitation raised soil moisture, which in turn increased the humidity in the rodent burrow. Elevated humidities may have increased larval and metamorphic survival, resulting in the adult population peaks observed. This hypothesis is consistent with known sensitivities of some flea larvae to various levels of humidity.

DECOMPOSITION OF 4-AMINOPYRIDINE IN SOIL. P. M. Betts and J. R. Fleeker. Dept. of Biochem., N. Dak. State Univ., Fargo, N. Dak. 58102

The decomposition of the avicide, 4-aminopyridine (4-AP) was studied in three N. Dak. soils. Each soil was incubated with 10 μg 4-AP-2- ^{14}C per g air-dried soil at 20 $^{\circ}\text{C}$ and 80% of field capacity in moisture. After 52 days, 4.5, 13.0 and 17.9% of the applied ^{14}C was recovered as $^{14}\text{CO}_2$ from Barnes loam, Towner sandy loam and Fargo clay, respectively. With all three soils, over 50% of the $^{14}\text{CO}_2$ recovered was collected during the last 10 days of incubation. Corn chops, 10/64 - 9/64 mesh, containing 3% 4-AP and in contact with moist soil, had a slightly longer period before spoiling compared to corn chops containing no 4-AP. Corn chops containing 3% 4-AP-2- ^{14}C were incubated on the surface of Barnes loam at 20 $^{\circ}$ and 80% field capacity. After 70 days, 14.5% of the applied ^{14}C was recovered as $^{14}\text{CO}_2$. A gram-positive coccoid bacteria, utilizing 4-AP as carbon source, was isolated from the soil after the 70 day incubation period.

ELECTROCARDIOGRAPHIC RESPONSES OF ICE DIVING SCUBA DIVERS. D. M. Allen Brennan, B. K. Ross and S. J. Brumleve. Dept. Physiol. and Pharmacol., University of No. Dak., Grand Forks 58202.

Diving mammals exhibit an adaptive mechanism referred to as the diving reflex or diving bradycardia. It has been observed to occur reflexly for the duration of apneic dives. The exact mechanism for this diving reflex has not been elucidated. However, it is thought to be an oxygen conserving mechanism. In this project six SCUBA divers of both sexes participated in laboratory and under-ice experiments. Each diver's EKG was recorded individually with a clinical, 4-lead EKG machine. The experimental sequence was a control in air, face only submerged in ice water with mask on, face submerged with mask off. Subjects were asked to hold their breath for 45 seconds for each trial. Preliminary data suggest that there is a marked bradycardia when the face is unprotected. With the mask on, the diving reflex is significantly inhibited. (Supported by NIH Training Grant No. 5 T01 HL 05939-02.)

A MOSASAUR FROM RANSOM COUNTY, NORTH DAKOTA. J. A. Brophy. Dept. of Geology, N. Dak. State Univ., Fargo, N. Dak. 58102

In September, 1974, fossil remains of a marine reptile of the mosasaur group were recovered from the Niobrara Formation (Upper Cretaceous Series) in a road-cut exposure in northwestern Ransom County. The specimen consists of well-preserved to highly fragmented skeletal elements, including teeth, palatal fragments, cranial fragments, quadrates, vertebrae, rib fragments, limb fragments, and phalanges. Studies of the remains and their distribution on the surface of the outcrop and in the adjacent undisturbed rock suggest that they represent a single individual with an overall length of about 4 to 5 meters. Finds of mosasaur fossils have been rare in North Dakota, consisting of a few bones and teeth. The present find is the most complete so far recorded in the state.

CHRONOLOGICAL PROFILES OF NITRATE REDUCTASE ACTIVITY IN SUNFLOWERS. Kenneth W. Chisholm and D. S. Galitz. Dept. of Botany, North Dakota State Univ., Fargo, N. Dak. 58102.

Sunflowers were grown in vermiculite under four nutrient regimes: .001M, .005M, .01M, and .10M with respect to NO_3 -nitrogen, identical with respect to other major and minor nutrients. Levels of NRA were monitored in leaf tissue using the in vivo assay. Two groups were sampled on alternating days. Growth measurements were recorded; proteins were determined at each sampling by the Lowry method, as well as nitrate concentrations via the cadmium reduction method. A diurnal study was used to determine peak periods of activity and for calculation of a daily conversion factor. Canopy profiles of activity were determined for all treatments, as well as the specific activity of each sample.

Maximum rates of NRA/gfw/hr were found in all treatments to occur in the primary leaves of 7-10 day-old plants. Highest levels of daily NRA (NRA/gfw/hr X total leaf wt. X 24 X diurnal conversion factor) were found to occur later in the season with leaf weight reaching its peak while levels of activity were lower.

Specificity of nitrate reductase for NADH vs. NADPH was determined; the effects of FAD and FMN in combination with the reductants were observed; the effects of PMS on activity were measured; the in vivo assay was compared to the in vitro assay for sunflower leaf tissue.

BREWER LAKE, 1974 PHOTOSYNTHESIS AND RELATED MEASUREMENTS.

G. W. Comita and D. L. Henegar. Dept. of Zool., NDSU, Fargo, N.D. 58102 and N.D. Game and Fish Dept., Bismarck, N.D. 58505

Weekly measurements of integral gross photosynthesis made in Brewer Lake, N.D. during its first summer (1974) as a filled water basin, were related (* = 5, ** = 1% levels, N = 22) with transparency, -0.44 *, community respiration, 0.58 **, integral net photosynthesis, 0.63 **, and chlorophylls -a, 0.60 **, -b, 0.66 ** and -c, 0.65 **. Other parameters measured simultaneously were solar radiation, ammonia, nitrite, nitrate, phosphate, dissolved oxygen and alkalinity, but none of these were significantly related with integral gross photosynthesis at the 5% level. When these relationships were examined at each of the upper five meter levels, few correlations derived were significant, and no pattern was demonstrated. Photosynthesis reduced to near zero for about one week during the early July fish-kill, then resumed the pre-kill rate after another week and continued to a new high for the season on August 13.

THE STEROLS OF NORMAL AND MALE-STERILE MAIZE TASSELS DURING DEVELOPMENT. J. J. Comita and H. J. Klosterman, Dept. of Biochemistry, N. Dak. State Univ., Fargo, N. Dak. 58102

Normal and male-sterile (Texas type) genotypes of maize were investigated to determine whether there are any differences in their steroids during tassel development. A bioassay for estrogens of the base soluble lipid fraction of the normal meiotic and postmeiotic tassels gave no positive results on mice which gave positive results with 800 pg estrone. Using gas-liquid chromatography and mass spectrometry for identification, it was found that cholesterol, campesterol, stigmasterol, sitosterol, and (iso)fucosterol are present in meiotic tassels. A quantitative investigation of the dominant sterols, free and esterified, during the premeiotic, meiotic, and postmeiotic stages, using GLC with 5 α -cholestane as an internal standard, found 300-400 μ g/g total sterols (ca 45% sitosterol, 30% stigmasterol, and 13% campesterol) in all classes analyzed except the premeiotic, with 500 μ g/g sterol esters. Significantly more free sterols were found in the normal meiotic and postmeiotic than in male-sterile tassels. After application of cholesterol-4- 14 C to the normal and male-sterile leaves for 3 days at meiosis, the distribution of label in the leaves analyzed by liquid scintillation spectrometry indicated the presence of free sterols and sterol esters while autoradiography disclosed only free sterols in the tassels.

SPECIES DIVERSITY AND DISTRIBUTION OF MUSSELS (BIVALVIA: UNIONACEA) IN LAKE ASHTABULA, SOUTHEASTERN NORTH DAKOTA. A. M. Cvanara, Dept. of Geol., Univ. N. Dak., and P. G. Freeman, 314 Belmont Road, Grand Forks, N. Dak. 58202

Lake Ashtabula, a man-made reservoir on the Sheyenne River in southeastern North Dakota, was surveyed for mussels by SCUBA during the summer of 1974. Belt transects, 20 m by 1.75 m and paralleling depth contours, were run at each meter depth to 6 m at six stations. Four species were found to inhabit the lake (numbers of live individuals recovered in parentheses): *Anodonta grandis* Say (81), *Lampsilis radiata* (Gmelin) (12), *Amblema plicata* (Say) (2), and *Lasmigona complanata* (Barnes) (1). Individuals of *A. grandis* were conspicuously smaller than those in the river. Most were about 3-4 years old (oldest specimen was about 5 years old). Individuals of *Lampsilis radiata*, however, were of a size comparable to those in the river. Most mussel individuals occurred at 3-m depth, fewest occurred at 6 m, and none was found alive at 5 m. Individuals of *A. grandis* generally decreased down the lake toward the dam. The average density of *A. grandis* in the lake (0.27 mussels/m²) was significantly ($P=0.10$) greater than that in the river below the lake (0.05), and about the same as that above the lake (0.32). The average density of *L. radiata* in the lake (0.05) was significantly ($P=0.10$) less than that above the lake (0.24), and about the same as that below the lake (0.07).

VARIATION OF SHELL DIMENSIONS AND SHELL AND BODY WEIGHTS OF ANODONTA GRANDIS SAY AND LAMPSILIS RADIATA (GMELIN) (BIVALVIA: UNIONIDAE), SHEYENNE RIVER, NORTH DAKOTA. A. M. Cvancara, J. P. Bluemle, and M. E. Bluemle. Dept. of Geol., Univ. of N. Dak. and N. Dak. Geol. Survey (JPB), Grand Forks, ND 58202

The mussels (Bivalvia: Unionidae) Anodonta grandis Say and Lampsilis radiata (Gmelin), the most widespread species in the Sheyenne River of eastern North Dakota, were collected from 27 stations (spanning 511 river miles) during the summers of 1966, 1973, and 1974. Most individuals were collected from seven stations in 1974. Shells (n=341) of A. grandis became significantly (P=0.01) longer, higher, wider, and more inflated (width/height ratio increased) downstream. Shells (n=262) of L. radiata became significantly (P=0.01) higher and more inflated downstream. Shells (n=211) of A. grandis showed a significant (P=0.01) increase in shell weight and in the shell weight/length ratio downstream, and live individuals (n=236) showed a similar significant increase in total weight and in the total weight/length ratio downstream. These weight relationships fit a quadratic regression. Shells (n=154) of L. radiata showed a significant (P=0.01) simple linear increase in shell weight and in the shell weight/length ratio downstream, and live individuals (n=176) showed a similar significant increase in total weight and in the total weight/length ratio downstream.

EFFECT OF DYMELOR ON EMBRYONIC SURVIVAL. M.E. Dhuyvetter*, J.E. Tilton, R.M. Weigl and M.L. Buchanan. Department of Animal Science, College of Agriculture, NDSU, Fargo, North Dakota.

Eighteen gilts were used to ascertain the influence of an oral hypoglycemic agent (Dymelor) on embryonic survival to 30 day post conception. Gilts were handmated two times, 24 hours apart on the day of estrus and were randomly assigned to a treatment group (4 gm/hd/day for 3 days) on a control group. An initial blood sample was collected prior to treatment with a final blood sample collected at slaughter. Blood glucose levels and glycogen content of liver muscle, fetal and endometrial tissues were analyzed and found similar. Corpora lutea counts were made to determine ovulation rate, number of viable embryos and detectable resorptions were established following excision after slaughter. Also, the mean fetal weights were determined. Ovulation rates were similar but number of embryos for treatment and control groups were 12.1 and 10.5, respectively. Average resorption rate was 0.6 for treatment level and 0.8 in the control group. Percent embryonic mortality was 9.35 for treated females and 23.18 in the controls. Fetal weights were heavier in treatment groups. Data indicate the addition of Dymelor to the diet had a beneficial effect on embryonic survival.

CHEMICAL RECLAMATION FOR SODIC STRIP-MINE SPOILS. E. J. Doering and W. O. Willis. Northern Great Plains Research Center, Agricultural Research Service-USDA, Mandan, N. Dak. 58554

Sodic strip-mine spoil can be chemically reclaimed by leaching with concentrated solutions of highly soluble calcium salts. Sodic soils disperse when treated with low-salt water, and their hydraulic conductivities often are so low that reclamation by leaching is impractical. When treated with high-salt water, however, hydraulic conductivity is established and maintained at a higher level. Laboratory tests showed that hydraulic conductivity for a strip-mine spoil was about 0.001 inch per day with saturated gypsum ($\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$) solution, but exceeded 1.5 inches per day with calcium chloride (CaCl_2) solutions greater than 0.5 normal.

Cation-exchange relationships were used to develop an equation for calculating the amount of CaCl_2 needed to chemically reclaim sodic soils. Chemical reclamation by leaching with high-salt solution is effective but not recommended for large-scale application on strip-mine spoils because of the cost. Rather, a better alternative is to apply a suitable thickness of nonsodic topsoil and subsoil on strip-mine spoils.

PHYTOPLANKTON RESPONSE TO HYDROPHYTE REMOVAL AND NUTRIENT ENRICHMENT IN LAKE METIGOSHE, NORTH DAKOTA. J. A. Downing and J. J. Peterka. Dept. of Zoology, NDSU, Fargo, N. Dak. 58102

Experimental chambers were used to determine the effect of hydrophyte removal and nutrient additions on phytoplankton in Lake Metigoshe. Additions of N as NaNO_3 and P as $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ were made in chambers with hydrophytes and in chambers with hydrophytes removed to determine effects on species and numbers. There were no increases in phytoplankton indicated in chambers containing hydrophytes regardless of nutrient addition. In chambers with hydrophytes removed Chlorophyte plankton increased to a mean volume of $11 \times 10^8 \mu^3 \text{l}^{-1}$ as compared to chambers with hydrophytes at a mean volume of $3 \times 10^8 \mu^3 \text{l}^{-1}$ within 4 days of defoliation. Phytoplankton in control chambers did not deviate significantly from that of the lake for at least 14 days. Supported by North Dakota Water Resources Research Institute grant A-034.

COMPARISON OF NITRATE REDUCTASE ACTIVITY IN SELECTED GRASS SPECIES. J. A. Dusky and D. S. Galitz. Dept. of Botany, North Dakota State Univ., Fargo, N. Dak. 58102

Levels of activity of the enzyme nitrate reductase (NADH-nitrate oxidoreductase E.C. 1.6.6.1) and optimal extraction and assay conditions were determined for eight grass species common to western North Dakota. Optimal pH for the extraction of the enzyme ranged from 7.0 to 9.5 and the assay pH was found to be 7.6 in all eight species. Optimal substrate (NO_3^-) concentrations were between 1.0 and 10.0 mM with high concentrations (100 mM) significantly inhibiting activity. Cofactor (NADH) concentrations were found to be optimal when final reaction mixture concentrations were between 0.05 mg/ml and 0.20 mg/ml. Optimal enzyme concentrations for assay and sulphhydryl (cysteine hydrochloride) concentrations for extraction as well as reaction rate, rate of decay and specific activity were determined for each species. Comparative studies were also made as to the ratio of in vivo to in vitro activity, NADH versus NADPH specificity and whether the addition of BSA (bovine serum albumin) during extraction as a protein protectorant will increase the measurable nitrate reductase activity. The significance of the levels of activity in these species will be discussed.

HORMONAL EFFECT ON THE DEVELOPMENT OF CHLOROPLAST PIGMENTS IN WATER STRESSED WHEAT. M. E. Duysen and T. P. Freeman. Dept. of Botany, N. Dak. State Univ., Fargo, N.D. 58102.

When dark-grown wheat seedlings are subjected to mild water deficits of -10 bars prior to illumination, plastid pigment accumulation is reduced in leaves during greening. Furthermore, the mild stress impairs wheat seedling growth, leaf unfolding, and increases the Chl a/b ratio in chloroplasts of stressed leaf segments. The application of N^6 -benzyladenine (BA) to etiolated stressed leaf sections increased the total chlorophyll (a+b) and carotenoid accumulations per unit leaf area to within 15% of the plastid pigment accumulations in nonstressed leaf sections during greening. BA had little effect on the stress impairment of leaf unfolding or leaf area but BA reduced slightly the Chl a/b ratio. The Chl (a+b) and carotenoid contents per unit leaf area of stressed sections were restored to the level of plastid pigments in nonstressed wheat leaf segments by dark application of BA+Indoleacetic acid (IAA). Leaf growth was unaffected by the combination treatment but the hormones reduced the Chl a/b ratio in stressed leaves.

PHYSICAL CHARACTERIZATION OF REACTIVE METAL SLURRIES PRODUCED BY THE METAL ATOM TECHNIQUE. Howard F. Efner and Kenneth J. Klabunde, Dept. of Chem. University of North Dakota, Grand Forks, N. D. 58202

The co-condensation of magnesium atoms with tetrahydrofuran (THF) or hexane at liquid nitrogen temperatures has been shown to give a highly reactive metal dispersion (K. J. Klabunde, *Et. Al., J. Organometal. Chem.*, **71**, 309, 1974). A detailed study has been made of the physical characteristics of the highly reactive and pyrophoric metal powders formed by the reaction of magnesium, nickel or iron atoms with either coordinating (THF and toluene) or non-coordinating (hexane) solvents. The particle size, average crystallite size, amount of adsorbed solvent, and surface areas have been investigated. Nickel atoms, for example, when deposited in a THF matrix, give a black slurry on warming to room temperature. Solvent removal under reduced pressure leaves nickel metal with a particle size of 0.5 to 2μ , average crystallite size < 100 , Å, and a THF to nickel mole ratio of 1:2 to 1:5. BET surface area measurements were unsuccessful due to structural rearrangements of the metal on solvent desorption. Approximate surface areas based on THF desorption are in the range of 300 to 900 m^2/g . Supported by NSF (grant 42376).

DIGESTIBLE ENERGY AND PROTEIN OF RAT RATIONS SUBSTITUTED WITH LOW LEVELS OF ALFALFA. D.O. Erickson, Gilberto Tenesaca and C.N. Haugse. Dept. of Animal Science, NDSU, Fargo, N.D.

The effects of adding low levels of alfalfa to commercial rat rations on the digestible dry matter and digestible protein were studied. Alfalfa was harvested at 2 physiological stages of maturity (prebloom and full bloom) and added at 3 levels (10, 20 and 30%) to the control commercial ration. Six weanling white rats were used per treatment. The digestible dry matter significantly decreased with additions of full bloom alfalfa ranging from 71% for the control down to 56% for the ration with 30% alfalfa, whereas adding 10, 20 and 30% prebloom alfalfa did not significantly reduce the digestibility. Protein digestibility values were similar for all rations except when 30% full bloom alfalfa was added dropping the digestibility of protein down to 60% compared to 66 to 70% for the other 6 rations. These data along with the rat performance data indicate that low levels of good quality alfalfa can be incorporated into non-ruminant rations.

PRELIMINARY EVIDENCE FOR THE VOLCANIC ORIGIN OF BENTONITE IN THE SENTINEL BUTTE FORMATION, NORTH DAKOTA. N. F. Forsman and F. R. Karner. Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58202.

Optical and X-ray studies show that the major petrographic evidence for the volcanic origin of the "blue bed" bentonite is the occurrence of common volcanic glass fragments (R.I. 1.502), biotite grains, and smectite clay minerals. Three types of glass grains occur: (1) Irregularly shaped grains with many closely spaced spheroidal or ellipsoidal vesicles. (2) Roughly equant to elongate grains containing many, oriented, string-like vesicles which often form wavy fluidal patterns and are oriented parallel to the direction of grain elongation. (3) Elongate grains containing relatively straight tubular vesicles oriented parallel to the direction of grain elongation. These three types of grains show gradational characteristics and may be part of a genetically related series. The evidence suggests that the "blue bed" contains partially altered volcanic ash from a rhyolitic source. Supported by NSF Undergrad. Research Participation Grant GY 11184.

ALTERATIONS IN POSTNATAL DEVELOPMENT OF RAT BRAIN IN RESPONSE TO ZINC DEFICIENCY. Gary J. Fosmire and Harold H. Sandstead. USDA, ARS, Human Nutrition Laboratory, Grand Forks, ND, 58201.

Zinc deficiency during neonatal life has severe adverse effects on growth and maturation of rat brain (Fosmire *et al.*, Ped. Res. 9, 89-93, 1975). The present work has been done to detail how different areas within the brain are affected by zinc deficiency during the first 21 days of postnatal life. Dams were fed a zinc deficient (<1.0 ppm) diet from parturition; supplemental zinc at 25 ppm was provided to the pair fed and ad libitum fed controls in their drinking water. At 6, 11, 16 and 21 days, pups were decapitated, brains were dissected into cerebrum, midbrain and cerebellum and analyzed for DNA, RNA, and protein. Zinc deficient dams displayed anorexia and a cyclical pattern of food intake. Pups from such dams demonstrated growth failure, impaired growth of cerebrum, midbrain, and cerebellum, a reduction in total DNA, RNA, and protein in the three brain regions and a decreased protein/DNA ratio in cerebrum and cerebellum. The above observations are consistent with a decrease in total number of cells in the three regions and a reduction in cell size in cerebrum and cerebellum. There was a variable pattern of response to zinc deficiency between the three regions with the cerebellum being most severely affected.

APPLICATIONS OF GEOLOGY TO STRIP-MINE RECLAMATION. G. H. Groenewold and S. R. Moran, N. Dak. Geol. Survey, Univ. Station, Grand Forks, N. Dak. 58202 and L. A. Hemish, Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dak. 58202

The stated goal of reclamation in North Dakota is the return of strip-mined land to a level of productivity equal to or greater than pre-mining conditions. This is achieved by separation and stockpiling of those materials in the overburden which are best suited for plant growth.

The first step in reclamation is to delineate the three-dimensional distribution of the various lithologic units in the overburden by detailed sampling and geophysical logging of all testholes in the proposed mining area. Combination of the lithologic information thus obtained with chemical analyses of the various materials allows for the delineation of "reclamation units." These units are bodies of material that are defined on the basis of their suitability for soil development and plant growth. The physical and chemical characteristics of a reclamation unit reflect its material composition, its position in the present groundwater flow system and its history relative to the evolution of landscape and groundwater flow during the past 40×10^6 years.

Because the properties of overburden materials vary within and between mining areas detailed study of each area is required to develop the best reclamation plan for that area.

POTENTIAL IMPACT OF LARGE-SCALE STRIP MINING ON GROUNDWATER SUPPLIES IN WESTERN NORTH DAKOTA. G. H. Groenewold and S. R. Moran, N. Dak. Geol. Survey, Univ. Station, Grand Forks, N. Dak. 58202 and C. A. Anderson, Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dak. 58202

Four potential modifications of groundwater supplies may result from large-scale lignite development in western North Dakota. (1) Water levels in shallow wells adjacent to mine excavations will be lowered; some wells may become completely dry. In most instances, this will be a temporary situation which will end once the excavation is filled. (2) Removal of lignite and overburden aquifers may cause permanent disruption of some wells by removing their source of water. (3) Mining is expected to cause changes in the vertical permeability of large areas which will result in either an increase or a decrease in groundwater recharge. This will cause corresponding changes in water levels throughout the part of the basin that is recharged through the mined area. Where water levels decline, wells will be affected; where water levels rise, areas of springs and seeps will increase. (4) Chemical quality of groundwater may decrease as a result of leaching of ions from spoil material or because of leakage of waste-management facilities.

Detailed studies are underway in each area of proposed lignite development to identify which of these impacts are likely to occur and to what extent they can be avoided or controlled.

RETENTION AND RECOVERY OF A HERPESVIRUS FROM NORTH DAKOTA NATURAL WATERS. B. R. Hanson and I. A. Schipper. Dept. of Vet. Sci., N. Dak. State Univ., Fargo, N. Dak. 58102.

Retention and recovery of an attenuated infectious bovine rhinotracheitis (IBR) virus from native waters were studied utilizing cotton and fiberglass filters. The distance traversed by the virus and its time of survival in water were also studied. Following addition of IBR virus to a flowing river, attempts were made to retain the virus in cotton or fiberglass filters located at distances up to one mile from the point of release. Experimental results showed that significant quantities of viable virus can be recovered up to one mile downstream. IBR virus was found to be viable in natural waters for at least 12 h at water temperatures between 5 C and 10 C. Therefore, IBR virus survives in native waters long enough (12 h) for the virus to be carried a significant distance downstream and expose susceptible animals and thereby spread and perpetuate the disease.

A PRELIMINARY STUDY OF THE OAK SAVANNA IN SOUTHEASTERN NORTH DAKOTA. J. D. Hanson and William T. Barker. Dept. of Botany, N. Dak. State University, Fargo, N. Dak. 58102

Ten stands of Quercus macrocarpa have been studied in the savanna of southeastern North Dakota during the 1974 season. This phytosociological study was carried out by use of the point frame, quadrat square and belt transect methods. The point frame was used to determine the basal cover and relative density of grasses, sedges and forbs. It was found that under the canopy the total basal cover ranged from 3.0% to 10.2% with the dominant species being Poa pratensis. In the surrounding prairie the total basal cover ranged from 8.6% to 18.8%. The dominant species of the prairie were Bouteloua gracilis, Poa pratensis and Stipa comata. A one-square foot quadrat was used to measure the number of plants per square foot, the percent frequency and the relative density of forbs and shrubs. The dominant species were Rhus radicans and Symphoricarpos occidentalis. A series of ten belt transects, each 180 square feet, was used in each stand to determine the density and basal area of Quercus macrocarpa. The density ranged from 217.92 to 823.24 trees per acre. The basal area was as much as 31,222.28 square inches per acre.

SEASONAL DISTRIBUTION PATTERNS OF DEER IN SOUTH CENTRAL NORTH DAKOTA. A. K. Harmoning. Dept. of Zoology, NDSU, Fargo, N. Dak. 58102

To determine seasonal distributions of white-tailed deer (Odocoileus virginianus) in the Coteau region, the movements of 19 radio-tagged animals were recorded. These deer were trapped during the winters of 1972-73 and 1973-74 in the Dawson State Game Management Area, south of Dawson, N. Dak. Spatial distributions and ranges of these deer were then measured during subsequent seasons. Distances between the centers of each deer's range were compared with a nearest neighbor technique. Deer differed significantly ($P < 0.05$) from a random distribution during winter and spring. The average nearest neighbor distances for summer closely approximated the distances expected from a random distribution. Distances computed for fall periods, although differing somewhat from distances expected from a random distribution, did not depart significantly. This research was funded by the N. Dak. Game & Fish Dept., NDSU, and Pittman-Robertson funds under project W-67-R-14 and W-67-R-15.

COMPARATIVE DISTRIBUTION OF IMMATURE BITTACOMORPHA CLAVIPES (FABRICIUS) AND PTYCHOPTERA QUADRIFASCIATA SAY (DIPTERA: PTYCHOPTERIDAE) IN SANDHILL STREAMS OF SOUTHEASTERN NORTH DAKOTA. S. C. Harris and R. B. Carlson. Dept. Entomology, N. Dak. St. Univ., Fargo, N. Dak. 58102

From March 1974 to December 1974, samples were collected monthly from eleven sites in two sandhill streams of southeastern North Dakota. Sampling sites were located in either open or shaded stream sections. Both streams were very hard (>280 mg/l CaCO_3) and both had a uniform sand substrate. Bittacomorpha clavipes was generally restricted to the headwaters of the stream where hardness ranged from 400-480 mg/l CaCO_3 . Ptychoptera quadrifasciata were widely distributed in both study streams. Both species were generally restricted to stream margins where water depths did not exceed 5 cm.

A COMPARISON OF BENTHIC FAUNA IN WEIR PONDS OF STREAMS DRAINING DEFORESTED, FERTILIZED, AND FORESTED WATERSHEDS. S. C. Harris and R. B. Carlson. Dept. Entomology, N. Dak. St. Univ., Fargo, N. Dak. 58102 and D. E. Samuel. Dept. of Wildlife Biology, W. Va. Univ., Morgantown, W. Va. 26506.

To assess the effects of various management practices on stream fauna, eleven quantitative samples of benthos were collected monthly in stream weirs of four different watersheds. The watersheds, within the Fernow Experimental Forest, Parsons, West Virginia, were previously treated in one of the following ways: deforested by clearcutting, partially deforested by clearcutting, urea fertilized, forested control. Both water chemistry and stream temperatures differed in the study weirs. The observed taxa in the four weirs were generally similar, but relative importance ratios of individual taxa and standing crops varied. The weirs draining the partially forested and forested control watersheds supported the greatest number of invertebrates; weirs draining the deforested and urea fertilized watersheds supported the lowest. Step wise multiple regression analyses using selected taxa indicated temperature generally had the most influence on numbers of individual invertebrates present in the weirs.

H \rightleftharpoons D EXCHANGE IN SOLID INSULIN DUE TO INTERACTION WITH D₂O VAPOR. W. S. Hnojewyj. Dept. of Physics, College of Sci. and Math., N.D.S.U., Fargo, N. Dak. 58102.

Data on the increase in the original weight of lyophilized solid Insulin (acidic form) are presented for a series of D₂O adsorptions followed by successive high vacuum desorptions. Increase of original weight is assumed to be a result of an exchange of labile Hydrogens in Insulin by Deuterium of D₂O. The maximum of this weight increase (H \rightarrow D effect) is reached when all labile \sim 87 Hydrogen atoms present in the functional groups of the solid Insulin Molecule are exchanged by Deuterium from the interacting D₂O vapor.

GLACIAL STRATIGRAPHY OF EASTERN NORTH DAKOTA. H. C. Hobbs, S. R. Moran, and M. Camara. Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58202

In two separate studies, six lithologically distinct units of glacial sediment have been recognized in eastern North Dakota and traced over an area of thousands of square miles. The upper three units, the Falconer, Dahlen, and Gardar Formations, have been recognized for several years. The present studies have extended the area known to be underlain by these units and have added considerable detailed lithologic information on them. The lower three units had not previously been recognized.

The Falconer Formation is restricted to the Red River Valley; the Dahlen and Gardar Formations are continuously present over eastern North Dakota. The Dahlen Formation is generally five to twenty feet thick over most of the area. The Gardar Formation is tens of feet thick east and west of the Pembina Escarpment and generally five to ten feet thick over the crest of the scarp. The three lower units are discontinuous along the crest of the Pembina Escarpment, being present only as fillings in buried valleys. East and west of the scarp these units are fairly continuous and widespread.

The composition of the upper three formations is strongly related to the underlying lithology. Both the Dahlen and Gardar Formations contain abundant shale along the crest of the Pembina Escarpment where the Gardar rests on shale bedrock over extensive areas. Toward the south and west, where the underlying units of glacial sediment are present, the shale content of the Dahlen and Gardar Formations is considerably lower.

LONGEVITY OF HERPESVIRUS (IBR) IN TYPICAL NORTH DAKOTA LIVESTOCK WATER SOURCES. A. B. Horsager and I. A. Schipper. Dept. of Vet. Sci., N. Dak. State Univ., Fargo, N. Dak. 58102.

Tests for this investigation were performed using infectious bovine rhinotracheitis (IBR) virus in a variety of water samples including distilled water (control), river water, and river water containing straw and manure. Water temperatures were maintained at 37 C, 22 C, and 2 C. One-ml aliquots were withdrawn from each water preparation at seven intervals from initiation through 48 h to test for virus concentration. Initially, all samples contained an average of 110 PFU/ml. After 1 h at the respective temperatures the samples containing straw and manure at 37 C and 22 C had dropped to 5 PFU/ml while the same preparation at 2 C contained 65 PFU/ml. Those samples containing only river water retained 32 PFU/ml at 37 C and 83 PFU/ml at 22 C while both the river water and distilled at 2 C were essentially unchanged. At 1 h only the river water and distilled water at 2 C contained virus, the amounts being 33 PFU/ml and 45 PFU/ml respectively. At 24 h only distilled water showed IBR present, the concentration being 6 PFU/ml. No virus was recovered at 48 h. Conclusions drawn from these preliminary results indicated the viability of IBR virus decreased at a lower rate as water purity increased and water temperature decreased.

LARGE SCALE EVAPORATIVE COOLING. L.D. Howlett, Consulting engineer, Rockford, Ill. 61111

Evaporation cooling systems are used in many industrial applications. Heat is transferred from the circulating water to the ambient air by convection and evaporation. Recent papers by Kolflat and Porter indicate that in some situations, modifications to the basic theory must be made to adequately predict heat dissipation performance. This paper develops a heat transfer model which incorporates the heat loss from the water, the mechanisms of heat transfer, and the heat gained by the ambient air. Calculated results from the model indicate that the capacity of the ambient air to absorb moisture is an important factor in thermal performance predictions at increased values of initial water temperature, water surface area, and convection coefficient and at decreased values of air mass flow.

FLOTATION AND SEDIMENTATION IN TREATING REFINERY WASTES. Yung-Tse Hung. Civil Engr. Dept., Univ. of N. Dak., Grand Forks, N. Dak. 58201

A pilot plant study was conducted to evaluate both coagulation-sedimentation and air flotation methods in removing oil and suspended solids from refinery wastes. Factors affecting oil and suspended solid removals, such as overflow rates, rise rates, recirculation ratios and chemical dosages, were investigated. Experimental results on waste from an API separator indicate that under the condition of optimum overflow rate, rise rate and chemical dosages, oil removal efficiency of 90-90% is attainable by either coagulation-sedimentation or air flotation process. For the coagulation-sedimentation process, the required operating conditions are: 1) an overflow rate of 390 gpd/sq ft (15,890 l/day/sq m); 2) chemical dosages of 2 mg/l polyelectrolyte, 100 mg/l CaCO_3 , and 50 mg/l Ca(OH)_2 . For the air flotation process, the required operating conditions are: 1) a rise rate of 1.9 gpm/sq ft (77.4 l/min/sq m); 2) 25% recycle; 3) chemical dosages of 2 mg/l polyelectrolyte and 50 mg/l Ca(OH)_2 . The selection of whether to use the coagulation-sedimentation or the air flotation process should be based on a cost comparison.

COMPARATIVE STUDIES OF THE WARBURG AND MARIAS METHODS FOR DETERMINING OXYGEN UPTAKE RATE CONSTANTS. Yung-Tse Hung, Civil Eng. Dept., Univ. of N. Dak., Grand Forks, N.Dak.58201
W. Wesley Eckenfelder, Jr. Environ. and Water Resour. Dept., Vanderbilt Univ., Nashville, TN.

The oxygen uptake rate constant is an important factor in determining the waste assimilation capacity of streams. The objectives of this study are: 1) to apply the Marias technique to the measurements of oxygen uptake rate constant (k) in the Houston Ship Channel waters; 2) to determine whether the k and the ultimate biochemical oxygen demand (BOD) values obtained by the Marias method and the Warburg method are comparable. The average k values were found to be 0.092 day^{-1} by the Marias method and 0.22 day^{-1} by the Warburg method. The average BOD values were 5.4 mg/l and 13.7 mg/l by the Marias method and Warburg method respectively. The Warburg method yields both higher k and ultimate BOD values than the Marias method. Correlations between the results obtained by the two methods are rather poor. The results indicate that the Marias method is more suitable for the determination of k values in the low oxygen uptake waters than the Warburg method.

THE USE OF RADIOACTIVE INULIN IN CONTROLLED INTESTINAL PERFUSION STUDIES. F.A. Jacobs and Carol Overvold, Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, ND 58202

Since the polysaccharide inulin is not diffused or transported across the biological membrane, it lends itself to the measurement of net water exchange within the confines of a given aqueous system. Our current perfusion studies differ from earlier studies in several ways: 1) inulin- ^3H is used instead of inulin- ^{14}C ; 2) the system is a recirculating one, rather than a single-pass system; 3) rats are not fasted, but are fed a complete liquid diet up to the time of perfusion; 4) the perfusate passes through a cannulated segment of the jejunum rather than the duodeno-jejunal area as in previous experiments. Since our measurement of amino acid absorption from the intestinal lumen depends upon a monitored flow system, ^{14}C -labelled amino acids are perfused for such measurements. The water exchange studies are made using inulin- ^3H contained in the same perfusate, and measured by liquid scintillation counting. Thus by the use of two β -emitting isotopes we measure amino acid absorption and evaluate net water exchange simultaneously, hence, allowing more direct control of the experimental conditions. (Supported in part by USPHS, NIH Research Grant MH 19235 ALC and an Institutional Grant No. 5 S01 RR05407, for General Research Support).

BAYESIAN STATISTICAL TECHNIQUES•USEFUL IN ESTIMATING FREQUENCY AND DENSITY. Douglas H. Johnson, Northern Prairie Wildlife Research Center, Jamestown, N. D. 58401

This paper presents some elementary applications of the Bayesian philosophy of statistics to problems faced by biologists and other scientists using counts of organisms. In particular, we indicate how the Bayesian approach can be used to sharpen inferences drawn from random samples of counts. For frequency of occurrence data, Bayesian confidence limits are shown to be generally superior to classical confidence limits. Frequency data can also be used to estimate population density if the species is sparsely distributed relative to the size of the sample plot.

For species more densely distributed, we develop confidence limits based on the normal distribution that employ the prior knowledge that the density is non-negative. This method insures that the lower confidence limit for a quantity--known to be positive--is non-negative. Bayesian limits are superior to classical confidence limits if the sample mean is small relative to its standard error. We briefly indicate how counts performed in earlier years can be used to sharpen inferences from a new sample.

GLYCOLATED PELLETS FOR SIMULTANEOUS X-RAY ANALYSIS OF CLAY AND NON-CLAY MINERALS. F. R. Karner and F. D. Wosick. Dept. of Geol. Univ. N. Dak., Grand Forks, N. Dak. 58202.

In this procedure a single diffraction analysis is used to determine the mineral content of clay-bearing materials. Qualitative, semi-quantitative, or quantitative results can be obtained. The method will probably be most useful for routine analysis of large numbers of samples augmented by special studies of specific mineral phases present. A one gram, ground sample of the whole rock or sediment is formed into a cellulose-jacketed pellet with a hydraulic press. The powder forming the upper surface of the pellet is initially saturated with ethylene glycol to form a slurry which is then compacted in the press producing a smooth upper surface with preferred orientation of clay minerals and glycolation of minerals of the smectite group. Sample preparation time is about 10-15 minutes. In a trial using two samples with approximately 80 percent and 40 percent clay minerals, the x-ray peak heights of the basal clay reflections were 3-4 times those of standard powder mounts while the peaks of non-clay minerals were reduced about 30 percent. Basal clay peaks for the 80 percent clay sample are about one-half the intensity obtained from clay-fraction mounts prepared by sedimentation on glass slides. Supported by NSF Undergrad. Research Participation Grant GY 11184.

EFFECTS OF INOCULATION POINT ON SORUS LOCATION IN BARLEY COVERED SMUT. R.L. Kiesling, Dept. of Pl. Path., Col. of Agric., N.D. State Univ., Fargo, N. Dak. 58102

Kernels of Odessa barley (C.I. 934) were dehulled, steeped in tap water for 2 hr and pregerminated for 24 hr at 20°C. Teliospores of race 6, Ustilago hordei, were placed on the base, middle, tip or over the entire surface of the coleoptile after pregermination. Inoculated seedlings were incubated for 24 hr at 20°C or 24°C and then planted in greenhouses held at the same temperatures as their respective incubations. The smut sorus production in the top four leaves, the number of plants infected and the number of seedlings killed were highest with basal inoculation and growth at 24°C. Smut sori developed in the top three leaves with middle inoculation and growth at 24°C. Inoculation over the entire coleoptile and growth at 24°C produced sori in the upper two leaves. Tip inoculation at 24°C produced no infected plants. Sori were produced only in flag leaves in the 20°C trials. The number of plants with sori in all tillers was highest with inoculation over the entire coleoptile and growth at 20°C. Inoculation in the middle of the coleoptiles and growth at 20°C produced the largest number of plants with sori in some but not all tillers.

PHOTOLYSIS OF METHYL 2-BENZIMIDAZOLECARBAMATE IN SUNLIGHT. H. M. Lacy and J. R. Fleeker, Dept. of Biochem., N. Dak. State Univ., Fargo, N. Dak. 58102.

The photolysis of the fungicide methyl 2-benzimidazole-carbamate (MBC) was investigated. Exposure of MBC-2-¹⁴C-C³H₃ in water (2 µg/ml) to sunlight for 16 hrs resulted in a loss of 5-10% of the fungicide. Similar solutions containing in addition riboflavin (5 mg/l), lost 80-90% of the MBC after 5 hrs in sunlight. The major radioactive photolysis products retained both ³H and ¹⁴C in the same ratio as in the parent compound. Carbomethoxyguanidine was one of the degradation products. No loss of ³H or ¹⁴C occurred when the radioisotopically labeled fungicide was streaked on silica gel TLC plates (5 µg/cm²) and exposed to sunlight 5 hrs each day for 6 days. However, 10-15% of the isotope label did not chromatograph as MBC. The exposure to sunlight was during the months of June and July.

THE POTAMOGETONACEAE IN NORTH DAKOTA. Gary E. Larson. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak. 58102

A study of the pondweed genus Potamogeton in 1973 and 1974 revealed that fourteen species occur in North Dakota. Included are the broad-leaved species P. amplifolius, P. gramineus, P. natans, P. nodosus, P. praelongus, P. richardsonii and P. spirillus, and the narrow-leaved species P. filiformis, P. foliosus, P. friesii, P. pectinatus, P. pusillus, P. vaginatus and P. zosteriformis. Of these species, P. filiformis represents a new record for the state. To facilitate identification of North Dakota species a diagnostic key has been devised utilizing such criteria as presence or absence of floating leaves, achene size and shape, leaf shape and venation and stipular characteristics. Analysis of prime characters showed that North Dakota plants deviate little from previous descriptions. As a result of summer field collecting and herbarium research, much information has accumulated concerning the distribution and habitat preferences of Potamogeton species in North Dakota. Water factors such as permanence, salinity, turbulence, depth and turbidity dictate the ranges of the species.

THE HEAT VALUE OF VARIOUS AGRICULTURAL CROP RESIDUES AND SEWAGE SLUDGE COMPOST. Jacob L. LaRue. Sophomore in Ag. Engr. at North Dakota State University, Fargo, ND 58102.

The fuel shortage that developed when the Arabian oil embargo was put into effect has emphasized the fact that alternate fuel sources will probably be needed within fifty years. Agricultural crop residues, livestock manure and sewage compost are renewable organic wastes that may adapt to this need. The objective of this experiment was to determine the heat value of several agricultural crop residues and sewage compost treated in different methods. These data were then compared with standard accepted values of known heat sources. Test methods used were based on the principles of thermal calorimetry. The apparatus used was an Emerson fuel calorimeter with an adiabatic jacket. The heat values determined were as follows: wheat straw 7600, flax straw 8100, sunflower hulls 8100, and sunflower stalks 8500 btu's per pound. Lignite coal contains about 6580 btu's per pound. Results for the sewage sludge compost ranged from 220 to 7600 btu's per pound. Mineral matter accounts for a large amount of this variation.

THE DESIGN OF A NEW METHOD FOR OBTAINING USEFUL POWER FROM THE WIND. R.S. Majkrzak. Dept. of Mechanical Engr., Univ. N. Dak., Grand Forks, N. Dak. 58201

The recent emergence of the "energy crisis" to public prominence has spurred the desire for a new form of power to provide energy for the world's needs and which would leave the environment intact. One candidate for such an alternate form of energy is the wind, and a new concept for obtaining this is discussed.

The idea arose to adapt or modify some existing water turbine to run on air, and the Banki water turbine was chosen for this. The Banki turbine is unique in that the working fluid flow is both inward and outward, which permits the fluid to impart two impulses rather than one during its passage through the rotor. It was further decided to mount the rotor on a vertical axis and to surround the rotor with a series of identical nozzles. This would give the device the much desired characteristic of being independent of wind direction, and also permits the rotor to be shielded from adverse weather such as sleet or hail.

A working model of this concept was built and tested in a wind tunnel, with power output measured by a small Prony brake. The results from the model tests were used to predict the performance of a large scale device, employing standard dimensionless numbers from the turbomachinery field.

THE EFFECT OF 6 DAY EXPOSURE TO NORMOXIC AND HYPEROXIC-HYPERBARIC ENVIRONMENTS UP TO 40 ATA UPON THE GROWTH AND TISSUE WATER CONTENT IN GUINEA PIGS. M. J. Mason, G. Kosel, and T. K. Akers. Dept. of Phys. and Pharm., Univ. N. Dak., Grand Forks, N. Dak. 58202.

Exposure to hyperbaric-hyperoxic environments has been reported to alter body weight. Growing guinea pigs were exposed to 20 and 40 ATA with pO_2 levels of 200 and 600 mm Hg for 2, 4, and 6 days, and compared with air controls. Body weight and individual tissue water content were recorded for each animal. The control group of animals averaged gains in body weight while the treated animals all lost weight. The life span of treated animals was significantly shortened. Lungs, kidneys, and adrenal glands of the treated animals showed alterations in water content after treatment while the same treatment did not alter the water content in skin, muscle, bone, brain, blood, and heart. Supported by ONR (Contract No. N00014-68-A-0499).

DROSOPHILA ASSAY OF FRACTIONATED CRUDE PLANT PART EXTRACTS FOR BIOLOGICAL ACTIVITY. Marvin P. Mattson, L. J. Schermeister and P. C. Sandal. Dept. of Agron., N.D. State Univ., Fargo, N.D.

Drosophila assay of 651 crude plant part extracts revealed 42% expressing biological activity.

Fifty Silica Gel Column separated fractions of each of five selected crude ethanol extracts gave the following results: Cynoglossum officinale leaves, crude extract prevented fly emergence; among fractions, four were toxic, two reduced progeny size and one caused death of flies on emergence: Fumaria officinalis leaves, crude extract delayed development time and interfered with metamorphosis; among fractions, eight accelerated emergence by one to two days: Eupatorium perfoliatum flowers, crude extract was toxic to adults placed on medium; among fractions, five accelerated fly development and one was toxic: Astragalus tenellus fruit, crude extract killed throughout the larval and pupal stages; among fractions, three delayed development time but did not cause death: Asclepias speciosa leaves, crude extract biologically inactive; fractions biologically inactive. Secondary metabolites present in all crude extracts were flavanones, coumarins, flavonoids, glycosides, alkaloids, tannins and amines.

RESPONSE OF HEPATIC PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) TO Fe^{2+} ACTIVATION AND QUINOLINIC ACID (QA) INHIBITION. J.R. Maxwell and P.D. Ray. Biochem. Dept., U.N.D., Grand Forks, ND 58202

Tryptophan (TRY) given to intact normal rats inhibits gluconeogenesis (GNG) and increases in vitro activity (ACT) of PEPCK. QA elicits like responses in perfused normal rat livers. As these effects cannot be shown in intact diabetic rats or their isolated livers, a basic regulatory mechanism may be lost. The elevated ACT of PEPCK of TRY-treated normal rats decays toward normal levels on storage; the naturally elevated ACT of PEPCK from diabetic rats does not. PEPCK from normal rats is activated in vitro by Fe^{2+} ; activated enzyme is inhibited by QA. PEPCK from TRY-treated rats is not Fe^{2+} -activated initially, but sensitivity to Fe^{2+} returns as enzyme ACT decays. PEPCK from diabetic rats is insensitive to TRY in vivo but does respond to Fe^{2+} and QA. GNG in perfused guinea pig livers is partially inhibited by QA; guinea pig cytosolic (C_s) PEPCK is Fe^{2+} -activated and the activated enzyme inhibited by QA. GNG in perfused rabbit and pigeon livers is not inhibited by QA; yet their PEPCK's are Fe^{2+} -activated although the activated enzymes are not inhibited by QA. QA-induced inhibition of GNG in perfused livers may be correlated with inhibition of Fe^{2+} -activated C_s PEPCK in vitro. However, a discrepancy exists in diabetic rats where QA does not inhibit GNG in perfused livers but does inhibit the Fe^{2+} -activated enzyme. (Support: NIH AM12705)

PIONEER VEGETATION ON VARIOUS TREATMENTS OF COAL OVERBURDEN.

Jon Morken and Warren C. Whitman. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak. 58102

Experimental plots in three replications have been established on 4 open pit mines in Mercer and Oliver counties in North Dakota. Within each replication treatments of leveled raw spoil material, 2" of topsoil over spoil material, and 12" topsoil have been selected. On Oct. 23 of 1973 the plots were disked and fertilized with 100 lbs P per acre. A mixture of common weed seeds was broadcast by hand and disked in at a rate of about 12 lbs per acre. In the fall of 1974 native grass seed was introduced into the weed mulch and the feasibility of establishing stands of native grasses by this method will be evaluated. Success of the pioneer weed species will be evaluated. Their success was determined by plant counts, plant heights, yield clippings, and root penetration studies. Sampling during the summer of 1974 resulted in differences in plant responses not only between treatments of 0", 2", and 12" topsoil, but also between the types of the spoil material on which the trials were placed. (Example: Kochia scoparius measured July 9, 1974 achieved an avg. height of 2.23" on raw spoils at one site and at the same time it was an avg. 6.32" at another mine site. On the same two mine sites the 12" topsoil treatments with K. scoparius avg. 6.84" and 12.84", respectively.)

PREPARATION AND REACTIONS OF A Ni/THF SLURRY. T. O. Murdock and K. J. Klabunde. Dept. of Chem., Univ. N. Dak., Grand Forks, N. Dak. 58202

An active form of nickel has been prepared by the cocondensation of tetrahydrofuran with nickel vapor (atoms) on the walls of a reactor at -196°C. A finely divided, black nickel slurry is formed on warmup, and is then reacted with an alkyl or aryl halide. The reaction of the nickel slurry with 2-iodopropane at 50 to 55°C for 24 hours yielded a 2:1 ratio of propane to propene in an overall yield of 25.9%; 3-iodopropene yields propene (75.8%), propane (3.1%), 1,5-hexadiene (13.0%) and an unidentified product (8.1%) in an overall yield of 96.9%; benzyl chloride gives a 42% yield of bibenzyl and a small amount of toluene. Numerous comparisons of this active form of nickel with the Raney form have been carried out. For example, catalytic asymmetric hydrogenation of acetophenone using the active Ni/THF slurry with L(+)-valine yielded the corresponding alcohol which had a small rotation. The results are similar to those obtained for the identical procedure using Raney Ni. (Angew. Chem. I. E., 10, 871. 1974). The rate of hydrogenation of olefins and aromatic compounds is currently under investigation comparing the Ni/THF catalyst to Raney Ni and a commercial hydrogenation catalyst. (Supported by NSF-42376 and an NSF Energy Related traineeship to T. O. M.)

INHIBITION OF TOBACCO EXTRACELLULAR NUCLEASE BY ATP AND OTHER NUCLEOTIDES. Arland E. Oleson. Dept. of Biochem., North Dakota State Univ., Fargo, N. Dak. 58102.

Suspension-cultured tobacco cells release into the growth medium a sugar-unspecific nuclease that hydrolyzes RNA, DNA and 3'-nucleotides at pH 5-7 (Oleson, Janski and Clark, *Biochim. Biophys. Acta* 366:89, 1974). A study of the effects of several nucleotides on the monoesterase and diesterase activities of this enzyme revealed that this phosphohydrolase is strongly inhibited by purine nucleoside 5'-di- and triphosphates. Approx. 80% inhibition of the DNase activity is produced by 5 μ M ADP, ATP, dATP or GTP. In contrast, 150 μ M AMP is required to produce this level of inhibition. High concentrations of some other nucleotides, P_i and PP_i were also inhibitory. 3':5' cAMP caused less than 10% inhibition at a concentration of 50 μ M. The inhibition of the DNase activity by ATP was found to be competitive in nature, with a K_i of approx. 0.5 μ M.

THE PURIFICATION OF LINDANE FROM BENZENEHEXACHLORIDE. Richard Payfer and Virgil I. Stenberg, Dept. of Chem., Univ. N. Dak., Grand Forks, N. Dak. 58202

The results of a series of experiments on the separation of the γ -isomer, 1,2,3,4,5,6-hexachlorocyclohexane (Lindane), from a mixture of isomers (benzenehexachloride) resulting from the photochlorination of benzene is described. The final separation involves the preferential extraction of the γ -isomer from the solid mixture with methanol held at constant temperature. After filtration, the mixture is allowed to cool and crystallize to give crystals of 61-70% purity of the γ -isomer. Recrystallization from methanol gives the final product in a nearly pure state.

XENOBIOTIC STIMULATION OF ASCORBIC ACID EXCRETION IN THE RAT.

R. L. Puyear, J. L. Sell, K. L. Davison and P. K. Yang, Depts. of Zoology and Animal Science, NDSU and USDA, ARS, Fargo, N.D. 58102

Experiments were performed to determine the relative importance of glucose as a precursor to ascorbic acid in the rat. The xenobiotics used in this study are known to stimulate gluconeogenesis and all but endrin have been observed to elevate urinary ascorbic acid excretion. Xenobiotics, o,p'-DDT (500 ppm), barbital (1000 ppm), heptachlor (40 ppm) and endrin (10 ppm) were fed to rats for 8 days. Urinary ascorbic acid values for a 24 hr collection period were: (1) control:2.2 mg, (2) o,p'-DDT:9.2 mg, (3) barbital:14.6 mg, (4) heptachlor:11.2 mg, (5) endrin:11.2 mg. Glucose-U-14C (4uc/4.2 mmole) was administered to each rat P.O. Radioactivity of the 24 hr urine sample was measured by liquid scintillation spectrometry. The recovery (in millions) of radioactivity in the urine was as follows: (1) control:3.7, (2) o,p'-DDT:3.5, (3) barbital:3.6, (4) heptachlor:3.6 and (5) endrin:2.5. Thin layer chromatography revealed that most of the radioactivity was in ascorbic acid. Specific activities (in millions) of urinary ascorbic acid for the various experimental groups are: (1) control:1.5, (2) o,p'-DDT:0.37, (3) barbital:0.25, (4) heptachlor:0.32 and (5) endrin:3.25. These data support the hypothesis that glucose is not the sole precursor of ascorbic acid in the rat.

RESISTANCE TO NEONATAL ZINC DEFICIENCY IN AN UNDESIGNATED STRAIN OF MICE. B.L. Reis and G.W. Evans. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

For the purpose of immunological studies, mice from the well-known C57BL/6J strain, and another undesignated, private strain (temporarily known as SM, "Super Mouse") were obtained. Pregnant dams previously maintained on Purina Laboratory Chow were placed on a zinc deficient diet (deionized H₂O, sprayed egg white diet <1 ppm - Zn) after parturition. Dams and litters were housed in cages that were thoroughly washed and deionized to eliminate traces of any metal and plastic grids were employed to elevate the cage bottom thus avoiding coprophagy. Nursing neonates of the C57BL/6J strain began showing symptoms of deficiency (i.e., weight loss, retarded development, lack of hair growth) by day 8 and were dead between days 11 and 12. Nursing neonates of the undesignated strain (dam being maintained on a zinc deficient diet) reached weanling age, 30 days, with no apparent symptoms of zinc deficiency. The experimental evidence strongly suggests a genetic related response, and various crosses are presently being studied to determine if this is a nuclear or cytoplasmic phenomenon. To date, it is not known if the observed anomaly in the undesignated strain is a primary zinc response or a secondary zinc response due to altered calcium metabolism.

THE PREPARATION OF METHYL SUBSTITUTED-TRIHAPTO-BENZYL PALLADIUM CHLORIDE EMPLOYING THE METAL ATOM TECHNIQUE. J. S. Roberts and K. J. Klabunde. Dept. of Chemistry, Univ. N. Dak., Grand Forks, N. Dak. 58202

We have recently reported the synthesis of benzyl palladium chloride [J. Organomet. Chem., 85, C13 (1975)]. The preparation of a number of methyl and trifluoromethyl benzyl palladium chloride compounds was undertaken in order to more conclusively prove the allylic nature of the benzyl-palladium bond. These compounds were prepared by the cocondensation of palladium atoms with a suitably substituted benzyl chloride at -196°C . The system is under a high vacuum. The palladium atoms were produced by resistively heating an aluminum oxide coated tungsten crucible which contained palladium metal. The compounds which were prepared in this manner are : 4-methyl benzyl palladium chloride, 3,4-dimethyl benzyl palladium chloride and α -trifluoromethyl benzyl palladium chloride. The bonding in these compounds appears to be allylic from NMR and UV data. These compounds are only slightly air and water sensitive. The use of 2-methyl benzyl chloride and 3-methyl benzyl chloride resulted in the exclusive production of palladium dichloride. This work was supported by NSF grant #42376.

POSTNATAL CHANGES IN THE CAT HEART. Brian K. Ross and Edwin W. House. Dept. Physiol. & Pharmacol., Univ. No. Dak., Grand Forks 58201, and Dept. Biology, Idaho State Univ., Pocatello 83201.

Analyses of age-related changes in dimension, tensile strength and compliance were undertaken on a total of 18 newborn (less than 72 hours old) and 30 adult domestic cats. There was no significant difference in the free-wall thickness of either the left or right ventricle of the newborn, while in the adult the left ventricular free-wall was significantly thicker than that of the right. The left ventricular free-wall showed a marked hypertrophy with age. In contrast, the adult right ventricular free-wall was not significantly thicker than that of the newborn. There were no differences in the tensile strengths of newborn left and right ventricular tissues, while the adult tissues showed marked differences. The compliance of the newborn left and right ventricles was not significantly different. In addition, the compliance of both ventricles did not change with increased pressure. The compliance of the adult right ventricle was greater than the left and the compliance of both ventricles decreased with increased pressure, the left ventricle showing the greater change.

COMPOSITION STUDIES OF BISMUTH TELLURIUM SULFIDE CRYSTALS. Ken Severson. Dept. of Physics, Univ. of N. Dak., Grand Forks, N. Dak.

Single crystals grown by the Bridgman technique from an original melt composition $\text{Bi}_8\text{Te}_7\text{S}_5$ do not have a uniform composition through the length of the crystal. The purpose of this investigation was to determine the magnitude of the composition gradient by two methods, X-ray fluorescence and density measurements. The results show that the bottom of a crystal, where solidification first occurs, has a composition of approximately $\text{Bi}_8\text{Te}_6.5\text{S}_5.5$. The middle and the top of a crystal tend to be closer to the melt composition. The very last part of the crystal to form does not have the same characteristics as the rest of the crystal. This portion was found to be very rich in tellurium, but low in sulfur. Work supported by National Science Foundation Grant No. GH-34561.

EFFECT OF VITAMIN A ON ZINC MOBILIZATION FROM CHICK LIVER. Mamduh Sifri and J. L. Sell. Animal Science Dept., N. Dak. State Univ., Fargo, N. Dak. 58102

Newly hatched White Leghorn chicks were fed a semi-purified diet (low in zinc and devoid of vitamin A) until 8 days of age. After this zinc- and vitamin A-depletion period, triplicate groups of ten chicks each were randomly assigned to four ration treatments; Basal (no vitamin A, 10 ppm Zn), Basal + 6000 IU vitamin A/kg, Basal + 70 ppm Zn and Basal + 6000 IU vitamin A + 70 ppm Zn. Deionized water and the rations were given ad libitum during the 21 day trial. Added Zn significantly ($P \leq 0.05$) reduced the concentration and quantity of vitamin A in liver. These results agree with previous reports that Zn was important for mobilization of vitamin A from livers of rats (Science 181:954, 1973 and J. Lab. Clin. Med. 84:692, 1974). In addition, it was observed in the current study that vitamin A supplementation of chick diets significantly ($P \leq 0.01$) decreased the concentration and total amount of zinc in liver, indicating that vitamin A enhanced the mobilization of and/or decreased the storage of Zn in the liver of chicks.

INFLUENCE OF ORALLY ADMINISTERED FLUIDS ON THE GASTROINTESTINAL ABSORPTION OF A NON-POLAR COMPOUND. C. B. Struble. Dept. of Zoology, NDSU and USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota

This study tested the influence of three dosing fluids (Triolein, Homogenized Whole Milk, and Distilled H₂O) on the gastrointestinal (GI) absorption of the non-polar insecticide carbaryl, [1-naphthyl-N-methyl (carbamate ¹⁴C)]. Carbaryl was incorporated into each dosing fluid (1X10⁻⁴M) and 7 gm. of the fluid was administered directly into the stomachs of fasted (24 hr.) rats. Two hours after dosing, the rats were sacrificed and ligatures were placed at the pyloric and ileocecal junctions. The luminal contents of the sections were separated, H₂O added, the mixture extracted with benzene, and assayed for [¹⁴C]. Benzene extracts were co-chromatographed with carbaryl. Maximum absorption, [(Total ¹⁴C dose)-(¹⁴C in benzene extract of lumen)/Total ¹⁴C dose] X 100, and minimum absorption, [(Total ¹⁴C dose)-(Total ¹⁴C in lumen)/Total ¹⁴C dose] X 100, values obtained were: Triolein 37.8 ± 1.5 to 25.8 ± 0.4; Milk 66.8 ± 16.4 to 43.7 ± 16.7; H₂O 99.2 ± 0.4 to 68.6 ± 68.6 ± 1.7. It appeared that the lipophilic nature of the dosing fluid may influence GI absorption directly. An indirect influence may result from altered gastric emptying rates.

AN AUTOMATIC METHOD FOR DETERMINATION OF TOTAL POLYMERIZATION TIME. Warren D. Sundet. Dept. of Elec. Engr., NDSU, Fargo, N. Dak. 58102

The time interval involved with the copolymerization of acrylamide and bisacrylamide in the presence of oxygen, has been found to have a direct relationship with the amount of oxygen in the sample. The accurate measurement of this time interval thus becomes very important. Previously, a manual method of mixing has been employed. With this method, the reactants were mixed by simply tipping the reaction vessel and observing with the naked eye the end point of the reaction. This paper deals with an automatic method of mixing and end point detection whereby, once the reactants are initially mixed, no more manual manipulations are involved. It also includes a practical application for determination of oxygen content in blood.

PERFORMANCE OF RATS FED VARIOUS LEVELS OF ALFALFA. Gilberto Tenesaca, D.O. Erickson and C.N. Haugse. Dept. of Animal Science, NDSU, Fargo, N.D.

White rats weighing an average of 84.33 g were used in an experimental design of 2x3 factorial to determine the rate of gain and feed efficiency when alfalfa cut at two stages (vegetative and full bloom) was fed at 5, 10 and 15% of the commercial rat chow. The experimental units were kept individually and feed and water were supplied ad libitum. Rats weights and feed consumption were recorded. Two trials of seven days each were conducted during this experiment. In the first trial, there were no differences in gain among control, vegetative and full bloom alfalfa, (45 g, 44.61 g and 45.67 g respectively). In the same trial the feed efficiency among control and 5% of either vegetative or full bloom alfalfa were similar, however when rats fed higher levels of alfalfa the feed efficiency decreased significantly. The data from second trial supports our findings in the first. These findings indicate that rats (non-ruminants) can be fed low levels of high quality alfalfa.

TOXIC CONCENTRATIONS OF NARCOTICS AND RELATED AGENTS WITH EVIDENCE FOR TOLERANCE IN PARAMECIUM SP. Michael Tofano, B. De Boer, and K.O. De Boer. Dept. Physiol. & Pharm., University of North Dakota, Grand Forks 58202.

Effects of several drugs were studied using mixed cultures of Paramecium sp. and rotifers. Other ciliate and flagellates were present in small numbers in some cultures. Long-term effects were observed by adding small amounts of the drug to cultures and observing them for time intervals up to 1-2 weeks. Toxicity was studied using a hanging drop of culture medium to which the appropriate concentration of the drug was added. The immediate effects of opiates was an increased activity in the cultures followed by depression of activity. Methadone was lethal in concentrations of approximately 0.05 mg/ml as compared to 2.5 mg/ml for meperidine HCl; 10.0 mg/ml of morphine SO₄ and 12.5 mg/ml of codeine SO₄. Rotifers were capable of withstanding doses greater than those used for Paramecium for each drug tested. Attempts to demonstrate the development of tolerance and cross tolerance were inconclusive. (Supported by NIH Training Grant No. 5 T01 05939-02.)

PALEONTOLOGY OF BRACKISH-WATER FAUNAS IN TWO TONGUES OF THE CANNONBALL FORMATION (PALEOCENE, DANIAN), SLOPE AND GOLDEN VALLEY COUNTIES, SOUTHWESTERN NORTH DAKOTA. J. B. Van Alstine. Dept. of Geol., Univ. of Minn., Morris, Minn. 56267

In June and July, 1972, four stratigraphic sections containing the Cannonball and Ludlow Formations were measured and described in Slope and Golden Valley Counties, North Dakota. The Cannonball consists of two tongues, in the upper part of the Ludlow (perhaps the Lebo Member), separated stratigraphically by about 30 m of Ludlow. The biota of the upper tongue of the Cannonball consists of three bivalves (Corbicula berthoudi?, Corbula (Bicorbula) subtrigonalis, and Crassostrea glabra) and the trace fossil Ophiomorpha. The biota of the lower tongue consists of two foraminiferids (Trochammina sp. and ?Haplophragmoides sp.) and three bivalves (Corbicula berthoudi?, Corbula (Bicorbula) subtrigonalis and ?Ostrea sp.). The Ludlow biota consists of 3 gastropods (Viviparus sp., Goniobasis cf. G. tenuicarinata, and ?Goniobasis sp.), 2 ostracods (Candona sp. and Ilyocypris sp.), and 1 charophyte (Sphaerochara sp.). No species are in common with the two formations, and only two species are in common with the two Cannonball tongues. The ostracods and the charophyte in the Ludlow, and the foraminiferids, ?Ostrea sp., and Ophiomorpha in the Cannonball tongues are here newly reported. ?Ostrea sp. is newly reported for the entire Cannonball formation.

Attempts to Eliminate Latent Herpes Simplex Virus by Chemical and Immunological Methods. J. Varani and J.J.Kelleher. Univ. of N. D., Grand Forks, ND 58201.

A latent herpes simplex virus infection was established in cultured rabbit kidney cells following preincubation of the virus-infected cells at 41C. Attempts were made to eliminate virus from the latent infection by treatment of the infected cells with various chemical agents, including 2-deoxyglucose, neutral red, acridine orange and primaquine. Treatment of cultures with 2-deoxyglucose apparently failed to affect latent virus. Virus was recovered from the same proportion of treated cultures as control cultures. Treatment of cultures with the two photosensitizing dyes, neutral red and acridine orange, and exposure to visible light, also failed to eliminate the latent virus. In fact, virus was recovered from a significantly higher proportion of the acridine orange-treated cultures than control cultures ($P < .01$). However, treatment of cultures with primaquine did reduce the rate of virus recovery by a significant amount ($P < .01$). Finally, attempts were made to eliminate latent virus by treatment of the cultures with high titer antiserum to herpes simplex virus and complement. This treatment failed to affect the latent virus and virus was recovered from the same proportion of treated cultures as control cultures.

GLUTATHIONE REDUCTASE AS AN INDEX OF RIBOFLAVIN (VITAMIN B₂) NUTRITURE. K.P. Vo-Khactu, R.H. Clayburgh, D.D. Hintz and R.L. Sims. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

Glutathione reductase (GR) is a vitamin B₂ dependent enzyme. Therefore the B₂ status can be determined by monitoring the in vitro stimulation of erythrocyte GR by the B₂ derivative FAD. Weanling male rats were divided into two groups: one was fed a diet deficient in B₂, while the other (controls) was fed the same diet fortified with B₂. After two weeks, the deficient animals were given B₂ for complete repletion. The enzyme reactions were monitored continuously to insure greater linearity. An activity coefficient (AC) is used as index for assessing vitamin B₂ status. AC value is defined as the ratio of the change in absorbance with FAD to the change in absorbance without FAD. The AC of the B₂ deficient group increased significantly ($P < .001$) from 1.13 ± 0.04 to 1.63 ± 0.13 on the 4th day. Following repletion on the 14th day, their AC decreased from 2.54 ± 0.31 to 1.3 ± 0.08 within 3 days. The mean control AC was 1.20 ± 0.12 .

This method has been automated in our laboratory. Automation was accomplished in triplicate, by use of the Diclan 240, where exacting hand procedures can be reproduced. It is therefore significant in application for nutritional survey and hospital routine.

GLUTAMIC OXALACETIC TRANSAMINASE (L-ASPARTATE: 2-OXOGLUTARATE AMINO TRANSFERASE E.C.2.6.1.1.) AS AN INDEX OF PYRIDOXIN (B₆) STATUS. K.P. Vo-Khactu and R.L. Sims. USDA, ARS, Human Nutrition Laboratory, Grand Forks, North Dakota, 58201.

We have reported (American Institute of Nutrition, April, 1975) on an in vitro stimulation of aspartate amino transferase (GOT) for assessing vitamin B₆ status. The activity of this enzyme has been somewhat unsuccessfully used by others as an index of B₆ status. We have found the activity coefficient (AC) of the GOT to be a reliable index of B₆ nutriture in rats. The change in absorbance when the enzyme was stimulated divided by the change in absorbance without stimulation represents the AC value. Animals were fed a B₆ deficient diet, while their controls were fed the same diet fortified with B₆. Weight gain was measured daily and found different ($P < .05$) between the two groups only on the 26th day. In contrast, the AC for GOT was significantly different ($P < .001$) between B₆ deficient and control animals on day 4. On the 26th day of the study, the deficient rats were given vitamin B₆ for complete repletion. The AC decreased from $2.19 \pm .09$ to $1.18 \pm .07$ in 2 days. The control AC values were $1.07 \pm .06$ throughout the experiment.

Other methods of assessing vitamin B₁ (transketolase) and vitamin B₂ (glutathione reductase) do not interfere with the assessment of vitamin B₆.

Repression of Biotin Transport in *Lactobacillus plantarum*. James R. Waller and David A. Gabrielson. Depart. of Microbiology, Univ. of North Dakota, Grand Forks, ND 58202.

Non-proliferating cells of *Lactobacillus plantarum* accumulate biotin to high levels by an active transport process, and to a level approx ten-fold lower by a non-active, mediated system. Part of the transport system is under metabolic-control since growth of *L. plantarum* in media containing excess biotin (6 ng/ml) causes a 90% reduction in transport activity. If cells grown in low biotin media are transferred to high biotin media under conditions allowing cell growth, the transport ability decreases with increasing growth. If cells with a maximum transport activity were allowed to initiate growth in a high biotin medium, transport activity dropped to 80%. After these cells doubled in number, they had only 40% of the original activity. Thus, doubling the cell population resulted in a halving of transport activity. If cells possessing maximum activity were incubated in high biotin at cell levels allowing little growth, only a 15% decrease in transport activity occurred. These data show that biotin transport in *L. plantarum* is controlled by repression. The activity remaining in fully repressed cells (10%) approximates the non-active transfer activity of fully active cells. This suggests that only the active portion of the biotin transport system is controlled.

Kinetics of Biotin Transport in *Bacillus coagulans*. James R. Waller and Duane C. Ulmer. Department of Microbiology, Univ. of North Dakota, Grand Forks, 58202.

Biotin transport into late log-phase cells of *Bacillus coagulans* was studied. Biotin uptake was followed by incubating cells in solutions of 1% glucose, 0.1M PO₄ buffer, and biotin at the desired pH and temperature. Cells were harvested by cold centrifugation and boiled for 10 min to release free biotin. Temperature and pH optima for biotin uptake were 37C and 5.8, respectively. Biotin uptake occurred at both 0C and 37C. The uptake process at both temperatures exhibited substrate saturation. The kinetics and extent of uptake at each temperature were markedly different. At 37C a maximum capacity of 65 ng biotin/mg cell dry wt was attained in 20 min. The apparent K_m and V_{max} values were approx. 6.0 ng/ml and 7 ng/mg/min, respectively. At 0C a maximum capacity of 3.5 ng biotin/mg cells was attained within 10 min. The apparent K_m and V_{max} of this reaction were approx. 1.3 ng/ml and 1.06 ng/mg/min, respectively. These data indicate that biotin uptake in *Bacillus coagulans* is carrier mediated. Also, the marked difference in transport kinetics at 0 and 37C suggest that two mediated transport systems or two manifestations of the same system are operable.

CLINICAL CYTOGENETIC FINDINGS IN NORTH DAKOTA OVER A SEVEN-YEAR PERIOD. W. A. Wasdahl, J. H. Saumur, L. M. Larson. Dept. of Pathology, Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201.

Since 1964 the Pathology Department of the University of North Dakota Medical School has provided the opportunity of chromosomal analysis on a referral basis to physicians of the state of North Dakota, western Minnesota and occasionally South Dakota. Methodology used in the laboratory has included peripheral blood cultures, buccal smear analysis, direct analysis of bone marrow, autopsy material, and chromosome banding techniques.

This is a report of the 327 cases studied over a seven-year period from 1968-1974 in which we have found a total of 100 abnormal karyotypes. The most common abnormality detected was Trisomy 21 which constituted 50 cases -- one half of the total. Other abnormalities found included Trisomy 18, Trisomy D, XXX, a number of translocation mongolism and carriers and a variety of other abnormalities of both sex and autosomal chromosomes.

Supported in part by Pathology Foundation and North Dakota State Department of Health.

CHEMICAL MEANS OF PREGNANCY DIAGNOSIS IN LARGE ANIMALS. R.M. Weigl*, J.E. Tilton, C.N. Haugse and M.L. Buchanan. Department of Animal Science, College of Agriculture, NDSU, Fargo, N.D.

Plasma progesterone levels for 45 ewes were determined at four periods post breeding in an attempt to diagnose pregnancy and fetal numbers. Blood samples were drawn on the day of mating, 10 days, 18 days, and 70 days post mating. The competitive protein binding assay was used to quantify progesterone. Plasma progesterone levels at 10 and 18 days post breeding (4.90 and 2.44 ng/ml). Pregnant ewes showed a similar rise in progesterone on day 10 (4.43 ng/ml) but the day 18 levels were also elevated (4.50 ng/ml). Ewes exhibiting this at day 18 difference were diagnosed as pregnant. Pregnancy was predicted at day 18 with an 80 percent accuracy. Fetal numbers were predicted using the 70 day post breeding plasma progesterone levels. Ewes having 0, 1, 2 or 3 lambs had progesterone levels of 1.48, 5.89, 8.01 and 9.80 ng/ml, respectively. Predictions were made utilizing these differences. Multiple fetuses were diagnosed with a 75% accuracy. Single lambs, twins and triplets were predicted with an accuracy of 75, 50 and 50 percent, respectively.

VASCULAR FLORA OF EMMONS COUNTY, NORTH DAKOTA. Richard P. Williams and William T. Barker. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak. 58102

Geologically Emmons County occupies a unique position. The Missouri River marks the approximate boundary between the glaciated area to the east and the unglaciated Missouri Plateau to the west. As a result the eastern half of the county is occupied largely by soils derived from glacial deposits while the western half of the county is characterized by soils developed predominantly from the Fox Hills, Pierre and Hell Creek formations of the Cretaceous period. The complex geology and soils is reflected by the flora of this transition zone. More than 500 species have been collected in the county. No less than 25 species reach their eastern limit of occurrence within this relatively narrow transition zone. Examples include: Yucca glauca, Sitanion hystrix, Lupinus pusillus and Oenothera caespitosa. About an equal number of species with eastern affinities reach their western limit of occurrence. This group includes: Tilia americana, Zizania aquatica, Delphinium virescens, Hydrophyllum virginianum and Oxalis violacea. In addition the study has recorded four new species to the flora of the state: Iris missouriensis, Najas guadalupensis, Juncus compressus and Carex nebraskensis.

AFFECT OF GLUCONEOGENESIS ON URINARY ASCORBIC ACID IN THE RAT. P.K. Yang, R.L. Puyear, J.L. Sell and K.L. Davison, Depts. of Zoology and Animal Science, NDSU and USDA, ARS, Fargo, ND 58102

This study was initiated to determine if gluconeogenesis contributed to urinary ascorbic acid. Male rats were adrenalectomized or sham operated. After a 7 day recovery period rats were given a single i.p. injection (10mg/100 g B.W.) of sesame oil containing o,p'-DDT, triamcinolone, o,p'-DDT+triamcinolone or just sesame oil. Urine was collected every 24 hours for 4 days and analyzed for ascorbic acid and glucose. Blood glucose and liver glycogen were determined at the end of the fourth day. Urinary or blood glucose did not change with treatment in the adrenalectomized or sham operated rats. There was an increase in urinary ascorbic acid in the adrenalectomized and sham operated rats following treatment with o,p'-DDT or o,p'-DDT+triamcinolone. Liver glycogen doubled in the adrenalectomized groups receiving o,p'-DDT, triamcinolone, and o,p'-DDT+triamcinolone. This data suggests that although gluconeogenesis was stimulated by triamcinolone and DDT, only DDT stimulates urinary ascorbic acid excretion.

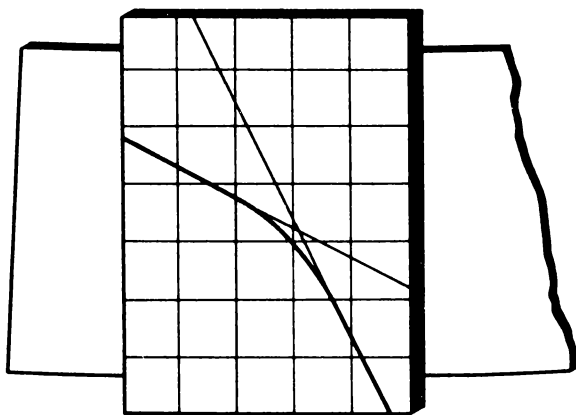
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EFFECTS OF INOCULATION POINT ON SORUS LOCATION IN BARLEY COVERED SMUT

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ABSTRACT

Kernels of Odessa barley (C. I. 934) were dehulled, steeped in tap water for 2 hr and germinated for 24 hr at 20°C. Teliospores of race 6, *Ustilago hordei*, were placed on the base, middle, tip or over the entire surface of the coleoptile after germination. Inoculated seedlings were incubated for 24 hr at 20°C or 24°C and then planted in greenhouses held at the same temperatures as their respective incubations. The smut sorus production in the top four leaves, the percent of plants infected and the percent of seedlings killed were highest with basal inoculation and growth at 24°C. Smut sori developed in the top three leaves with middle inoculation and growth at 24°C. Inoculation over the entire coleoptile and growth at 24°C produced sori in the upper two leaves and the head. Tip inoculation at 24°C produced no infected plants. Sori were produced only in flag leaves and in florescences in the 20°C trial. No leaf sori were formed in the leaves below the flag leaf in the 20°C trial. The number of plants with sori in all tillers was highest with inoculation over the entire coleoptile and growth at 20°C. Inoculation in the middle of the coleoptiles and growth at 20°C produced the highest percent of plants with sori in some but not all tillers.

INTRODUCTION

When susceptible spring barley varieties infected with race 6, *Ustilago hordei* (Pers.) Lagerh. are grown under field conditions, smut sori will develop in their inflorescences only. Sorus formation is reported, however, in culm, leaf, and node tissues (Fischer and Holton, 1957). Sori developed in the leaves and spikes of Odessa barley when infected plants were grown at 20°C or 24°C (Kiesling, 1953 and Schafer, Dickson, and Shands, 1962). Infections of the coleoptiles of Jet barley caused the coleoptiles to recurve which separated the infected coleoptile tissues from the growing shoots (Kiesling, 1953). These separations prevented further infection of the seedlings. Onion seedlings also escaped infection by the smut fungus, *Urocystis cepulae* Frost when only the tip of the cotyledons were infected (Evans, 1937).

This study was conducted to determine the effect on symptom development of placement of teliospores on specific areas of the coleoptiles of a susceptible barley cultivar.

Kernels of Odessa barley (C. I. 934) were dehulled, steeped in tap water for 2 hr and germinated for 24 hr at 20°C. Sixty seedlings per treatment were used in the study at 24°C and one hundred seedlings per treatment in the 20°C. Teliospores of race 6, *U. hordei* were placed on the base, middle, tip or over the entire surface of the coleoptiles after the seedlings germinated. Inoculated

seedlings were incubated in petri dishes at 20°C or 24°C and then planted in greenhouses held at the same temperatures as their respective incubations. After the plants were mature, the location of the sori in the plants was recorded for both temperature treatments. In the trial at 20°C, the number of infected tillers was also recorded. An infected tiller was any tiller which had visible smut sori in its tissues.

RESULTS AND DISCUSSION

The percent of plants with sori in the top three or four leaves as well as the inflorescences was highest when inoculations were made at the base of the coleoptiles in the 24°C treatment (Table 1). Inoculations of the middle of the coleoptiles resulted in sori forming in the top three leaves and inflorescences of infected tillers, but tip inoculations produced no infection (Table 1). Sori developed in the top two leaves and inflorescences of infected tillers of the seedlings inoculated over their entire coleoptile surfaces (Table 1). In the 20°C trial, sori were produced in the flag leaves only.

General inoculation produced the highest percent of plants with one or more infected tillers, but the highest percent of infected plants with all tillers infected developed from basal inoculations in the 20°C trials (Table 2). When the main tiller was not infected, the number of tillers not infected also increased. The highest percent of plants with healthy main tillers and one or more healthy tillers occurred with general or middle inoculations (Table 2). As in the 24°C trials, the tip inoculations did not produce a significant amount of infection. The one plant in the tip inoculation treatment which developed infection in its main tiller and all of its tillers may have resulted from contamination of the basal coleoptilar area with teliospores.

The location of the leaves bearing sori on the tillers and the number of tillers containing sori in their leaves and inflorescences provides evidence of the rate and extent of colonization of the meristematic areas of the seedling as well as an approximation of the time elapsed between penetration of the coleoptile and colonization of the meristematic areas of the crown and shoots. The covered smut fungus penetrates and develops generally perpendicular to the longitudinal axis of the seedling (Kiesling, 1953). The fungus must traverse the tissues of the coleoptile and any leaves which lie between its point of penetration and the meristematic areas of the crown (Kiesling, 1953). Since barley leaves elongate by basal growth, the further the infection point on the coleoptile is from the base of the coleoptile the greater the probability that the infection will be swept away from the growing point by leaf and internode elongation.

In wheat the primordium of the fourth leaf is present in the embryo; and within 14 days following germination, the internode above the coleoptile begins to elongate (Briggle, 1967). This initial elongation is followed by a period of axillary bud development and little or no elongation of the axis. However, new leaves continue to emerge sheathed by the previously emerged leaf, and these leaves elongate which increases the probability that infections distal to the coleop-

TABLE 1. The effect of the inoculation point on the production of infected or dead plants and location of smut sori in Odessa barley inoculated with race 6 *Ustilago hordei* and grown at 24°C.

Inoculation point on coleoptile	Inoculated plants infected (Percent)	Inoculated plants dead (Percent)	Sori in heads only	Infected Plants (Percent)				
				Flag leaf	2nd leaf	3rd leaf	4th leaf	Sori in leaves
General	38	8	30	43	27	0	0	0
Base	60	20	3	25	31	25	16	16
Middle	35	13	11	16	58	15	0	0
Tip	0	11	0	0	0	0	0	0

TABLE 2. The effect of inoculation point on the production of infected tillers or dead plants in Odessa barley inoculated with race 6 *Ustilago hordei* ad grown at 20°C.

Inoculation point on coleoptile	Inoculated plants infected (Percent)	Dead (Percent)	Infected Plants (Percent)					
			Main culm infected			Main culm not infected		
			All tillers infected	No tillers infected	All tillers infected	1 tiller not infected	2 tillers not infected	3 tillers not infected
General	85	8	71	1	8	14	6	0
Base	68	3	76	2	5	8	7	2
Middle	46	1	38	2	26	20	12	2
Tip	2	0	50	0	0	50	0	0

tilar node may be lost. A similar developmental pattern occurs in spring barley.

Plants of Odessa barley grown in the greenhouse at $22\pm 2^{\circ}\text{C}$ and a 12 hr photoperiod produced eight to nine leaves per main culm. The tips of the fourth leaf (sixth from the top) were emerging and leaves from axillary buds were 10 to 20 mm long 14 days after the inoculation and planting of Odessa seedlings. The tips of the sixth leaf (fourth from the top) were emerging and tillers had two to three leaves 24 days after the Odessa seedlings were planted.

Early colonization of the shoot apices and secondary stem buds is evidenced by more leaves per culm and more culms per plant developing sori. Infections resulting from basal inoculations in the 24°C study colonized the shoot apices before the fourth or fifth leaf primordia had developed. The greater efficacy of inoculum applied to the basal area of the coleoptile in the 24°C study is also evidenced by the increased number of infected plants. Basal inoculation and incubation at 24°C also resulted in increased percentage of dead seedlings. No explanation is offered for the increased fatalities following such inoculation of a susceptible variety.

Colonization of the shoot apices was not as fast in the 20°C trial as in the 24°C trial which was demonstrated by the presence of sori in the inflorescences and flag leaves only in the 20°C trial. Basal and general inoculations in the 20°C trial resulted in a thorough colonization of axillary buds and main culm apices as demonstrated by the high percentage of infected plants in which the main culm and all tillers were infected. Basal inoculation at 20°C , however, produced fewer infected plants than the general inoculation (Table 2). Inoculations in the middle areas of the coleoptiles failed in many seedlings to reach the apices of the main culms and some tillers in the 20°C trial, which may indicate the colonization of these meristematic tissues was delayed perhaps by elongation of leaves. Tip inoculations were also ineffective in the 20°C trial.

Kiesling (1953) reported that mycelium of race 6 penetrated tissues of the second internode and third leaf of Odessa barley seedlings incubated for 14 days at 20°C after inoculation. In the present study, colonization of the sixth leaf probably occurred between the 14th and 24th day of seedling growth following basal inoculation in the 24°C trial. Colonization of the apices of axillary buds which resulted in sorus formation in tillers probably occurred after the 24th day in the 20°C trials because smut sori were found in the inflorescences and flag leaves only.

Basal inoculation resulted in quicker and more thorough colonization of infected plants, but general inoculation and incubation at 20°C produced the largest number of plants with smut sori. These data indicate that in testing segregating populations general inoculation and incubation at 20°C would promote maximum infection probabilities.

ACKNOWLEDGEMENT

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VARIATION OF SHELL DIMENSIONS AND
WEIGHT OF *ANODONTA GRANDIS* SAY AND
LAMPSILIS RADIATA (GMELIN) (BIVALVIA: UNIONIDAE)
SHEYENNE RIVER, NORTH DAKOTA

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ABSTRACT

The mussels (Bivalvia: Unionidae) *Anodonta grandis* Say and *Lampsilis radiata* (Gmelin), the most widespread species in the Sheyenne River of eastern North Dakota, were collected from six lake stations and 21 river stations, spanning 818 river km, during the summers of 1966, 1973, and 1974. Most individuals were collected from seven stations in 1974, and usually the larger individuals collected were measured and weighed. Shells were measured for length (greatest distance parallel to the hinge line), height (greatest dorsoventral distance at right angles to the hinge line), and width (greatest distance across both valves at right angles to a plane passing between them). The shells (N = 341) of *A. grandis* became significantly ($P = 0.05$) longer, higher, wider, and more inflated (width/height and width/length ratios increased) downstream. A significant ($P = 0.05$) increase in shell weight (N = 211), the shell weight/shell length ratio (N = 211), total (body and shell) weight (N = 236), and the total weight/shell length ratio (N = 236) occurred downstream. These weight relationships fit a quadratic regression. Shells (N = 262) of *L. radiata* became significantly ($P = 0.05$) higher and more inflated downstream. A significant ($P = 0.05$) increase in shell weight (N = 154), shell weight/shell length, total weight (N = 176), and the total weight/shell length ratio occurred downstream. Both the dimension and weight relationships fit a simple linear regression. Possible causes for the observed trends are: 1) increase in nutrients downstream, 2) increase in soluble salts downstream, 3) differences in stream velocity and discharge, 4) variation in bottom sediment type, and 5) taxonomic differences.

INTRODUCTION

That the shells of mussels (Bivalvia: Unionacea) vary with location in a stream has been known for some time. Ortmann (1909:192) first pointed this out for *Obovaria subrotunda* (Rafinesque) from the Ohio River drainage in Pennsylvania. This species was relatively compressed in the smaller streams and

relatively swollen (inflated) in the larger streams. In 1920, Ortmann summarized earlier work on mussel shell variation with stream position and provided quantitative data that showed that in many species "obesity" ("diameter", or width, divided by length) tend to increase downstream. Several species, however, did not follow this "law." Ortmann also pointed out that a decrease in obesity (upstream) is commonly associated with an increase in shell length, and a few species with tubercles in larger streams tend to lose them in the headwaters. Ball (1922) analyzed obesity statistically for several species of mussels, attempted to correlate this variable with stream-flow measurements, and arrived at essentially the same conclusions as Ortmann. In addition, other workers have found that the shells of certain species become longer (Baker, 1922:27, 1926:109; Grier and Mueller, 1926:22), higher (Grier and Mueller, 1926:22), thinner (Meek and Clark, 1912:14), and heavier (Baker, 1926:109; Utterback, 1915:42) in a downstream direction. Eager (1948) provided a useful summary of the literature dealing with variation in the shells of fresh-water mussels in regard to habitat.

Cvancara, Norby, and Van Alstine (1976) summarized the distribution and ecology of the mussels in the Sheyenne River of eastern North Dakota, and Cvancara and Freeman (1978) discussed the mussels in Lake Ashtabula. The purpose of the present paper is to describe the variations of selected shell dimensions and shell and total (body and shell) weights of two species of mussels in the Sheyenne River — *Anodonta grandis* Say and *Lampsilis radiata* (Gmelin) — and suggest possible causes for these variations.

Originating in Sheridan County of central North Dakota, the Sheyenne River flows east, south, and northeast, and joins the Red River about 13 km north of Fargo (Figure 1). It flows primarily through the undulating to hilly Drift Prairie, but in its lower reaches (below about kilometer 242) it flows across the dominantly flat Red River Valley.

The bottom sediment in the river is derived from glacier-related and glacial-lake sediments and shales of Late Cretaceous age into which the river cuts. From kilometer 884 to kilometer 242, the river is cut into bouldery sand, silt, and clay (glacial till), sand and gravel (glacial melt water deposits), and marine shales, exposures of which are common from kilometer 616 to kilometer 276 (Bluemle, 1965, 1973; Bluemle et al., 1967; Bluemle, unpublished map of Ransom County; Carlson and Freers, 1975; Kelley and Block, 1967; Merritt, 1966; and Rude, 1966). Above kilometer 242, the bottom sediment is mostly pebbly sand and sandy pebble gravel. Shale is common to abundant in the coarse fraction at many places. From below kilometer 242 to kilometer 122, the Sheyenne cuts into sand and silt of the glacial Sheyenne delta (Baker, 1967). Within the area of the delta, the bottom sediment is primarily well-sorted sand. From below kilometer 122 to the mouth, the river cuts into silt and clay of glacial Lake Agassiz (Klausing, 1968); here, the bottom sediment is muddy sand and mud. The bottom sediment generally becomes finer downstream, although it varies considerably in response to the local surficial geology as described above. Turbidity also generally increases downstream as the bottom sediment becomes finer (Cvancara, 1970:83).

The Sheyenne River, from its mouth to Sheyenne Lake, is 878 km long. It extends westward another 36 km as a highly intermittent stream. Its basin upstream from gaging station 595 (Figure 1) has an area of about 22,973 km² (U.S. Geological Survey, 1964: 54). The average gradient of the river is 0.28 m/km.

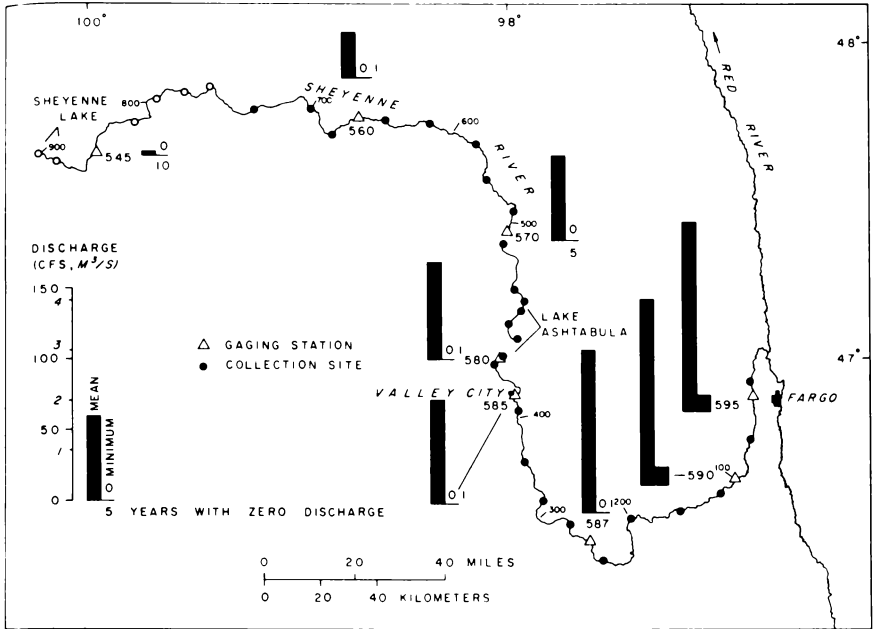


FIGURE 1. Location map showing mussel collecting stations and discharge values for eight USGS gaging stations on the Sheyenne River. Discharge values are for the 10-year period from October 1, 1955 to September 30, 1965 except for gaging station 587; here, the period covered is October 1, 1957 to September 30, 1966. Discharge data were taken from U.S. Geological Survey (1961-1966, 1964). Cfs signifies cubic feet per second. Small numbers on the Sheyenne River refer to river kilometers above the mouth.

Discharge data for eight gaging stations are given in Figure 1. Flow is generally intermittent above kilometer 487, where years in which zero discharge occurs is common. A substantial increase in discharge occurs where the river crosses the Sheyenne delta, due primarily to groundwater inflow from the sandy delta deposits (Paulson, 1964). Selected aspects of water chemistry are summarized in Table 1. Specific conductance, pH, chloride, and sulfate content tend to increase downstream whereas potassium shows no apparent trend for the river.

TABLE 1. Specific conductance and five chemical factors for five U.S. Geological Survey gaging stations on the Shyenne River (gaging stations are shown on Fig. 1)^a

USGS gaging station	Specific conductance (micromhos/cm)	pH	Total chlorides (mg/l)	Total sulfates (mg/l)	Total bicarbonates (mg/l)	Potassium (mg/l)	Period of sampling
560	1760 ^b	8.5	24	264	844	15	Oct. 1, 1955- Sept. 30, 1965
	625	7.7	12	79	294	7.0	
	234	7.1	0.6	28	102	1.9	
570	1080	8.0	26	162	521	11	Oct. 1, 1959- Sept. 30, 1960
	787	7.5	16	124	356	6.7	
	213	6.5	0.1	29	8	6.2	
587	1330	8.3	84	447	459	15	Oct. 1, 1957- Sept. 30, 1965
	782	7.5	34	144	276	11.0	
	295	6.7	8	39	104	5.0	
595	1050	8.0	51	193	344	9.5	Sept. 16, 1969- Sept. 15, 1970
	856	7.6	35	155	305	7.8	
	467	7.4	15	85	172	6.9	
606 ^c	1320	8.5	86	260	420	11	July 16, 1969- Sept. 28, 1971
	913	7.9	43	185	303	7.0	
	426	7.1	17	76	143	0.0	

^aData from U.S. Geological Survey 1959-1965; 1970-1973.

^bUpper number is the maximum, middle number is the mean, and lower number is the minimum for the interval of time sampled.

^cStation 606 is between station 595 and the mouth of the Shyenne River.

MATERIALS AND METHODS

Anodonta grandis Say and *Lampsilis radiata* (Gmelin) were chosen for this study because they are the most widespread mussels in the Sheyenne River (Cvancara, Norby, and Van Alstine, 1976). Individuals were collected from 21 stations (spanning 818 km) in the river (Figure 1) by handpicking during the summers of 1966, 1973, and 1974. Most, however, were collected from seven stations in 1974 when an attempt was made to collect 30 individuals of each species from each station; individuals collected during 1966 and 1973 were only representative specimens for a reference collection. Individuals were collected from six stations in Lake Ashtabula by scuba diving primarily during the summer of 1974 (Cvancara and Freeman, 1978).

Generally, the larger individuals from the river were measured and weighed. Shells were measured (to the nearest millimeter) for length (greatest distance parallel to the hinge line), height (greatest dorsoventral distance at right angles to the hinge line), and width (greatest distance across both valves at right angles to a plane passing between them). The total weight (body and shell) and shell weight were recorded to the nearest tenth of a gram. Only males of *Lampsilis radiata* were measured and weighed. The ratios of shell weight/shell length and total weight/shell length were calculated to minimize variability resulting from differences in age. Most of the live individuals were returned to the water after measuring and weighing. Voucher specimens used are encompassed within accession numbers A106-111, 142-143, 145-150, 159, 161-165, 167-168, 981-986, 1061-1062, and 1201-1222 of the Department of Geology, University of North Dakota.

RESULTS

Results of the analysis of shell measurements and shell and total (body and shell) weights are summarized in Table 2. Shells of *Anodonta grandis* became significantly longer, higher, wider and more inflated (width/height and width/length ratios increased, Figure 2) downstream. A significant increase in shell weight and in the shell weight/shell length ratio (Figure 4) occurred downstream, and a similar significant increase in total weight and in the total weight/shell length ratio (Figure 5) occurred downstream. These weight relationships fit quadratic regressions. The shells from Lake Ashtabula were decidedly smaller than those for the river; the mean length ($N = 82$) was 55 mm, with a standard error of ± 1.0 and a range of 38-84 mm.

Shells of *Lampsilis radiata* became significantly higher and more inflated (Figure 3) downstream; they also showed a significant increase in shell weight and in the shell weight/shell length ratio downstream. A similar significant increase in total weight and in the total weight/shell length ratio occurred downstream. Both the dimension and weight relationships fit simple linear regressions. The shells from Lake Ashtabula were about the same size as those from the river; the mean length ($N = 9$) was 90 mm, with a standard error of ± 8.4 and a range of 37-112 mm.

TABLE 2. Statistical data of shell dimensions, shell dimension ratios, and shell and total weights and their ratios with shell length for *Anodonta grandis* Say and *Lampsilis radiata* (Gmelin) from the Sheyenne River (21 stations) and Lake Ashtabula (6 stations).

Species and variable against downstream distance	Correlation coefficient	Significant (P = 0.05)	Number	Mean \pm S.E.	Range
<i>Anodonta grandis</i> Say					
Length (mm)	+0.43	Yes	341	84 \pm 1.2	38-156
Height (mm)	+0.46	Yes	341	48 \pm 0.7	23-87
Width (mm)	+0.48	Yes	341	30 \pm 0.5	12-60
Height/length	+0.09	No	341	0.58 \pm 0.002	0.51-0.74
Width/length	+0.24	Yes	341	0.36 \pm 0.001	0.29-0.42
Width/height	+0.17	Yes	341	0.62 \pm 0.002	0.49-0.74
Total weight ^a (g)	+0.96 ^a	Yes	236	71.6 \pm 5.0	5.2-355.3
Shell weight (g)	+0.48 ^a	Yes	211	14.0 \pm 1.0	1.1-146.9
Total weight/length	+0.95 ^a	Yes	236	0.74 \pm 0.04	0.14-2.50
Shell weight/length	+0.43 ^a	Yes	211	0.16 \pm 0.01	0.03-0.94
<i>Lampsilis radiata</i> (Gmelin)					
Length (mm)	+0.04	No	262	91 \pm 0.7	37-122
Height (mm)	+0.28	Yes	262	53 \pm 0.5	23-85
Width (mm)	+0.06	No	262	31 \pm 0.3	10-46
Height/length	+0.06	No	262	0.59 \pm 0.002	0.48-0.79
Width/length	+0.51	Yes	262	0.34 \pm 0.002	0.25-0.40
Width/height	+0.43	Yes	262	0.58 \pm 0.003	0.40-0.76
Total weight ^a (g)	+0.19	Yes	176	93.0 \pm 2.8	4.3-233.2
Shell weight (g)	+0.28	Yes	154	45.2 \pm 1.9	1.3-123.1
Total weight/length	+0.26	Yes	176	1.00 \pm 0.02	0.12-1.91
Shell weight/length	+0.32	Yes	154	0.47 \pm 0.02	0.04-1.05

^aFits a quadratic regression.

^bWeight of body and shell.

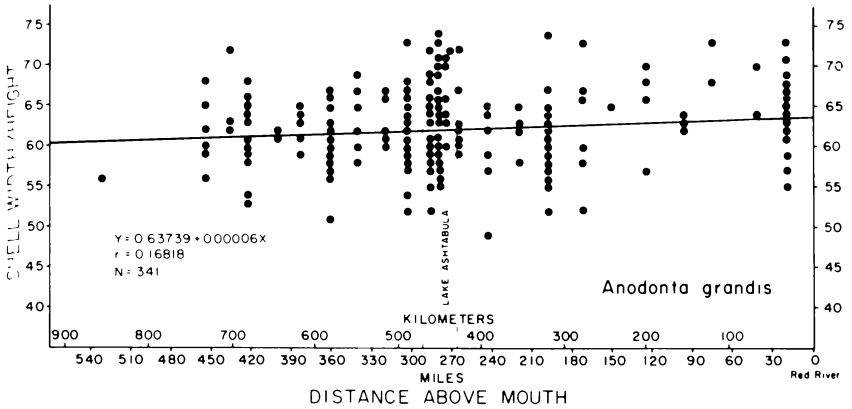


FIGURE 2. Scatter plot of the shell width/shell height ratio against river distance above the mouth of the Sheyenne River for *Anodonta grandis* Say.

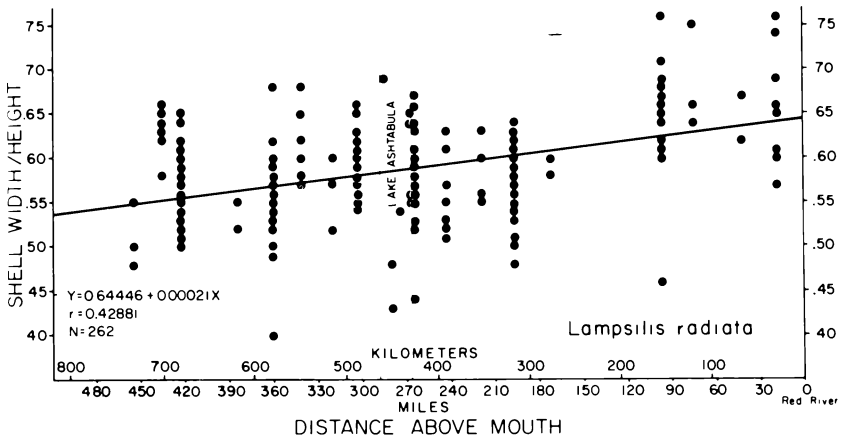


FIGURE 3. Scatter plot of the shell width/shell height ratio against river distance above the mouth of the Sheyenne River for *Lampisilis radiata* (Gmelin).

DISCUSSION

All the data analyzed for *A. grandis* indicate that this species generally became larger downstream. This trend was also reported by Baker (1922:27) for this species in the Big Vermilion River, Illinois. The trend of more inflated shells downstream, to our knowledge, has not been reported previously for this species. The weight data imply not only that *A. grandis* became larger but also the shells

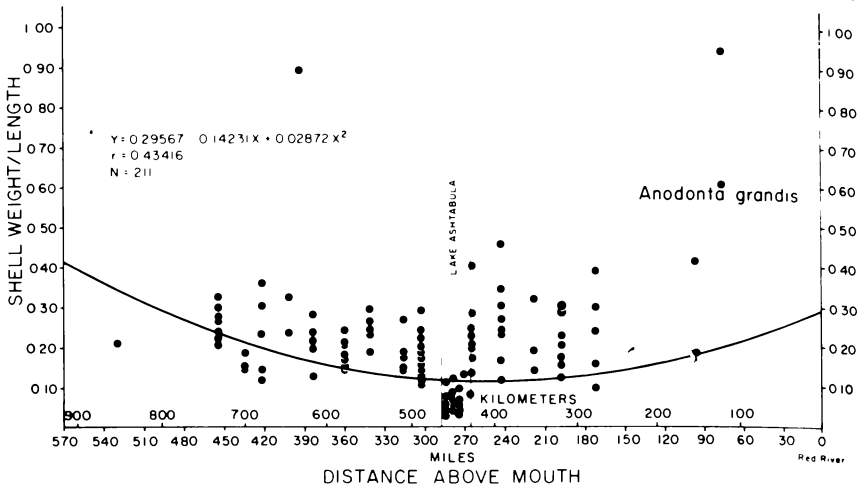


FIGURE 4. Scatter plot of the shell weight/shell length ratio against river distance above the mouth of the Sheyenne River for *Anodonta grandis* Say.

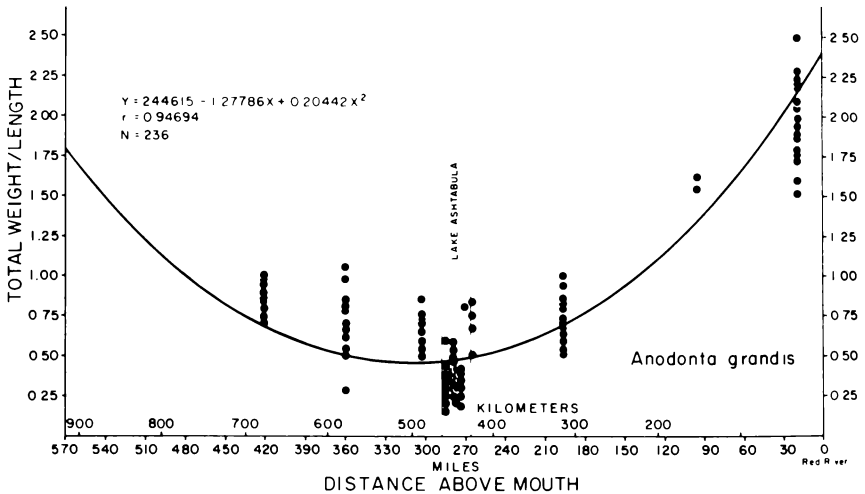


FIGURE 5. Scatter plot of the total weight (body and shell)/shell length ratio against river distance above the mouth of the Sheyenne River for *Anodonta grandis* Say.

became thicker downstream. Heavier shells downstream for certain species has been reported by Baker (1926:109) and Utterback (1915:42); however, thinner shells may also occur downstream (Meek and Clark, 1912:14) in certain species.

Lampsilis radiata did not generally show a size increase downstream in the Sheyenne River, although shell and total weight did increase. Baker (1922:27), however, showed an increase in size downstream for this species. Grier and Mueller (1926:22) observed that *L. radiata* became shorter in a downstream direction. The increase in inflatedness downstream, to our knowledge, has not been documented previously for this species. Using the width/length ratio as an index of inflatedness, Ortmann (1920:308) observed no relationship between this ratio and size of stream for this species; Grier and Mueller (1926:26) suggested that *L. radiata* becomes more inflated ("convex") in the lower reaches of streams.

Possible causes for the observed trends in shell dimensions and shell and total weights are: 1) increase in nutrients downstream, 2) increase in soluble salts downstream, 3) differences in stream velocity and discharge, 4) variation in bottom sediment type, and 5) taxonomic differences. A downstream increase in nutrients, which seems likely because of cumulative contribution from the drainage basin, might result in more available food for mussels, which is primarily organic detritus and animal plankters (Fuller, 1974:221). The increased food supply could account for the generally larger size of *Anodonta grandis* downstream; for *Lampsilis radiata*, the relationship is less convincing because length and width did not increase downstream although height, shell weight, and total weight did. Inflatedness might also be related to an increased food supply downstream but this is more doubtful; a species may increase in size with increasing inflatedness (Baker, 1926:109) or decreasing inflatedness (Ortmann, 1920:309), and all species presumably do not become more inflated downstream (Ortmann, 1920:308).

A general increase in certain soluble salts downstream (Table 1) may account for the trend of increasing shell weights downstream in both species. This trend, reflecting an increase in shell thickness, is most readily explainable by a greater availability of calcium salts. One is tempted to suggest that the total weight/shell length and shell weight/shell length ratios for *Anodonta grandis* decreased in the vicinity of Lake Ashtabula (Figures 4-5) because the specific conductance and various salts are generally of lower concentrations in the lake, at least than in the river above the lake (Johnson et al., 1974; 17-19). However, the same ratios did not decrease for *Lampsilis radiata* in the vicinity of the lake. Furthermore, there is no assurance that the quadratic regressions would not best fit the ratio data if the lake were not in existence. The low grouping of points for Lake Ashtabula individuals probably is because these individuals were relatively young, most of which had two and three rest rings. It is unfortunate that we do not have long-range chemical data on the river water for each station the mussels were collected. Even though we know that a general increase in soluble salts occurs downstream, we need additional data to safely generalize about how the trend operates.

Differences in stream velocity and discharge might be related to variations in shell inflatedness and shape, but the relationship is unclear. Previous work in-

icates that not all species show the same shell variations downstream whereas stream velocity and discharge generally portray the same relationships in most streams. Ball (1922) attempted to correlate inflatedness with stream-flow measurements, but questioned (p. 117) whether stream size is the cause of the variation in inflatedness. Grier and Mueller (1926:27) suggested that less inflated shells upstream might enable the animal to orient itself better in relation to the current. The lower (less high) shells of both of the species studied in the Sheyenne River may also be related to better adaptability to the current in the upper reaches.

Variation in bottom sediment type might be considered a factor in affecting shell dimensions and shell and body weights, but the relationship is uncertain. Although bottom sediment generally becomes finer downstream in the Sheyenne River, considerable local variation occurs in response to the geology. Sediment type also varies in response to stream velocity and discharge, so any gross effect sediment might have on mussel shape and weight is probably indirect.

Although many mussel species exhibit gradual variations in their physical characteristics in streams, all do not exhibit the same variations. This suggests that taxonomic differences are partly responsible for certain observed trends. Ortman (1920:311) suggested that nearly all the species he studied that became more inflated downstream are of "rather primitive structure," implying greater exhibition of a trend for species grouped taxonomically. One of the species that he said (p. 308) did not become more inflated downstream is *Lampsilis radiata*, which clearly did so in the Sheyenne River. The present study also suggests that different species exhibit different trends within a river system.

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CLINICAL CYTOGENETIC FINDINGS IN NORTH DAKOTA FROM 1968 - 1974

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ABSTRACT

Since 1964 the Pathology Department of the University of North Dakota Medical School has provided the opportunity for chromosomal analysis on a referral basis to physicians of the state of North Dakota, western Minnesota and occasionally South Dakota. Methodology used in the laboratory has included peripheral blood cultures, buccal smear analysis, direct analysis of bone marrow, autopsy material, and chromosome banding techniques.

This is a report of the 327 cases studied over a seven-year period from 1968-1974 in which we have found a total of 100 abnormal karyotypes. The most common abnormality detected was Trisomy 21 which constituted 50 cases — one half of the total. Other abnormalities found included Trisomy 18, Trisomy D, XYY, a number of translocation mongolism and carriers, and a variety of other abnormalities of both sex and autosomal chromosomes.

INTRODUCTION

The Department of Pathology of the University of North Dakota has offered the service of chromosome analysis to area physicians since 1964. The cytogenetics laboratory has functioned not only as a diagnostic unit within the Department of Pathology, but also as a learning experience for university students in medicine, medical technology, cytotechnology, biology and residents in pathology.

Services offered by the laboratory have included peripheral blood cultures for karyotype analysis, buccal smear analysis, occasional tissue analysis such as spleen from autopsy material, direct analysis of bone marrow and banding techniques for individual chromosome identification. In this report we enumerate and describe the abnormalities which have been found from January, 1968, through December, 1974.

MATERIALS AND METHODS

Patient Selection. — Patients have been referred by physicians throughout North Dakota, western Minnesota, the Grand Forks Air Force Base, and the Child Evaluation Center of the University of North Dakota. An occasional case has come from South Dakota. They have included newborns, children and young adults. Forms were provided for a pertinent family history of the patient and physical and medical history by the physician. These were required with each referral.

Blood Culture. — Peripheral blood was cultured according to a modified method of Hungerford (1965) using phytohemagglutinin (PHA) for stimulation of DNA replication of the lymphocytes.

Air-dried slides were prepared and stained with 2% Giemsa stain at pH 7.0. Photography was done with a Zeiss Photomicroscope on Kodak high contrast copy film (ASA 12). Karyotypes were arranged according to the Chicago Conference (1966) and the Paris Conference (1971).

Because of the number of patients in a wide geographical area, a kit was sent to the physician consisting of media prepared without the addition of PHA for inoculation by the physician, a sterile tube containing the anticoagulant (heparin), and slides for buccal smears. Upon return to our laboratory, fresh culture bottles containing PHA were inoculated from the blood and PHA was added to the mailed culture bottles. The cultures were incubated at 35-37°C for 68-72 hours. If care was taken to protect the materials and blood from extreme cold or heat, the results obtained from mailed specimens were excellent. The heparinized specimen was stored at 4°C and was used for up to one week if additional cultures were necessary (Ying, 1969).

Bone Marrow Direct Study. — We have examined bone marrow specimens by a direct method in a variety of diseases, the most common of which was chronic myelogenous leukemia in which the Philadelphia Chromosome is of diagnostic and prognostic importance. Bone marrow preparations were made without stimulation by PHA according to the method of Lam-Po-Tang, (1968). These were harvested and examined by the procedure described for whole blood cultures.

Splenic Cultures. — The spleen, as an excellent source of lymphocytes, was minced freeing a heavy suspension of blood and lymphocytes which was cultured by the peripheral blood method. This method has the advantage of achieving a culture not available before death of a severely malformed infant.

Buccal Smear Analysis. — Buccal smears were examined prior to culturing of patients referred for potential abnormalities of sex chromosomes. Scrapings of the buccal mucosa were smeared on glass slides coated with Mayer's albumin. These were stained with cresyl echt violet according to the method of Lynch et al. (1969).

G-Banding. — G-bands were achieved by a modification of the Seabright (1971) technique. We used a 0.025% trypsin solution on air-dried slides (aged for three days at room temperature) for approximately 10-12 minutes. After rinsing with tap water, the slides were stained with Giemsa for 3-4 minutes. The timing varied with the age and quality of the slides used.

Slides were examined, photographed and karyotyped as previously described for routine work.

Fluorescent Studies: Chromosome Q-Banding and Y-Body Analysis of Buccal Smears. — Standard air-dried slides for chromosome analysis were used for Q-banding of chromosomes and standard buccal smear preparations fixed with hair spray or other spray fixative were used for Y-Body analysis of interphase nuclei. The standard procedure was as follows: 5 minutes in each of 70% ethanol, 50% ethanol, deionized water, 15 minutes in 0.5% quinacrine dihydrochloride, and 3 rinses in 0.06 M phosphate buffer pH 7.0. The slides were mounted in buffer and the coverslip sealed with Vaseline. The preparations were examined using an

HBO 200 excitor lamp with BG 12 primary filter and 500 nm secondary filter. Photographs were made using Tri-X film (ASA 400).

RESULTS AND DISCUSSION

Jacobs (1974) reported the incidence of chromosomal aberrations in 11,680 consecutive newborn infants. In this survey male sex chromosome anomalies occurred with a frequency of 0.3% and female sex chromosome anomalies were 0.18%. These are almost always numerical in nature, with an increase or decrease in the number of X or Y chromosomes. The incidence of autosomal trisomies, or a numerical increase of one autosome, the great majority of which were of the No. 21 chromosome, was 0.17% and of structural rearrangements of autosomes was 0.23%. When the data was combined with that of five other surveys, a total of 43,000 infants, the overall incidence of chromosome abnormalities in consecutive newborns detectable by non-banded techniques was 0.56%.

Our findings for a seven-year period from January, 1968, through December, 1974, are summarized in Table 1. Of a total of 327 case studies, 100 chromosomal aberrations have been identified. A statistical comparison of the frequencies was, of course, impossible due to the selection of our patients who were referred for diagnostic purposes, both medical and mental retardation.

Autosomal Anomalies. — The most common abnormality found in our studies is Trisomy 21 (Down's syndrome) of which we found a total of 24 males and 26 females. One patient presented phenotypically as a Down's syndrome but the peripheral blood culture revealed a 46,XY karyotype. We were unable to obtain additional tissues for a study for mosaicism in this case. Other autosomal trisomies identified were one 18-Trisomy and one D-Trisomy which is undoubtedly Trisomy 13.

Structural abnormalities include Robertsonian translocations of D and G groups in three families, one *de novo* D/G translocation and one *de novo* G/G translocation. The karyotype 45,XY,+t(Dq Dq) has been reported to be the most common form of structural rearrangement of chromosomes (Sergovich, 1969). This abnormality was found in one instance, and banding demonstrated that chromosomes 13 and 14 were involved in the translocation.

The most unusual karyotype found was one with 47 chromosomes, the extra one being a minute metacentric chromosome, smaller than an F group chromosome. Identification of tiny chromosomes of this nature is impossible; however, it is probably an isochromosome of the No. 18 (47,XY,+18 pi) as described by Condrón et al (1974).

Sex Chromosome Abnormalities. — A total of 14 cases of Klinefelter's syndrome, one XYY syndrome, two cases of 46,XY testicular feminization, 12 cases of Turner's syndrome, 45,XO, one Turner's syndrome which was a mosaic of 45,XO/46,XXqi, and one Triple X (47,XXX) have been identified.

Bone Marrow Studies for Philadelphia Chromosome. — We have identified the Philadelphia Chromosome (Ph¹) in the bone marrows of four individuals with the diagnosis of chronic myelogenous leukemia. This chromosome appears as a deletion of the long arm of the No. 22 chromosome (22q-) in non-banded

TABLE 1. Karyotypes of individuals studied from January, 1968, through December, 1974.

KAROTYPE	TOTAL
<u>Normal</u>	
46,XY*	105
46,XX*	122
<u>Sex Chromosome Anomalies</u>	
45,XO	12
45,XO/46,XXqi	1
47,XXY	14
47,XXX	1
46,XY testicular feminization	2
47,XXY	1
<u>Autosomal anomalies</u>	
47,XY,+21	24
47,XX,+21	26
46,XY, phenotypic Down's syndrome	1
47,XX,+18	1
47,XX,+D	1
46,XX,-D,-G,+t(Dq Gq)	2
46,XY,-D,-G,+t(Dq Gq)	2
45,XX,-D,-G,+t(Dq Gq)	2
45,XY,-D,-G,+t(Dq Gq)	3
46,XX,+t(Gq Gq)	1
45,XY,+t(13q 14q)	1
47,XY,+18 pi	1
Bone marrow for Philadelphia chromosome	4
TOTAL	327

*These figures include relatives of patients referred for possible translocation.

preparations. However, after trypsin banding it has recently been shown to be a translocation to the telomere of the long arm of No. 9 (Rowley, 1973).

With the routine use of the new banding techniques it is felt that structural abnormalities previously unrecognizable, will be found. Such data will be presented in the future.

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COMPOSITION STUDIES OF BISMUTH TELLURIUM SULFIDE CRYSTALS

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ABSTRACT

Single crystals grown by the Bridgman technique from an original melt composition $\text{Bi}_8\text{Te}_7\text{S}_5$ do not have a uniform composition through the length of the crystal. The purpose of this investigation was to determine the magnitude of the composition gradient by two methods, X-ray fluorescence and density measurements. The results show that the bottom of a crystal, where solidification first occurs, has a composition of approximately $\text{Bi}_8\text{Te}_6.5\text{S}_5$. The middle and the top of a crystal tend to be closer to the melt composition. The very last part of the crystal to form does not have the same characteristics as the rest of the crystal. This portion was found to be very rich in tellurium, and low in sulfur.

INTRODUCTION

Bismuth tellurium sulfide has composition near $\text{Bi}_8\text{Te}_7\text{S}_5$ (Soonpaa, 1960). Its structure is characterized by a stacking of quintuple layers of the form



The Y layers contain a suitable mixture of tellurium and sulfur to satisfy the crystal composition. The bonds are primarily covalent except for a Van der Waals bond between Y layers, where the crystal can be easily cleaved.

The ease of cleavage is dependent on the strength of the Van der Waals bond, and the bonding strength is dependent on the composition of the Y layers. The crystals grown by the Bridgman technique (Brice, 1965) appear to have slightly different cleavage characteristics in different parts of the crystal, which is suggestive of a composition gradient through the length of the crystal. The last part of the crystal to solidify forms a cap with a different crystal structure, perhaps due to a radically different composition than that of the bulk of the crystal. Measurement of the density of a crystal by Soonpaa and preliminary X-ray fluorescence measurements by Bale indicated that the crystals probably do not have the melt composition (Wilhelm and Bale, 1975).

The purpose of this investigation was to determine the composition and the composition gradient of crystals obtained from a melt of $\text{Bi}_8\text{Te}_7\text{S}_5$. X-ray fluorescence and density measurement techniques were chosen because of their usefulness in calculating the concentration of elements which are primary components of the sample.

MATERIALS AND METHODS

X-ray fluorescence measurements. — In X-ray fluorescence, an incident beam of X-rays creates a vacancy in one of the lower extra-nuclear energy states

of an atom. The transition of an electron to fill the vacancy results in the emission of an X-ray photon with energy characteristic of the element (Compton and Allison, 1935). The intensity of the emitted radiation is proportional to the concentration of the element. Thus by comparing the fluorescent intensities of the unknowns to those of standard samples, the compositions of the unknowns may be determined.

Five standards were prepared with compositions of the form $\text{Bi}_8\text{Te}_{12-x}\text{S}_x$, the original melt composition corresponding to $x = 5$. High purity bismuth, tellurium, and sulfur of the desired proportions were placed in clean quartz crucibles. The crucibles were then evacuated and sealed. The elements were mixed mechanically at 750°C . After cooling, the standards were powdered and pressed onto Bakelite backings.

The unknowns were selected from two crystals grown by the Bridgman technique. Both had original melt composition $\text{Bi}_8\text{Te}_7\text{S}_5$, which has a melting point of 630°C . They were grown in tapered quartz crucibles which were slowly lowered through a temperature gradient. The gradient had temperatures above the melting point at the top end and below the melting point at the lower end, causing the crystal to grow from the bottom upward when the crucible was lowered. The bottom of each crucible was tapered into a narrow tip for seeding single crystals. The resulting crystals were oriented with the cleavage plane parallel to the axis of the crucible.

When the growth was complete, there was a horizontal line in the crystal above which the growth appeared more random. This part of the crystal will hereafter be referred to as the cap. After the cap was removed with the aid of a razor blade, the remaining crystal cleaved readily. One-gram samples were taken from the bottom, middle, and top portions of each crystal, then pelletized through the same procedure as the standard samples. The cap (with a mass of .6 grams) of the first crystal was also prepared for analysis. The cap of the second crystal was too small to make analysis practicable. The total mass of each crystal was approximately 20 grams.

Density measurements. — The densities of two segments of a third crystal were determined through the use of Archimedes principle. One sample was cleaved from the bottom of the crystal, the other included portions of both the middle and the top. Both samples were weighed in air and in a liquid (KF8126 by 3M, density = 1.88 grams/cm^3). The densities of the samples were calculated from those weights.

The density for a crystal with composition $\text{Bi}_8\text{Te}_{12-x}\text{S}_x$ was determined as follows. The volume of a hexagonal unit cell is given by the expression $3a^2c/(2\sqrt{3})$, where $a = 4.1965\text{\AA}$ and $c = 29.4500\text{\AA}$ for this type of crystal (Soonpaa, 1960). Since there are fifteen atoms per unit cell the mass associated with each unit cell will be $.75 [8M_{\text{Bi}} + (12-x)M_{\text{Te}} + xM_{\text{S}}] / N$ where N is Avogadro's number. The value of x , and hence the composition, can be determined from the experimental density.

RESULTS

The results of the X-ray fluorescence analyses are summarized in Table 1. Five standard samples were examined in order to estimate the expected precision of the fluorescence analysis of the unknowns. Figure 1 is a graph of the ratio of sulfur $K\alpha$ intensity to bismuth $M\alpha$ intensity versus the sulfur subscript in the molecular weight formulas of the standards. The figure shows that to a good approximation, the ratio of the sulfur intensities to the bismuth intensities depended linearly on the sulfur concentration. Similarly the tellurium to bismuth intensity ratio depended linearly on the tellurium concentration. Standard deviations for the compositions of the unknowns were based on an error analysis of a linear least square fit of these results.

In the next set of experiments, the X-ray intensities of the samples from the top, middle, and bottom regions of the two crystals were determined. The three standards with compositions $\text{Bi}_8\text{Te}_7\text{S}_5$, $\text{Bi}_8\text{Te}_{6.5}\text{S}_{5.5}$, and $\text{Bi}_8\text{Te}_6\text{S}_6$ were analyzed in order to establish a calibration curve.

The last fluorescence experiments were designed to estimate the composition of the cap from the first crystal. The $\text{Bi}_8\text{Te}_{10}\text{S}_3$ and $\text{Bi}_8\text{Te}_9\text{S}_3$ standards were used for calibration.

The density measurements on the third crystal assume a composition of the form $\text{Bi}_8\text{Te}_{12-x}\text{S}_x$, i.e., the cation/anion ratio = 2/3. The determination also assumes that the crystal does not contain an appreciable number of defects, which would cause the density to differ from that which was calculated from crystallographic data. The value of x (the sulfur subscript) was $5.59 \pm .06$ for the sample from the bottom of the crystal and $5.42 \pm .04$ for the sample that included the middle and the top of the same crystal.

TABLE 1. Composition of Samples of $\text{Bi}_8\text{Te}_y\text{S}_x$

Tellurium subscripts

	Bottom	Middle	Top	Cap
1st crystal	6.5 \pm .21	6.74 \pm .21	6.98 \pm .21	10.7 \pm .4
2nd crystal	6.65 \pm .21	6.71 \pm .21	7.09 \pm .21	—

Sulfur subscripts

	Bottom	Middle	Top	Cap
1st crystal	5.41 \pm .15	5.26 \pm .15	5.15 \pm .15	2.2 \pm .2
2nd crystal	5.43 \pm .15	5.30 \pm .15	5.02 \pm .15	—

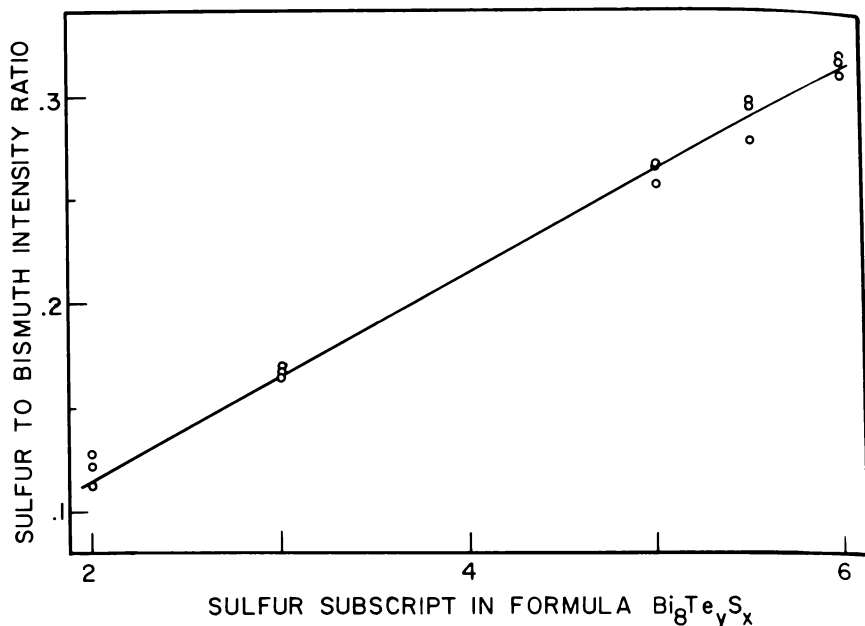


FIGURE 1. Intensity ratio (S-K α /Bi-M α) vs sulfur subscript for three series of standard samples.

DISCUSSION

The results of both the density and fluorescence measurements indicate that the crystals have a concentration gradient in the direction of growth of the crystal. The crystal starts growth rich in sulfur and low in tellurium relative to the melt composition, then the sulfur concentration decreases as the crystal grows while the tellurium concentration increases to a point where the composition is near the original melt composition $\text{Bi}_8\text{Te}_7\text{S}_5$ in the top region adjoint to the cap. The cap itself is very rich in tellurium and low in sulfur.

The fluorescence analysis indicates that the compositions of all three segments of both crystals satisfy the relation $\text{Bi}_8\text{Te}_{12-x}\text{S}_x$ expected for the known structure. The cap composition, however, seems to deviate from the above relation by more than the experimental uncertainty, indicating excess tellurium and sulfur in comparison to the amount of bismuth. This implies that the main part of the crystal has a net excess of bismuth. Crystals with similar lattice structures with melt composition Bi_2Te_3 also have excess bismuth (Satterthwaite and Ure, 1957). This could be caused by substitution of bismuth atoms for tellurium atoms, interstitial bismuth, or vacancies of tellurium in the crystal lattice. A small bismuth excess in

the main part of the crystal would not be resolvable within the experimental error of the fluorescence analysis.

The composition values determined by X-ray fluorescence and density measurements appear to differ by larger amounts than can be attributed to errors of the measurements. The difference might be caused in part by some type of systematic error in the preparation of the pellets for fluorescence study. It seems more likely, however, that the density measurement leads to too high a value for the sulfur content. There does not appear to be sufficient sulfur in the melt to produce the resulting crystal. Error in the compositions as determined through density measurements could be a consequence of defects such as layer separations parallel to the cleavage plane or to a large number of point defects.

ACKNOWLEDGMENTS

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APPLICATION OF THE WARBURG AND THE REAERATION TECHNIQUES IN THE DETERMINATION OF OXYGEN UPTAKE RATE CONSTANTS

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ABSTRACT

The oxygen uptake rate constant is an important factor in determining the wastes assimilation capacity of streams. The objectives of this study were: 1) to apply the reaeration and Warburg techniques to the measurements of oxygen uptake rate constants (k_1) in the Houston Ship Channel waters; 2) to determine whether the k_1 and the ultimate biochemical oxygen demand (BOD) values obtained by the reaeration method and the Warburg method are comparable. The average k_1 values were found to be 0.092 day^{-1} by the reaeration method and 0.22 day^{-1} by the Warburg method. The average BOD values were 5.4 mg/l and 13.7 mg/l by the reaeration method and the Warburg method respectively. The Warburg method yielded both higher k_1 and ultimate BOD values than the reaeration method. Correlations between the results obtained by the two methods were rather poor. The results indicated that the reaeration method is more suitable for the determination of k_1 values in the low oxygen uptake waters than the Warburg method.

INTRODUCTION

Dissolved oxygen (DO) is one of the most important water quality parameters of river waters. In order to develop a dissolved oxygen model for rivers, several factors should be considered: the amount of incoming wastewater flow, the amount of incoming fresh water flow, and the incoming organic loading. One parameter required for any dissolved oxygen model is the oxygen uptake rate at which the wastewaters are biologically degraded once they enter the river.

The biochemical oxygen demand (BOD) dilution method as described in the Standard Method (APHA, 1971) has long been used to determine the ultimate BOD and k_1 values of river waters. But it has several drawbacks: (1) in the dilution method the BOD exertion is under the quiescent condition rather than the turbulent condition actually occurring in the rivers, and (2) it requires a large number of BOD bottles for the BOD test because after the dissolved oxygen measurement is completed by titration the sample cannot be used for other tests. To circumvent these problems, the Warburg respirometric technique was introduced to the field of sanitary engineering by Caldwell and Lngelier (1948) for

determinations of BOD and k_1 values of wastewaters. Another method was the reaeration method such as the one reported by Marais (1968) which combined the use of a galvanic cell oxygen analyzer (Ingols, 1955; Lynn and Okun, 1955) and a reaeration technique (Theriault, 1931; Heukelekian, 1947; Kittrell and Kochtitzky, 1947; Orford et al, 1953; Elmore, 1955) to determine the BOD and k_1 values of wastewaters.

The studies reported herein were undertaken to apply the reaeration technique to the determination of k_1 values of the Houston Ship Channel waters and to compare results obtained with those of the Warburg method.

MATERIALS AND METHODS

This study consisted of two phases: (1) characterization of the Houston Ship Channel waters, and (2) determination of k_1 of the channel waters and Galveston Bay waters. The location of sampling stations in the Houston Ship Channel is shown in Table 1. Water samples were also taken from two stations in Galveston Bay. For characterization of channel waters six sampling stations were selected for testing. These stations were Mile points 0, 4, 10, 12, 16, 20 and 24. The mile points referred to the distance in miles upstream from the mouth of the ship channel to the location of the sampling station. The water samples were collected once a month for an eight-month period from June 1968 to April 1969. During each sampling run the water samples were taken from the middle of the Houston Ship Channel and at four depths: four feet below the surface, 1/3 of the total depth, 2/3 of the total depth and near the bottom of the channel. Since this task involved only the characterization of ship channel waters, the bottom sediment samples were not taken during the sampling run. The purpose of this phase of the study was to collect data on the composition of channel waters before the determination of k_1 of channel waters was carried out. Water samples collected during the last

TABLE 1. Location of Sampling Stations in the Houston Ship Channel

Sampling Station Ship Channel Mile point *	Description of Location
0	Morgan's Point
4	Near Baytown Tunnel
10	Near Battleship Texas
12	Junction with Patrick Bayou
16	Junction with Green's Bayou
20	Near Washburn Tunnel
24	Turning Basin

*Distance in miles upstream from the mouth of the Houston Ship Channel

sampling run in April 1969 were used in the determination of k_1 . The sampling stations were Mile points 0, 4, 10, 16, 20 and 24 in the Houston Ship Channel and two stations at Texas City Dike and Dickinson Bay. Only the composite water samples were used in the laboratory determination of k_1 . For the compositing procedure, equal amounts of water taken from the four depths mentioned previously at the sampling stations were put together to form composite samples. All samples collected for characterization and k_1 study were iced down after collection and transported to the laboratory immediately after the completion of the sampling run. During the sampling runs water temperature, dissolved oxygen (DO), pH, conductivity, salinity, and current velocity were measured at the sampling sites to provide information on the field characteristics of the ship channel.

Characterization of channel water samples, including the following measurements, was performed in the laboratory in accordance with the procedures described in "Standard Method for the Examination of Water and Wastewater" (APHA 1971): 5-day BOD, chemical oxygen demand (COD), chloride, pH, total suspended solids (TSS), volatile suspended solids (VSS), total phosphate and nitrogen. A Beckman Carbonaceous Analyzer was used to determine the total organic carbon (TOC) content in the water samples. The principle of the TOC test was that heated oxygen will oxidize any organic material to yield an amount of carbon dioxide. The water samples were first acidified and purged with nitrogen gas for a few minutes to strip off inorganic carbon contents in the samples. A small volume of water samples (20 microliters) was then injected into the combustion tube with a hypodermic syringe. Cobalt oxide impregnated asbestos packing in the combustion tube dispersed the sample and catalyzed the oxidation. The combustion tube was maintained at a temperature of 950°C. The carbon dioxide produced was carried along with the carrier gas oxygen through the Model IR-315 Infrared Analyzer. The recorder registered the resultant output signal which was proportional to the concentration of carbon dioxide in the oxygen stream and to the total organic carbon content in the water sample.

For the k_1 determination both the Warburg method and the reaeration method described below were employed. The laboratory experiments using both techniques were conducted at the same time. A Warburg apparatus manufactured by Precision Scientific Company was used in this study. It accommodated 18 standard Warburg manometers. The constant volume respirometer was the basic unit of the Warburg apparatus and each respirometer operated as a completely independent unit. The respirometers were placed in a common constant temperature water bath and were mounted on a common shaking mechanism. A shaking rate of 99 oscillations per minute was used during the experiment. The flasks used were 125 ml Erlenmeyer flasks which had been modified by the addition of three sample ports located on the sides of the flasks. Serum caps were inserted into the sample ports to make the flasks gas tight. A 3 ml center well fused to the bottom of the flasks held the 20% KOH solution for carbon dioxide absorption. During earlier tests it was discovered that alkali solution spilled out of the center well. To alleviate this problem, a small test tube of 0.6 cm outside diameter and 5 cm height were put into the center well of each flask to prevent

alkali spillage. A folded filter paper was inserted inside the test tube and the alkali solution was then added to it. The purpose of the filter paper was to provide a larger surface area for the carbon dioxide absorption. The Brodie's solution with a specific gravity of 1.033 was used as the manometer fluid. The operation of the Warburg apparatus followed the detailed techniques of "Direct Method" described in "Manometric Techniques" (Umbreit, Burris and Stauffer, 1964). In this paper the "Direct Method" was referred to as the Warburg Method. The sample size used was 25 ml and the water bath temperature was maintained at 20°C. In order to prevent photosynthesis the Warburg apparatus was covered with a black plastic sheet. The water samples were added to the Warburg flask. As the biochemical oxidation proceeded the dissolved oxygen in the water was consumed and carbon dioxide was produced and in turn was absorbed by the strong alkali solution in the test tube. A decrease in the partial pressure of oxygen in the flask was measured on the manometer and recorded. Thermobarometers were used to compensate the pressure change due to barometric pressure and temperature change. Duplicate samples were used in the Warburg test for a period of 108 hours.

For the reaeration method, water samples of 300 ml were added to the BOD bottles and were aerated for 10 minutes to bring the DO content to a level above 6 mg/l. The experiment was conducted in a 20°C constant temperature room. Magnetic stirrers were used to keep the bottle contents well mixed to facilitate the biochemical oxidation. Single samples were used in this test since each single sample required one magnetic stirrer. The DO contents in the samples were first measured at the beginning of the experiment and were also determined at various time intervals. When the DO level dropped to 1 mg/l the sample was reaerated to maintain an aerobic condition in the water samples. The experiment lasted for 108 hours.

In the field of sanitary engineering the BOD exertion is commonly expressed as first order reaction as indicated below:

$$Y = L(1 - 10^{-kt})$$

in which

Y = amount of BOD exerted at time t (mg/l)

L = the ultimate BOD (mg/l)

k = the reaction rate constant for BOD exertion (day⁻¹)

t = time (day)

Consequently, the oxygen consumption data collected in this study was analyzed based on the first order reaction kinetics. The Reed-Therault method (Therault, 1927) was used to compute k, and L of water samples.

RESULTS AND DISCUSSION

For the eight monthly sampling runs conducted between June, 1968, and April, 1969, channel waters were analyzed for different water quality parameters. The results are listed in Table 2. The range, mean and standard deviation for each

TABLE 2. Summary of results of Houston Ship Channel water analysis

Sampl. Station	TOC mg/l	BOD ₅ mg/l	COD mg/l	Chloride mg/l	pH	TSS mg/l	VSS mg/l	Total P mg/l	Org-N mg/l	NO ₃ -N mg/l	NO ₂ -N mg/l	NH ₄ -N mg/l
0	6-12* 9±2**	1.2-19 5.6±5.6	32-135 53±34	3112-9090 6326±2192	6.9-8.1 7.6±0.4	80-163 124±30	18-163 73±49	0.49-2.08 1.10±0.54	0.36-1.43 0.82±0.32	0 0	0.13-1.53 0.46±0.49	0.10-3.33 1.52±1.21
4	8-14 11±6	1.2-5.6 3.4±1.6	32-36 34±2	2658-4650 3520±755	6.8-7.7 7.2±0.3	58 58	17 17	0.92-2.88 1.75±0.74	0.05-0.73 0.55±0.29	0 0	0.17-0.67 0.32±0.21	0.84-2.20 1.33±0.52
10	7-16 13±3	3.3-21 7.3±5.3	27-63 46±11	1330-5770 3520±1545	6.6-7.8 7.4±0.4	49-68 56±7	11-56 38±16	2.12-4.50 2.97±0.92	0.65-5.08 2.18±1.58	0 0	0.06-0.50 0.24±0.14	0.64-6.49 3.93±1.96
12	9-16 12±4	3.7-11 6.2±2.8	29-62 46±12	2488-5970 4280±1135	7.7-6 7.4±0.2	34-76 53±17	8-60 35±21	2.16-10.40 5.61±3.20	0.80-4.34 1.68±1.36	0 0	0.06-0.50 0.25±0.16	2.52-6.78 4.10±1.63
16	9-27 14±5	3-9.4 5.7±2.4	30-61 46±10	1610-5500 3388±1284	6.8-7.6 7.2±0.3	38-79 60±18	13-79 42±27	1.71-5.70 4.22±1.41	0.60-5.88 1.70±1.73	0 0	0.04-0.50 0.23±0.14	2.53-16.10 5.81±4.44
20	6-21 14±5	2.2-10.9 5.4±2.8	39-58 47±6	1400-5600 3089±1649	6.8-7.6 7.3±0.3	49-60 54±4	12-38 25±11	1.65-9.17 3.98±2.50	0.75-1.60 1.12±0.31	0 0	0.05-0.30 0.19±0.09	2.25-5.15 3.27±0.94
24	5-17 11±4	4.1-8 5.4±1.4	28-60 45±11	386-4250 2385±1646	6.8-7.7 7.4±0.3	48-176 93±59	20-40 27±9	1.48-3.60 2.76±0.82	0.63-1.41 0.95±0.25	0 0	0.04-0.23 0.15±0.16	1.93-3.65 2.93±0.75
Average of all stations												
12	5.6	45	3787	7.4	71	37	3.19	1.29	0	0.26	3.27	

*Range of value of parameter analyzed of 8 sampling runs from June 1968 to April 1969

**Mean and standard deviation of value of parameter analyzed of 8 sampling runs from June 1968 to April 1969.

parameter were determined based on the laboratory data of that parameter for the eight sampling runs. The TOC and COD values were considerably higher than 5-day BOD values and also the calculated ultimate BOD values based on a k_1 value of 0.09 to 0.22 as reported in this study indicating that a large portion of organic material was non-biodegradable. The chloride concentration increased with distance downstream of Mile 24 (Port of Houston) due to the fact that the amount of sea water mixed with fresh water increased as the channel water traveled toward the mouth of the ship channel at Mile 0 (Morgan's Point). In most parts of the channel, a large portion of the total solids was total dissolved solids, and only a small portion is total suspended solids. The value of total suspended solids averaged 71 mg/l and the volatile suspended solids averaged 37 mg/l indicating less than half of the total suspended solids was volatile suspended solids. The average values of organic nitrogen and phosphate contents of channel waters were 1.3 and 3.2 mg/l, respectively indicating that the channel water was not nutrient deficient of N and P. The ammonia nitrogen content of channel water averaged 3.3 mg/l. Nitrification of channel water was insignificant since the nitrate and nitrite-nitrogen content of the channel water were low.

The oxygen uptake rate study was conducted on water samples taken at eight sampling stations of the Houston Ship Channel water and Galveston Bay using both the Warburg method and the reaeration method as described before. The total oxygen consumption versus time is shown in Figures 1 and 2. For all eight water samples the Warburg method tended to yield higher values of total oxygen consumption. The water sample size used in the Warburg method was only 25 ml compared to a 300 ml sample volume used in the reaeration method. For channel waters with a low oxygen uptake rate, the sample volumes used in the Warburg method were not large enough to produce a significant reading in the manometric measurement. The oxygen uptake rate (k_1) and ultimate BOD values (L) at 20°C were calculated using the Reed-Therriault method and the results are listed in Table 3. The oxygen uptake rate values ranged from 0.095 to 0.427 day⁻¹ with an average value of 0.22 day⁻¹ for the Warburg method, and varied from 0.027 to 0.14 day⁻¹ with an average value of 0.092 day⁻¹ for the reaeration method. The ultimate BOD values varied from 7.28 to 22.2 mg/l with an average value of 13.74 mg/l for the Warburg method, and ranged from 2.59 to 12 mg/l with an average of 5.37 mg/l for the reaeration method. The reaeration method appeared to be more reliable than the Warburg method since the ultimate BOD values by the reaeration method were closely related to the actual BOD values of the channel waters as listed in Table 2. The Warburg method tended to give both higher BOD and k_1 values than the reaeration method. The correlation of k_1 values and that of L values obtained by the two methods are rather poor as indicated by the coefficients of linear correlation of 0.24 and 0.318 for k_1 and L values, respectively.

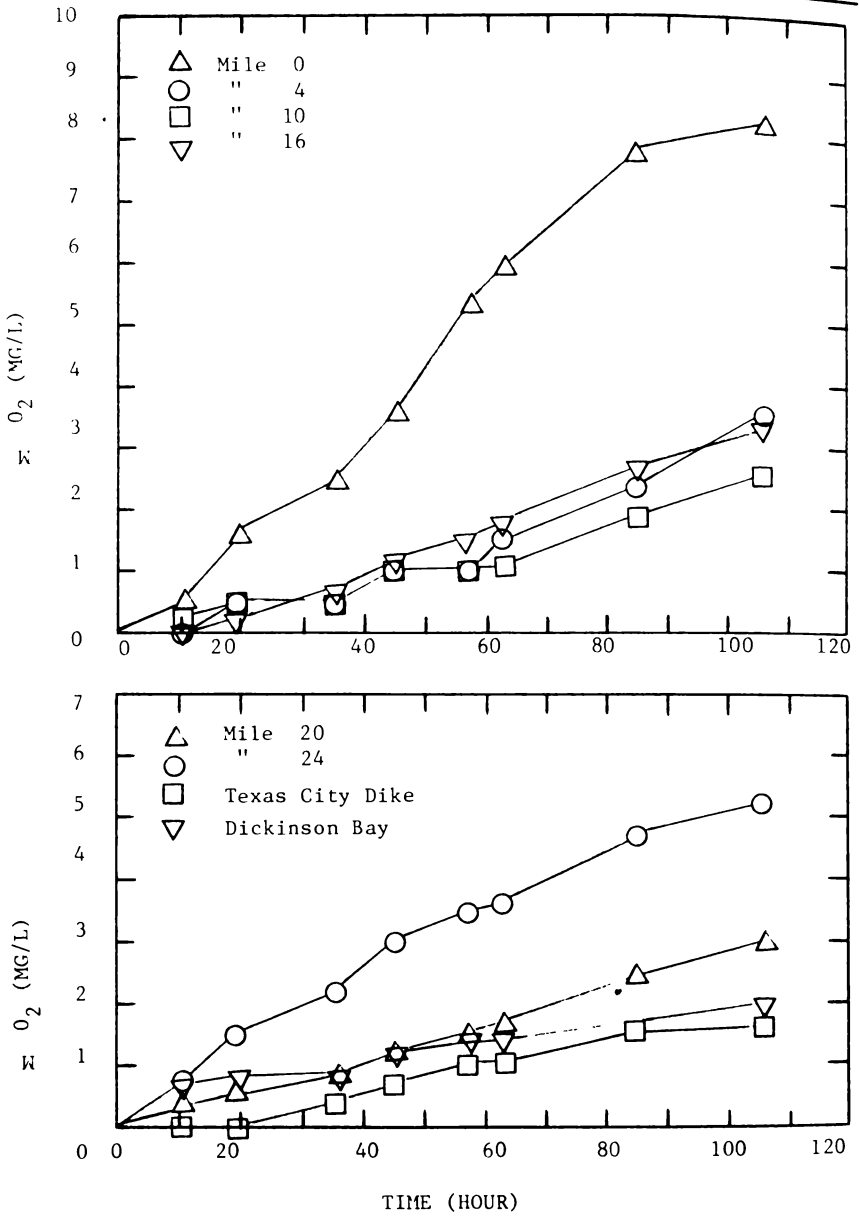


FIGURE 1. Total oxygen consumption vs. time by reaeration method

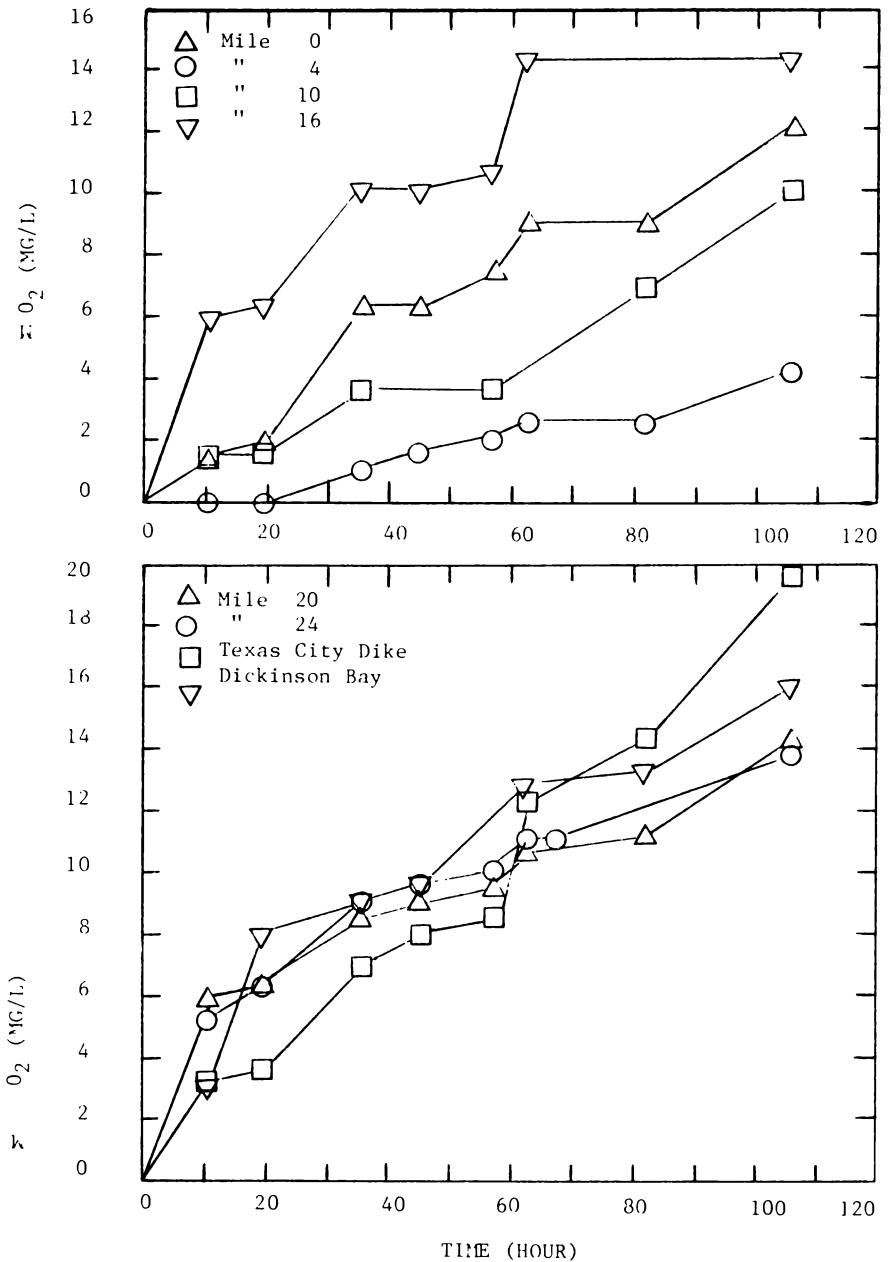


FIGURE 2. Total oxygen consumption vs. time by Warburg method

TABLE 3. Summary of k and L values by Warburg and Reaeration method

Sampling Station	$(k_1)_{20}$ (day^{-1})		L_{20} (mg/l)	
	Warburg	Reaeration	Warburg	Reaeration
Mile 0	0.196	0.130	11.50	12.00
Mile 4	0.097	0.027	7.28	2.59
Mile 10	0.095	0.057	12.80	3.85
Mile 16	0.278	0.105	14.60	3.67
Mile 20	0.268	0.044	14.10	6.88
Mile 24	0.427	0.094	11.90	8.66
Texas City Dike	0.138	0.140	22.20	2.57
Dickinson Bay	0.263	0.140	15.50	2.73
Average Value	0.22	0.092	13.74	5.73

CONCLUSIONS

1. In this study the oxygen uptake rate constant k_1 for the Houston Ship Channel waters was found to vary from 0.027 to 0.14 day^{-1} with an average value of 0.092 day^{-1} by the reaeration method.
2. The correlation of k_1 and L values obtained by the Warburg method and the reaeration method were poor.
3. The reaeration method was more suitable than the Warburg method for the determination of k_1 values in the low oxygen uptake rate waters, such as in the case of the Houston Ship Channel waters.

ACKNOWLEDGMENTS

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A MOSASAUR FROM THE NIOBRARA FORMATION (CRETACEOUS) OF RANSOM COUNTY, NORTH DAKOTA

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ABSTRACT

In September, 1974, fossil remains of a mosasaur were recovered from the Niobrara Formation (upper Cretaceous) in a road-cut exposure in northwestern Ransom County, North Dakota. The remains consist of well-preserved to highly-fragmented skeletal elements, including teeth, palatal fragments, cranial fragments, quadrates, vertebrae, rib fragments, limb fragments, and phalanges. Studies of the remains and their distribution on the surface of the outcrop and in the adjacent undisturbed rock suggest that they represent a single individual. Attempts were made to identify the genus of the specimen based on comparisons of the quadrates and teeth with published descriptions. No precise match was found, but the closest genera seem to be *Clidastes* and *Platycarpus*. Finds of mosasaur fossils are rare in North Dakota, consisting of a few bones and teeth. The present find is the most complete so far recorded in the state.

INTRODUCTION

The mosasaurs were large marine lizards that evolved from primitive stocks in middle Cretaceous time and became extinct at the end of the Cretaceous Period, about 70 million years ago. From their osteology, the distribution of their fossilized remains and the rock types in which they occur, it is clear that they were active, near-surface predators, living in the warm, shallow, epicontinental seas of the late Cretaceous. Adults of various species were from 2 meters to over 15 meters long (Russell, 1967). Mosasaur remains are known from every continent in the world except Antarctica, and in North America their fossils have been recovered from upper Cretaceous rocks from New Jersey to California and from Mexico to the Northwest Territories (Russell, 1967). According to Russell (1967), most of the well-preserved mosasaur fossils found in North America have come from the upper Cretaceous chalky Niobrara Formation in the northern Great Plains, and it was this unit in which the Ransom County specimen was found.

Only three records of mosasaur remains from North Dakota appear in the literature. A hind paddle of the genus *Platycarpus* is listed by Williston (1902) as probably from the Niobrara Formation near Milton, Cavalier County. Teeth of the genus *Mosasaurus* have been found in the Fox Hills Formation near Bismarck (Leonard, 1912, p. 2), and unspecified mosasaur bones were reported by Laird (1951) from the Niobrara of the Pembina Hills.

In addition to these, the author found a vertebra and rib fragments of a mosasaur in the bottom of a road ditch below an outcrop of Niobrara Formation in the SE $\frac{1}{4}$ SE $\frac{1}{4}$ sec. 35, T. 137 N., R. 58 W., Barnes County, about four miles northwest of the Ransom County find.

THE RANSOM COUNTY FIND

The Ransom County mosasaur (NDSU Specimen Z-11) was found by participants in an Audubon Club-sponsored geology field trip led by the author in September, 1974. Mrs. Nicholas Frank made the initial discovery on the side of a road ditch in the NW $\frac{1}{4}$ NW $\frac{1}{4}$ sec. 17, T. 136 N., R. 57 W., Ransom County. The remains were partly on the surface and partly enclosed in undisturbed Niobrara chalky clay. No detailed stratigraphic studies of the Cretaceous of this area have been done, but the presence of Pierre Shale at slightly higher elevations a few miles to the north suggests that the outcrop at the site of the find is near the top of the Niobrara. All of the bones lay within an area of about four square meters and several skeletal elements such as teeth and vertebrae showed a crude alignment. This configuration, along with the compatibility of size and lack of duplication of the various skeletal elements, suggests that the remains are of a single individual.

Of the several hundred bones and bone fragments recovered, the following are well enough preserved to be identified.

1. *Teeth.* Parts of at least 20 teeth (based on intact tips) were found. All but one had been broken away from the jaw. Bony tooth bases with broken stubs of teeth and parts of jaw bone attached are common. One fragment of palatal bone with the stub of a palatal tooth was found.
2. *Quadrates.* These bones are parts of the skull that serve to articulate the lower jaw. Both left and right quadrates are present, though parts of them are missing.
3. *Vertebrae.* Parts of seven vertebrae were found, including the atlas intercentrum and cervical, dorsal and caudal vertebrae. All but the atlas intercentrum have parts broken away.
4. *Phalanges.* One complete distal phalanx and parts of five other phalanges were recovered.
5. *Ribs.* Eight rib fragments were found.

IDENTIFICATION

Attempts to identify Specimen Z-11 were based entirely on comparisons of the quadrates and marginal teeth with the written descriptions and drawings of those parts in Russell's comprehensive paper on American mosasaurs (1967). The quadrates and marginal teeth were used because they were reasonably well-preserved and are often of diagnostic value in identification.

The quadrates (Figure 1) were reconstructed from several broken fragments and some parts are missing. The ventral extremities and parts of the medial and lateral surfaces of both quadrates are missing as is the tip of the suprastapedial process of the left quadrate. The following description was derived from the remaining parts:

Maximum width from anterior surface of shaft to posterior surface of suprastapedial process, 56 mm. Medial boundary of anterior surface of quadrate shaft concave laterally. Suprastapedial process large, extending for a vertical distance of about 30 mm below base of stapedial pit, and apparently not fused with infrastapedial process. Sides of suprastapedial process very slightly constricted medially, expanded distally and bluntly terminated. Muscle attachment pit on distal surface of suprastapedial process roughly circular with short, neck-like extension dorsally. Stapedial pit elliptical.

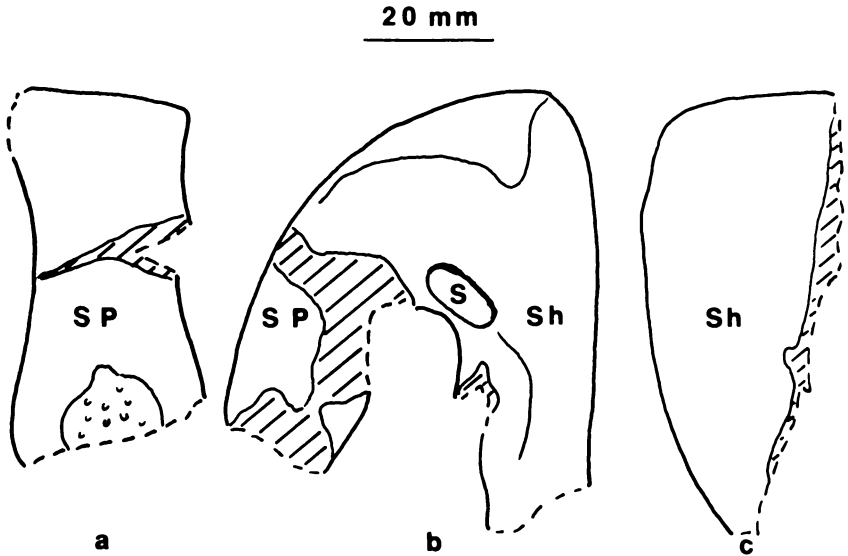


FIGURE 1. Ransom County Mosasaur (NDSU Specimen Z-11), Niobrara Formation. Left quadrate, showing posterior face of suprastapedial process (a), medial aspect (b), and anterior face of shaft (c). SP = suprastapedial process, S = stapedial pit, Sh = shaft. Dashed lines and shaded areas indicate parts of bone broken away.

Several marginal teeth (Figure 2) are well preserved and have the following characteristics:

Crown divided into lingual and buccal surfaces by well-developed anterior and posterior carinae. Lingual surface with many fine vertical striae; buccal surface with fewer striae and moderately faceted. Cross-section of tooth base elliptical with lingual portion more rounded and buccal portion slightly flattened. Crown axis curved with posteriointernal inclination. Dimensions of four intact teeth 16 mm - 19.8 mm high and 8.6 mm - 10.2 mm in diameter at crown base.

20 mm

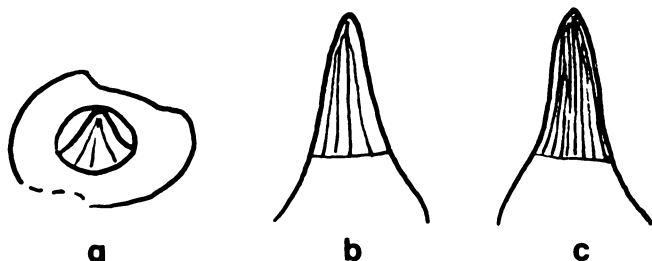


FIGURE 2. Ransom County Mosasaur (NDSU Specimen Z-11), Niobrara Formation. A marginal tooth showing top view (a), lateral view (b), and medial view (c).

The quadrates and teeth of Z-11 were compared with the thirteen North American and three foreign genera described by Russell (1967) on the basis of the following characteristics:

1. Size and shape of the suprapedial process.
2. Whether the supra- and infrapedial processes are fused or separate.
3. Shape of medial boundary of anterior surface of quadrate shaft.
4. Shape of stapedial pit.
5. Whether the teeth are bicarinate or not bicarinate.
6. Whether the tooth enamel is smooth or striated.

These characteristics were selected because they are clearly evident on the Z-11 material and they are frequently listed in Russell's generic diagnoses.

Comparisons were made first with known Niobrara genera, then with other genera of the North American interior, then with remaining North American genera, and finally with foreign genera.

Niobrara genera.—

Clidastes. There was a reasonably good match between Z-11 and this genus in the first five characteristics. However, Z-11 has abundant striations on the teeth, whereas *Clidastes* has smooth tooth enamel.

Mosasaurus. Z-11 matches this genus in characteristics 2, 4, 5 and 6, but fails to match in 1 and 3. The suprapedial process is relatively short in *Mosasaurus* and long in Z-11, and the medial boundary on the anterior face of the quadrate shaft is straight in *Mosasaurus* and concave laterally in Z-11.

Platicarpus. Z-11 matches this genus in all characteristics except number 3. *Platicarpus* has a straight medial boundary on the anterior surface of the quadrate shaft.

Ectenosaurus. Z-11 matches this genus only in the nature of the teeth. The *Ectenosaurus* quadrate is distinctly different from that of Z-11, having the supra- and infrastapedial processes broadly fused and a rectangular stapedial pit.

Halisaurus. No data are given by Russel for characteristics 3 through 6 for this genus. Like Z-11, the suprastapedial process is relatively long and the supra- and infrastapedial processes are separate.

Tylosaurus. Quadrate characteristics of *Tylosaurus* are distinctly different from those of Z-11. The suprastapedial process is distally pointed, the stapedial pit is rectangular and the medial boundary of the anterior surface of the shaft is straight. The teeth of this genus are bicarinate and vertically ridged, but lack the faceting noted in Z-11.

Other genera of the North American interior.—

Globidens. This genus has been found in the lower Pierre Formation. No data on its quadrates are listed by Russell, but its unique spherical teeth are quite different from those of Z-11.

Plioplatecarpus. This genus has been found in the upper Pierre Formation. Z-11 matches this genus in characteristics 2, 5 and 6, but differs in the shape of the stapedial pit which is reniform in *Plioplatecarpus*. The shape of the suprastapedial process in Z-11 is close to that described for this genus but Z-11 has a slight medial constriction as opposed to parallel sides in *Plioplatecarpus*.

Prognathodon. Fossils of *Prognathodon* have been found in the upper Pierre. Z-11 is a nearly complete mis-match with this genus. The only one of the six characteristics they have in common is bicarinate teeth.

Other North American genera.—

Amphekepubis. This genus is based on post-cranial remains found in northern Mexico. No data are available on quadrates or teeth.

Liodon. This genus has been found in the upper Cretaceous rocks of New Jersey. No data on quadrates are available, but the teeth have smooth enamel, thus differing from those of Z-11.

Plotosaurus. This genus is known from the upper Cretaceous rocks of California. The teeth of Z-11 are similar to those described for *Plotosaurus*, but the quadrates differ in characteristics 1 and 3. The suprastapedial process in *Plotosaurus* is relatively short and the medial boundary of the anterior face of the shaft is straight.

Plesiotylosaurus. This genus was found in the upper Cretaceous rocks of California. Supra- and infrastapedial processes are fused in this genus making the quadrate distinctly different from that of Z-11. *Plesiotylosaurus* teeth have smooth enamel as compared with the striated enamel of Z-11.

Foreign genera.—

Compressidens. This genus has been found in the upper Cretaceous rocks of Belgium. No data are available on its quadrates. The teeth of *Compressidens* are described as identical to those of *Clidastes* and would therefore be smooth enameled as opposed to the striated enamel of Z-11.

Taniwhasaurus. This genus was found in upper Cretaceous rocks of New Zealand. Its teeth are striated like those of Z-11, but lack carinae. No data are available on quadrates.

Dollosaurus. This genus is from the upper Cretaceous rocks of southern European Russia. No data are available on the quadrates. The teeth are bicarinate but the enamel is not described.

Hainosaurus. This genus is from the upper Cretaceous rocks of Belgium. The only quadrate characteristic described is the shape of the stapedial pit which is longitudinally compressed ellipse. While the stapedial pit of Z-11 is elliptical, it is not longitudinally compressed. The teeth are not described.

In summary, the teeth and quadrates of specimen Z-11 are not a precise match with those of any of the genera described by Russell. Of the Niobrara genera for which teeth and quadrates are adequately described, Z-11 shares a large number of characteristics with both *Clidastes* and *Platecarpus*, but fails to match in one important characteristic in each case. At least one major point of difference also exists between Z-11 and all of the other genera except the Niobrara genus *Halisaurus*, the Mexican genus *Amphikepubis* and the Russian genus *Dollosaurus* for which present data are inadequate for comparison. Pending further data the genus of Z-11 remains unknown.

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THE EFFECT OF 6-DAY EXPOSURE TO NORMOXIC AND HYPEROXIC-HYPERBARIC ENVIRONMENTS UP TO 40 ATA UPON THE GROWTH AND TISSUE WATER CONTENT IN GUINEA PIGS

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ABSTRACT

Exposure to hyperbaric-hyperoxic environments has been reported to alter body weight. Growing guinea pigs were exposed to 20 and 40 ATA with pO_2 levels of 200 and 600 mm Hg for 2, 4 and 6 days, and compared with air controls. Body weight and individual tissue water content were recorded for each animal. The control group of animals averaged gains in body weight, while the treated animals all lost weight. The life span of treated animals was significantly shortened. Livers, kidneys, and adrenal glands of the treated animals showed alterations in water content after treatment, while the same treatment did not alter the water content in skin, muscle, bone, brain, blood, and heart.

INTRODUCTION

Alterations in growth rate, tissue water content, and tissue morphology have been reported to accompany hyperbaric and hyperoxic environmental conditions. Bitter and Nielsen (1) in 1972 showed that rats exposed to 5, 10, 20, or 30 atmospheres absolute (ATA) conditions responded to the hyperbaric stress with increased excretion of corticosterone. Changes have been reported concerning water balance in rats under hyperbaric conditions by Boelkins, Syftestad, and Brumleve (2).

The purpose of these experiments was to examine changes in water content of various tissues at 20 ATA and 40 ATA normoxic, and at 20 ATA and 40 ATA hyperoxic, environmental conditions. Alterations in growth rate and tissue morphology due to treatment were also examined.

MATERIALS AND METHODS

Young, male guinea pigs in a rapid-growing phase, which weighed between 185-415 grams, were the subjects of these experiments. Treated animals were housed in individual plastic cages inside chambers of the High Pressure Life Laboratory at the University of North Dakota. Each chamber held 4 cages, and 12 chambers were used on both of the 2 treatment periods. Twenty-four animals were kept as controls in similar groups of 4 individual cages housed in a 1 ATA air chamber.

All of the guinea pigs were given free access to water and Ralston Purina guinea pig food. The cages were lighted in 12-hour time periods (0800-2000 hr) and kept dark for 12 hours (2000-0800 hr) each day.

The 40 ATA He-O₂ groups were kept at 90°F and relative humidity of 40%. The 20 ATA He-O₂ groups were maintained at 84°F and relative humidity of 40%, while the control groups were at 80°F and 40% relative humidity.

The guinea pigs could be observed through windows on the top of each chamber. The animals that were treated for 6 days had their cages and chambers cleaned and resupplied with food and water after 3 days.

The animals were placed in the chambers, sealed off, and the pressure gradually increased at a rate of 20 ATA per hour. There were 5 different sets of conditions for this study, which included: 20 ATA normoxic, 40 ATA normoxic, 20 ATA with pO₂ level at 600 mm Hg, 40 ATA with pO₂ level at 500 mm Hg, and an air control group. In 4 of these groups, there were 24 animals, while the other group contained only 20 animals. The O₂ level was maintained by being frequently monitored with a gas chromatograph. The chambers were decompressed using a 7-hour decompression schedule.

Immediately after decompression, the animals were anesthetized by an injection of phenobarbitol in a dosage of 32 mg/kg. Final weights were taken and autopsies performed on the treatment and control animals. Samples of bone, blood, skin, muscle, and whole tissues of brain, heart, lungs, kidneys, adrenals, and liver were taken from each animal. These were weighed immediately and frozen in liquid nitrogen. The tissues and organs were kept in a freezer for a few days and then freeze-dried and reweighed.

Wet-dry ratios for the tissues and initial and final body weights in grams were analyzed, using a 2-tailed student t-test for unrelated means. A 2-way analysis was applied in comparing treatment animals with the air controls and within each treatment condition. Levels of significance were recorded for $p < .05$, $p < .01$, and $p < .001$.

RESULTS

Values for wet-dry ratios for whole tissue samples of adrenal glands, lung, liver, and kidney, as well as initial and final total body weights in grams can be seen in Table 1.

It was found that animals under pressure, both at 20 ATA and 40 ATA environments, lost weight, whether under normoxic or hyperoxic conditions. The decrease in body weight compared to air controls sacrificed after the same length of time was significant under each of the treatment conditions except the 2-day exposure to 20 ATA pressure at normoxic levels. Weight losses at 4 and 6-day exposures to 20 ATA at normoxic level were statistically significant when compared with 4 and 6-day air controls at $p < .001$. Two-day exposures to 40 ATA at normoxic level were significant at $p < .01$, while 4-day exposures to 40 ATA at normoxic level were significant at $p < .01$. Two-day exposures to 20 ATA with hyperbaric levels (pO₂ = 600 mm Hg) significantly decreased body weights at $p < .01$ when compared with 2-day air controls, while 4-day exposures to the same conditions lower body weights at a significance level of $p < .001$. Exposure for both 2 and 4 days to 40 ATA and hyperoxic level (pO₂ = 500 mm Hg)

TABLE 1. Summary of Data for Experimental Groups

Control	0 Day	2-Day	4-Day	6-Day
Adrenal, wet/dry		1.090 ± 0.537	1.400 ± 0.255	1.619 ± 0.424
Lung, wet/dry		4.346 ± 0.494	4.085 ± 0.221	4.266 ± 0.302
Liver, wet/dry		2.285 ± 0.465	2.684 ± 0.459	2.499 ± 0.050
Kidney, wet/dry		3.396 ± 0.179	3.184 ± 0.320	3.201 ± 0.219
Body Weight (grams)	322.125 ± 81.393	327.250 ± 80.768	332.750 ± 48.566	380.500 ± 40.345
	319.375 ± 45.503			
	343.500 ± 50.931			
20 ATA Normoxic				
Adrenal, wet/dry		2.110 ± 0.740	2.580 ± 0.741	2.650 ± 0.446
Lung, wet/dry		4.315 ± 0.216	4.277 ± 0.261	4.085 ± 0.129
Liver, wet/dry		2.492 ± 0.143	2.911 ± 0.436	2.575 ± 0.072
Kidney, wet/dry		3.725 ± 0.289	3.744 ± 0.264	3.627 ± 0.269
Body Weight (grams)	320.500 ± 36.235	276.375 ± 49.779	228.875 ± 43.587	232.250 ± 36.176
	297.750 ± 43.361			
	282.250 ± 43.996			

20 ATA High Pressure O ₂	0 Day	2-Day	4-Day
Adrenal, wet/dry		2.372 ± 0.529	2.284 ± 0.675
Lung, wet/dry		4.212 ± 0.235	4.345 ± 0.330
Liver, wet/dry		3.018 ± 0.485	2.912 ± 0.390
Kidney, wet/dry		3.777 ± 0.521	4.303 ± 0.543
Body Weight (grams)	253.333 ± 27.741	231.833 ± 32.003	187.700 ± 19.448
	238.700 ± 19.464		
40 ATA Normoxic			
Adrenal, wet/dry		1.660 ± 0.395	2.155 ± 0.430
Lung, wet/dry		4.227 ± 0.168	4.060 ± 0.171
Liver, wet/dry		2.375 ± 0.240	2.883 ± 0.335
Kidney, wet/dry		3.514 ± 0.206	3.732 ± 0.161
Body Weight (grams)	282.875 ± 19.349	227.250 ± 22.621	218.500 ± 15.317
	268.833 ± 14.669		
40 ATA High Pressure O₂			
Adrenal, wet/dry		1.804 ± 0.616	2.197 ± 0.464
Lung, wet/dry		4.450 ± 0.355	4.349 ± 0.172
Liver, wet/dry		2.183 ± 0.231	2.725 ± 0.123
Kidney, wet/dry		3.318 ± 0.294	3.495 ± 0.298
Body Weight (grams)	304.454 ± 29.742	251.182 ± 23.837	264.667 ± 36.253
	331.000 ± 57.111		

decreased body weight significantly, at $p < .05$, when compared with air controls of 2 and 4 days.

Figures 1 and 2 demonstrate the depressed growth rate caused by treatment. While the air control groups showed steady increases, all of the treated groups showed a sudden loss in weight after the first 2 days. The normoxic groups were able to reverse this trend and began to regain some weight; however, the hyperoxic groups continued to lose. Between the 2 hyperoxic groups, the 20 ATA animals were unable to even slow their rate of loss.

In the 40 ATA groups, both normoxic and hyperoxic, only 1 animal had died after days. In the 4-day groups for both of these conditions, one-half of the animals had died. In the 20 ATA groups, normoxic and hyperoxic, no animals were dead after 2 days. Out of the 4-day groups, 3 out of 20 animals had died. In the only treated group that went for 6 days, the 20 ATA normoxic, one-half of the animals had died.

The adrenal glands were found to be hydrated to a significant degree under each treatment condition when compared with air controls. Data concerning wet-dry ratios for the adrenal glands are shown in Table 2. Under the 20 ATA-normoxic condition, the wet-dry values were increased significantly at $p < .05$ for

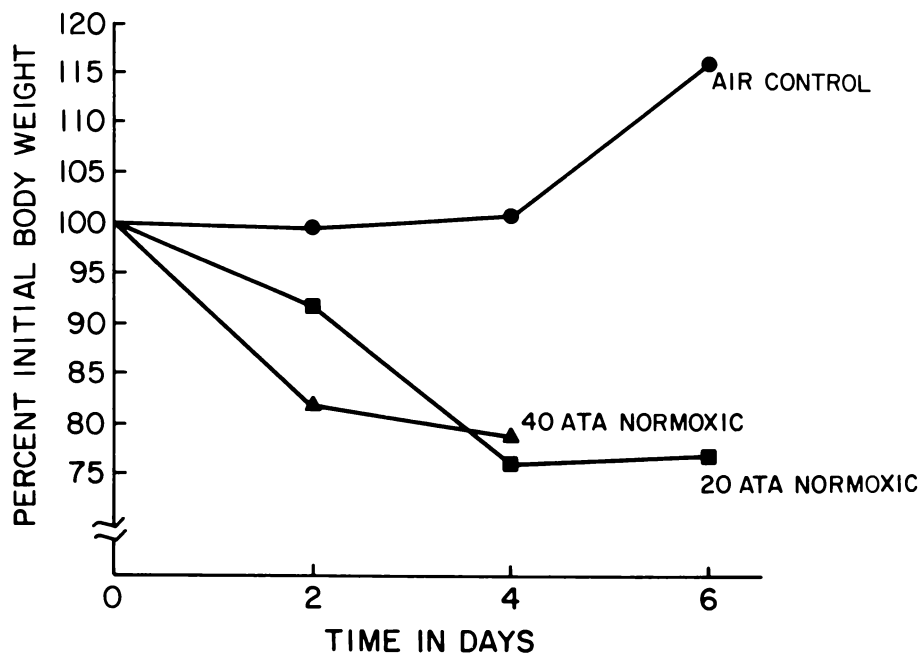


FIGURE 1. Growth Curve. Final weight compared with initial weight. Normoxic conditions and air controls.

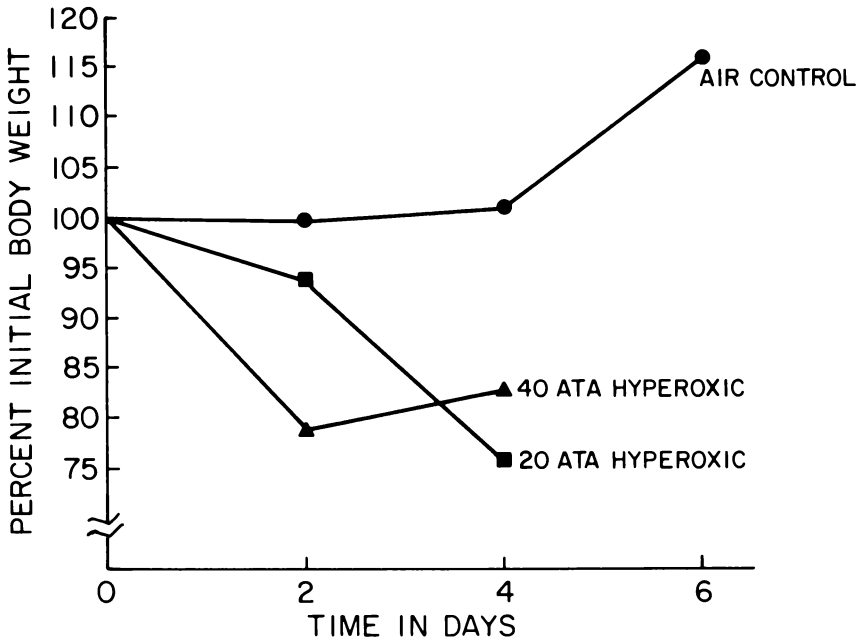


FIGURE 2. Growth Curve. Final weight compared with initial weight. Hyperoxic conditions and air controls.

2-day exposure and at $p < .01$ for 4 and 6-day exposure. Significance at $p < .001$ was determined for both 2 and 4-day exposure to 20 ATA with hyperoxic conditions. Under the 40 ATA-normoxic environment, 2-day exposure yielded a significant difference at $p < .05$, whereas 4-day exposure was at $p < .01$. Exposure to a 40 ATA-hyperoxic environment yielded similar increases with significance at $p < .05$ for 2-day and at $p < .01$ for 4-day.

The wet-dry ratios of the kidneys were found to be increased by both hyperbaric and hyperbaric-hyperoxic treatment, especially during 4 and 6-day exposure (Refer to Table 1). Under 20 ATA-normoxic conditions, the kidneys were significantly hydrated compared with air controls at $p < .05$ for 2 and 6-day exposure and at $p < .01$ for 4-day exposure. Exposure to 20 ATA-hyperoxic conditions for 4 days was significant at $p < .001$. At 4-day exposure to 40 ATA-normoxic conditions, significance was found to be at $p < .01$. Significant increases of hydration in the kidneys at $p < .05$ were found when comparing 4-day exposure at 20 ATA-hyperoxic with 4-day exposure at 20 ATA-normoxic conditions.

Treatment increased hydration in the liver but was found to be a significant alteration only under the 2-day 20 ATA-hyperoxic condition where the significance was at $p < .001$ when compared with 2-day air controls. The livers of the 2-day hyperoxic animals with pressure at 20 ATA were also found to be more

TABLE 2. Adrenal Glands. Wet-dry ratios of treatment conditions compared with air controls.

Treatment	2-Day	4-Day	6-Day
Control	1.090 ± 0.537	1.400 ± 0.255	1.619 ± 0.424
40 ATA, high O ₂	1.804 ± 0.616*	2.197 ± 0.464**	
40 ATA, normoxic	1.660 ± 0.395*	2.155 ± 0.430**	
20 ATA, high O ₂	2.372 ± 0.529***	2.824 ± 0.675***	
20 ATA, normoxic	2.110 ± 0.740*	2.580 ± 0.741**	2.650 ± 0.446**

*p < .05

**p < .01

***p < .001

hydrated than the livers of the 2-day normoxic animals under the same pressure with significance at $p < .05$.

Other tissues (i.e., brain, heart, lung, bone, blood, muscle, and skin) did not show significant alterations under the treatment conditions.

DISCUSSION

The definite weight loss experienced under all treatment conditions substantiates prior work done by Boelkins, *et al.* (2).

Although Figures 1 and 2 show that in the case of the normoxic group, the 40 ATA animals stopped losing weight and the hyperoxic 40 ATA animals greatly reduced their rate of loss after 4 days, as a group these animals do not represent a successful adjustment to the treatment. For 4-day animals, both of these groups had a 50% death rate. The 20 ATA animals under both hyperoxic and normoxic conditions had a death rate of only 15% for 4-day animals, but 50% of the 20 ATA-normoxic 6-day group died. High pressure treatment thus greatly shortens the life span of the guinea pigs with 40 ATA pressure having considerably faster lethality.

The decrease in body weight growth, along with significant hydration of the adrenal glands and kidneys, is indicative of the stressful conditions produced by exposure to both hyperbaric and hyperbaric-hyperoxic environments. Bitter and Nielsen (1) found that body weight losses generally corresponded to the extent of

diuresis. It was reported by Boelkins, *et al.* (2) that rats exposed to a 20 ATA He-O₂ environment for 2 to 12 weeks consumed more water and produced larger volumes of dilute urine throughout the 12-week exposure period than did comparable air control rats.

Because the treated guinea pigs had decreased body weight and an increased amount of urine production, it might be expected that some tissues would be dehydrated. On the contrary, none of the tissues or organs removed were found to be dehydrated, and in the case of the kidney and adrenals, hydration had occurred.

Prior work had indicated that body size may play an important role in animal responses to 1 ATA HE-O₂ as well as to responses at increased He-O₂ pressures. This may account for some of the variation in significance of altered hydration in kidney and liver, since not all of the groups were identical in weight.

The adrenal glands were observed to experience nearly a twofold increase in the wet-dry ratio values under treatment conditions when compared to air controls. It was also found that the alterations in adrenal hydration were generally more profound under the 20 ATA treatment than under the 40 ATA.

When removed from treated animals, the adrenal glands were observed to be a lighter buff color and almost twice as large as the adrenal glands of the air control animals.

The increased hydration of the kidneys at high levels of oxygen with identical pressure may indicate an altered response to oxygen levels by the kidneys. A corresponding significant alteration was not observed at 40 ATA.

Kidneys were found to be paler in color and spongier following the hyperbaric and hyperbaric-hyperoxic treatments.

SUMMARY

Decreases in body weights and increased levels of hydration in the adrenal glands, kidneys, and livers were found for guinea pigs exposed to hyperbaric (20 and 40 ATA) and hyperbaric-hyperoxic (pO₂ = 600 mm Hg) environments. The animals were exposed to treatment for 2, 4, and 6 days. It is proposed that these alterations were due to the animals' response to stress produced by the treatment. Gross morphological changes in lungs, adrenal glands, kidneys, and liver were noted.

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EMBRYONIC MORTALITY IN SWINE AS INFLUENCED BY AN ORAL HYPOGLYCEMIC AGENT

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ABSTRACT

Eighteen gilts were used to ascertain the influence of an oral hypoglycemic agent (Dymelor) on embryonic survival to 30 days post conception. Gilts were hand mated two times, 24 hours apart during estrus and were randomly assigned to a treatment group receiving 4 g./hd/day of Dymelor for 3 days, or a control group. An initial blood sample was collected prior to treatment with a final blood sample collected at slaughter. Blood glucose levels and glycogen content of liver muscle, fetal and endometrial tissues were analyzed and found similar between groups. Corpora lutea counts were made to determine ovulation rate. The number of viable embryos and detectable resorptions were established following excision of the uterus after slaughter. Also, the mean fetal weights were determined. Ovulation rates were similar but the number of embryos for treatment and control groups were 12.1 and 10.5, respectively. Average resorption rate was 0.7 for the treated and 0.8 for control groups. Percent embryonic mortality was 9.2 for treated females and 26.3 in the controls. Fetal weights were heavier in treatment groups. These results indicate that the addition of Dymelor to the diet had a beneficial effect on embryonic survival.

INTRODUCTION

The swine industry has incurred serious economic losses because of the high percentage of embryonic mortality that occurs with each pregnancy. In swine nearly 100 percent of all eggs ovulated are fertilized (Spies, 1959). However, not all fertilizations result in live pigs at birth (Perry, 1954; Lerner, 1957). Nutritional levels play a major role in the number of fetuses surviving to parturition (Robertson, 1951). Various additives have been experimentally tested to evaluate their influence on embryo survival. Previous research at this station has indicated a beneficial effect on embryonic survival of feeding an oral hypoglycemic agent to laboratory animals (Kubik, 1974). The purpose of this study was to determine the effect and mode of action of this same compound, Dymelor, on embryonic mortality in pigs.

MATERIALS AND METHODS

Experimental Procedure.— Eighteen virgin female pigs were used to determine the effects of the oral hypoglycemic drug, Dymelor, on embryonic mortality in swine. These females were allotted by breed into either a control or a Dymelor-fed group prior to mating. The males were exposed to the females twice daily. Each female was again remated 24 hours later to a different male to eliminate the effect of sire on embryonic mortality. On the day of first mating, a 24 hour fasted

blood sample was collected to analyze for glucose concentration. Following this initial bleeding, individual feeding of all females was completed to insure each gilt obtained her feed allotment. All females had received 1.81 kg. per day of the basal ration prior to mating. Following mating the treatment group received 4 g. Dymelor per day for 30 days mixed with the basal ration. Thirty days post-mating all females were slaughtered with a final blood sample collected for subsequent glucose analysis. Maternal liver, muscle, and endometrial samples were collected, placed in individual tubes containing 1 ml of 30 percent potassium hydroxide (KOH), and refrigerated (16°C) until analyzed for glycogen content. The reproductive tracts were removed and the number of ovulations determined by counting the corpora lutea on each ovary. The number of viable and resorbed fetuses were counted and fetal weights determined. Fetal tissue samples were collected and stored (16°C) in 1 ml of 30 percent KOH for subsequent glycogen analysis.

Methods of Analysis.— Blood serum glucose concentrations were determined using a quantitative colorimetric determination (Meites and Bohman, 1963). Following the method of Seifter (1950), the tubes containing previously collected tissue samples were analyzed for glycogen content using anthrone reagent.

Oral Hypoglycemic Agent.— Dymelor is an acetohexamide produced by Eli Lilly. This drug is a sulfonylurea characterized by the presence of an acetyl group in the para-position of the phenyl ring and the incorporation of a cyclohexyl group on the urea moiety ($p\text{-CH}_3\text{COC}_6\text{H}_4\text{-SO}_2\text{-NHCONH-C}_6\text{H}_{11}$).

Statistical Analysis.— Statistical analysis of the data was completed by analysis of variance.

RESULTS AND DISCUSSION

Blood Glucose Levels.— Blood glucose levels were extremely variable with some deviation from the accepted range of 75 to 150 mg/100 ml of blood. The final mean values were more normal but were not significantly different. The effect of Dymelor was not long-term as indicated by higher treatment glucose levels (Table 1).

Tissue Glycogen Levels.— Values observed when the various tissues were analyzed for glycogen content are presented in Table 2. Liver and muscle

TABLE 1. Influence of dymelor on blood glucose levels in gilts

	<u>Blood Glucose Levels*</u>	
	<u>Initial + SD</u>	<u>Final + SD</u>
Control	68.89 ± 20.43	78.57 ± 14.92
Treatment	113.33 ± 33.35	90.83 ± 9.20

*mg/100 ml

TABLE 2. Influence of dymelor on tissue glycogen levels in gilts

	Liver	Muscle	Endometrium	Fetus
Control	49.84	24.45	10.41	9.32
± SD	21.87	14.51	6.92	5.60
Treatment	35.95	19.34	8.07	18.25
± SD	22.80	5.78	2.10	12.17

glycogen levels were not significantly different. Treatment means for endometrial glycogen content were slightly reduced but the difference was not statistically significant. Treatment with Dymelor also elevated fetal glycogen levels but small numbers of observations limited attainment of statistical significance. These results, although not significant, are comparable to previous research reported by Kubik *et al.* (1974) and can be theoretically explained by increased permeability of the placenta allowing a greater transfer of nutrient materials across the placental barrier.

Reproductive Performance.—Ovulation rates for the two groups of pigs were identical (Table 3). Because treatment was administered after ovulation, no significant differences were expected. The resorption rates were similar across groups and were observed to be quite low. Number of viable embryos was slightly larger in the treatment groups, possibly indicative of a beneficial treatment influence (Table 4). Total weight differences between groups were small and insignificant. Percent embryonic mortality was less in the treatment group than the control females, but failed to be statistically significant, most likely because of a lack of sufficient experimental units. These results, although not statistically significant, are consistent with trends reported in earlier work. Some beneficial effect on percent embryonic survival and number of viable embryos were found.

TABLE 3. Effect of dymelor on ovulation rate and fetal reabsorption rates of gilts.

	Ovulation Rate	Reabsorption Rate
Control	13.33	0.78
± SD	1.87	1.20
Treatment	13.33	0.67
+ SD	1.87	0.71

TABLE 4. Influence of dymelor on number of viable embryos, percent embryonic mortality and fetal weight of gilts

	Number of Embryos	Percent Mortality	Fetal Weight
Control	10.56	26.3	1.42
± SD	2.96		.21
Treatment	12.11	9.2	1.40
± SD	1.83		.20

SUMMARY

Dymelor was added to the diet of pigs to study its effect on embryonic survival rates. Blood glucose and tissue glycogen content were assayed to study possible modes of action of the oral hypoglycemic drug. Differences were not statistically significant but followed patterns noted in earlier experiments with laboratory animals. Resorption rate was not altered but larger numbers of viable embryos were observed. Embryonic mortality was not significantly decreased.

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DISTRIBUTION OF *BITTACOMORPHA CLAVIPES*
(FABRICIUS) AND *PTYCHOPTERA QUADRIFASCIATA*
SAY (DIPTERA: PTYCHOPTERIDAE) IN A SANDHILL
SPRINGBROOK OF SOUTHEASTERN NORTH DAKOTA

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ABSTRACT

The distribution of *Bittacomorpha clavipes* (Fabricius) and *Ptychoptera quadrifasciata* Say in a sandhill springbrook of southeastern North Dakota was investigated from March - November, 1974 and 1975. *Bittacomorpha clavipes* was restricted to headwaters of the springbrook by a combination of physical factors, including streamflow and depth, and chemical factors, including iron and dissolved solids. Both species predominately occurred in organic matter and detritus along the stream margin. *Bittacomorpha clavipes* and *P. quadrifasciata* were univoltine, with four larval instars. Adults of both species emerged in July and August.

INTRODUCTION

Although ptychopterids have been extensively studied in Britain and Europe (Brindle, 1962; Kamil, 1971; Stubbs, 1972), the biology of the family is poorly known in North America. Two ptychopterids, *Bittacomorpha clavipes* (Fabricius) and *Ptychoptera quadrifasciata* Say, occur in sandhill springbrooks of southeastern North Dakota. This paper reports on the distribution and biology of the two species in Iron Springs, one such springbrook.

DESCRIPTION OF STUDY AREA

Iron Springs is located in the Sheyenne delta, Richland County, of southeastern North Dakota (T153N: R53W; S7-8, 17-19). The delta consists predominately of fine sand and some gravel to depths of 18 m (Baker, 1967).

Iron Springs is 4.9 km long with an average width of 2.2 m and average depth of 18 cm. The stream is formed by spring discharge of the helocrene type and is fed by seepage from the Sheyenne aquifer over its length. Water from this aquifer is hard and relatively high in dissolved solids (Baker, 1967). The substrate of Iron Springs is primarily sand and fairly uniform. The margin of the springbrook is characterized by seepage areas with high amounts of organic matter and detritus. Iron Springs flows northward emptying into the Sheyenne River.

MATERIALS AND METHODS

Permanent sampling stations were established at eight locations along Iron Springs. Stations I and II, located at the stream headwaters, were characterized by slow streamflow and shallow water (Table 1). Ferric hydroxide precipitated in the seepage areas of the stream margin. *Bidens cernua* L. and *Zanichellia palustris* L.

TABLE 1. Physical characteristics of sampling stations in Iron Springs, Richland County, North Dakota.

Station	Elevation ¹ (m)	Site Length (m)	Bank		Depth ² (cm)	Width ² (m)	Discharge ² (m ³ /sec)	Flow ² (m/sec)
			Vegetation Type	Type				
I	325	26	Wooded	Wooded	9.6	1.9	0.07	0.23
II	325	54	Open Prairie	Open Prairie	8.3	2.7	0.08	0.24
III	323	60	Open Prairie	Open Prairie	23.3	1.8	0.14	0.38
IV	323	48	Wooded	Wooded	30.5	2.5	0.10	0.30
V	318	58	Open Prairie	Open Prairie	22.3	1.3	0.18	0.47
VI	315	71	Wooded	Wooded	14.7	2.7	0.16	0.35
VII	303	91	Wooded	Wooded	17.5	2.0	0.15	0.37
VIII	301	51	Wooded	Wooded	14.3	2.1	0.18	0.49

¹Estimated from a U.S. Geological Survey topographic map of Southeastern North Dakota.

²Expressed as means for monthly readings, March - November, 1974 and 1975.

lined the stream channel. Stations III and V were located in the mid-reaches of the springbrook in open prairie. These stations were characterized by high stream banks and swift streamflow (Table 1). *Zanichellia palustris* was common at these stations with luxuriant growths in the summer. Station IV was located at a beaver pond. The pond encompassed 42 sq. m at its greatest size. Aspen and cottonwood grew on the stream banks. Stations VI, VII, and VIII were located in a wooded area, primarily ash and oak, near the stream mouth. Streamflow was swift at these stations (Table 1) and detritus accumulated at the stream margin.

Sites within the stations were sampled at approximately monthly intervals from March, 1974 to November, 1975. Collecting during the winter was not as intensive as during other seasons because of inclement weather and inaccessibility to stations.

A Neil-type, cylinder sampler which enclosed 0.06 m² of substrate to a depth of 6 cm, was used in collections. At each sampling station, two samples were taken from both the stream margin and stream center. Samples were screened (No. 50 Tyler) and the remaining substrate materials preserved in 70% ethyl alcohol. Ptychopterid larvae were removed by sugar flotation (Anderson, 1959) and stored in 70% ethyl alcohol.

Water samples and measurements of streamflow, stream depth and width, and temperature from each site were taken at the time of sampling. Streamflow was determined using a pygmy flow meter (manufactured by Scientific Instruments, Wisconsin). Water chemistry was analyzed in the field whenever possible, or in the laboratory within eight hours of collection, using a Hach DR-EL Laboratory. Water samples were also returned to the laboratory, refrigerated, and analyses for pH and conductance carried out within 48 hours of the time of collection. The pH was determined with a Corning pH meter (Model 10). Specific conductance was measured with a Lab-Line Solu-bridge (Model Mc-1).

RESULTS AND DISCUSSION

While *P. quadrifasciata* was widely distributed in the springbrook, *B. clavipes* appeared to be restricted to the stream headwaters (Table 2). The two stations at the headwaters of Iron Springs yielded 95 percent of all *B. clavipes* collected. The species was particularly abundant at Station I, situated near the spring source in a wooded area. The physical and chemical stream characteristics of the headwaters of Iron Springs show marked differences from those of other stations (Tables 1 and 3).

At Stations I and II, streamflow rates on any given sampling date were approximately one-half the rates observed at other Iron Springs stations. Chemical differences are reflected in values of hardness, conductance, and iron. Iron levels were two to three times higher at the headwaters than at other stations. Mackay (1968) found *B. clavipes*, often in association with *P. quadrifasciata*, in areas of high iron concentration in a small Quebec stream.

Immatures of both species were primarily collected along the margins of the springbrook. Only three larvae of *B. clavipes* and 25 larvae of *P. quadrifasciata* were collected away from the stream bank, and these mainly in patches of

TABLE 2. Total numbers of immature Ptychopteridae collected in Iron Springs, Richland County, North Dakota from March - November, 1974 and 1975.

Station	<i>Ptychoptera quadrifasciata</i>	<i>Bittacomorpha clavipes</i>
I	191	126
II	46	19
III	148	4
IV	162	2
V	39	0
VI	269	2
VII	195	0
VIII	72	0

Zanichellia palustris. Although both species most often occurred in the rich organic matter and detritus, characteristic of the stream margin, habitat differences were observed (Table 4). *Bittacomorpha clavipes* was collected at sites having a lower average streamflow and depth and higher average iron and dissolved solids levels than was *P. quadrifasciata*. Both species probably feed on decaying vegetation and associated microorganisms (Hodkinson, 1975).

Hodkinson (1973) reported that width of head capsules is a reliable means of distinguishing instars in *Ptychoptera*. Based on head capsule widths of larvae collected in Iron Springs and several other localities in the sandhill region, *B. clavipes* and *P. quadrifasciata* appear to have four larval instars (Table 5).

Ptychopterids often have multivoltine life histories. In Michigan, *B. clavipes* was reported to have three generations per year and *P. quadrifasciata* two (Rogers, 1942). In the sandhills, both species appeared to have a single generation per year with adult emergences in the summer, based on shifts from a predominance of III - IV instar larvae to I - II instar larvae in collections (Table 6). Pupae of both species were also collected in July and August, with larvae most abundant in the spring and fall. In the summer of 1975, the springbrook was flooded following high amounts of precipitation. Following this period of high water, only one larva of *P. quadrifasciata* and no specimens of *B. clavipes* were collected in the springbrook.

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TABLE 3. Mean water quality characteristics of sampling stations in Iron Springs, Richland County, North Dakota from March - November, 1974 and 1975.

Station	I	II	III	IV	V	VI	VII	VIII
pH	7.8	7.8	8.0	8.0	8.0	8.0	8.0	8.1
Oxygen (mg/l)	9.5	10.4	10.9	10.8	11.4	10.6	10.9	11.4
Total Hardness (mg/l)	387	364	319	295	297	292	275	291
Ca Hardness (mg/l)	207	204	194	182	187	179	180	184
Mg Hardness (mg/l)	180	156	123	113	110	113	95	107
Specific Conductance (mhos/cm)	837	813	627	618	611	576	551	567
Iron (mg/l)	2.94	3.32	0.99	0.98	0.77	0.71	0.89	0.75
Bicarbonate Alkalinity (mg/l)	432	409	330	323	318	306	301	297
Temperature (°C)	14	13	12	12	12	12	11	10

TABLE 4. Characteristics of sampling sites where Ptychopteridae were collected in a sandhill springbrook of southeastern North Dakota (data expressed as means).

	<i>Bittacomorpha clavipes</i>	<i>Ptychoptera quadrifasciata</i>
Streamflow (m/sec)	0.04	0.10
Stream Depth (cm)	3.7	8.6
Width of Stream Margin (cm)	101.4	57.3
Iron (mg/l)	3.4	1.7
Total Hardness (mg/l)	378.0	309.6
Specific Conductance (mhos/cm)	811.3	636.9

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TABLE 5. Means and ranges of head capsule widths in mm for instars of Psychoteridae in sandhill and springbrooks of southeastern North Dakota.

Instar	<i>Psychoptera quadrifasciata</i>			<i>Bittacomorpha clavipes</i>		
	Mean	Range	Number Collected	Mean	Range	Number Collected
1	0.21	0.18 - 0.23	21	0.41	0.37 - 0.45	4
2	0.29	0.28 - 0.33	130	0.66	0.58 - 0.72	47
3	0.41	0.37 - 0.45	379	0.94	0.87-1.02	57
4	0.62	0.55 - 0.72	892	1.08	1.02 - 1.17	51

TABLE 6. Seasonal percentages of larval instars of Ptychopteridae in collections from sandhill springbrooks of southeastern North Dakota. (N = number of larvae collected)

Instar	<i>Ptychoptera quadrifasciata</i>								
	Mar. 1974	Apr.	May	July	Aug.	Sept.	Nov.	Jan. 1975	Mar.
I	0	0	0	21	4	0	0	0	0
II	0	2	0	63	15	4	8	7	7
III	48	34	18	13	54	20	22	29	38
IV	52	64	82	3	27	76	70	64	55
	N=54	N=44	N=71	N=82	N=98	N=361	N=178	N=302	N=142

Instar	<i>Bittacomorpha clavipes</i>								
	Mar. 1974	Apr.	May	July	Aug.	Sept.	Nov.	Jan. 1975	Mar.
I	0	0	0	0	10	0	0	5	0
II	19	38	20	6	67	25	50	11	33
III	43	0	30	68	17	25	25	33	25
IV	38	62	50	26	6	50	25	51	42
	N=21	N=13	N=10	N=38	N=30	N= 4	N=12	N=17	N=12

H \rightleftharpoons D EXCHANGE IN SOLID INSULIN DUE TO INTERACTION WITH D₂O VAPOR

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ABSTRACT

Data on the increase in the original weight of lyophilized solid insulin (acidic form) are presented for a series of D₂O adsorptions followed by successive high vacuum desorptions. Increase of original weight is assumed to be a result of an exchange of labile hydrogens in insulin by deuterium of D₂O. The maximum of this weight increase (H \rightarrow D effect) is reached when all labile (~ 87) hydrogen atoms present in the functional groups of a unit of the solid insulin molecule are exchanged for deuterium from the interacting D₂O vapor.

INTRODUCTION

Earlier studies showed that after several adsorption-desorption cycles of D₂O vapor on solid adsorbents such as carbohydrates, proteins, and various synthetic compounds, a replacement of their labile hydrogens by deuterium occurred to an extent which depended on the adsorbent. For example, on β -lactoglobulin (1) it reached nearly seventy percent, on enzyme lysozyme (2) about ninety percent, on 4-chloro-3-dimethylphenolformaldehyde heptamer (3), and on hemoglobin (4) it reached complete H \rightarrow D exchange. In several cases the reversibility of the exchange was studied and established. A rise in temperature of the adsorbents seems only to accelerate the rate of exchange (kinetic effect). The importance of the H \rightleftharpoons D exchange effect in contributing to the possible clarification of the molecular structure of adsorbents provided the motivation to extend such studies to the natural proteins and synthetic polymers with well-defined molecular structures.

The present work is a part of a broad study concerned with the hydrogen-deuterium exchange on solid lyophilized insulin as the model substance of well known structure (5). The data on the H \rightarrow D exchange experimentally obtained are discussed in relation to its structure.

EXPERIMENTAL

Materials.— Insulin of high purity, in acid form, with the surface developed through lyophilization, was provided by Eli Lilly Company of Indianapolis. It was used after many adsorption-desorptions of water, in vacuum, until a constant and reproducible weight was obtained. Triply distilled H₂O and D₂O were used as in previously described preparations (1, 2).

Apparatus.— This is a combination of a high-vacuum system with a gravimetric sorption system, in which the sample of insulin hangs in a bucket from a fine quartz-helical spring. A detailed description of the system was given

elsewhere (2), except that the sensitivities of the springs used were equal to 0.920 and 1.498 mg/mm.

During desorption as well as adsorption the change of position of the sample bucket was measured by a traveling microscope accurate to ± 0.002 mm. The weights of the samples used were in the range of 180 to 230 mg. The separate water jackets surrounding the helical spring at 40° , and the samples at the experimentally required temperatures, were controlled to $\pm 0.02^\circ$.

PROCEDURE AND RESULTS

Determination of dry weight of insulin.— From preliminary tests it was established that amounts of adsorbed H_2O or D_2O exceeding $\sim 30\%$ of the weight of insulin caused contraction of its surface, which diminished accessibility to labile H-atoms and lowered the $H \rightleftharpoons D$ exchangeability.

To effect additional purification-degassing of samples, some preliminary adsorptions of H_2O vapor followed by complete desorptions were carried out. Then a prolonged desorption of insulin was continued at $27^\circ C$ and high vacuum (10^{-7} mm of pressure) for about seven days during which a constant weight was established. Subsequent adsorption-desorption cycles of H_2O at $27^\circ C$ did not alter the weight. However, it was observed that a rise of temperature of the samples to $37^\circ C$, and a continuation of desorption, caused a constant loss in weight at the rate of 0.0004% per hour. A markedly higher, but also a constant rate of loss of weight was observed at $47^\circ C$. These losses of weight were suggested to be due to decomposition or sublimation of insulin, and to avoid this the sorptions were conducted at $27^\circ C$.

Hydrogen Deuterium exchange on insulin.— The process of exchange is based on an interaction between the protein and the "sorbed" D_2O , the D-atoms of which replace the labile H-atoms of proteins, and give a gain in weight of dry protein (as determined after a complete desorption). Only a fraction of labile hydrogens is expected to exchange during the first adsorption-desorption run. This may be expressed by the following equation for the first such run:



where n = the total number of labile hydrogens in this protein, and m = the number of D_2O molecules that underwent exchange. Succeeding runs may be expressed by similar equations using the proper amounts to show further exchange.

Finally, when an adsorption-desorption run showed no further exchange, the product could be designated as Prot- D_n (fully deuterated protein). Its gram-molecular weight will be greater than the original protein by $n \times 1.0066$ g/atom (atomic weight difference, $D-H=1.0066$).

The first adsorption of D_2O was carried up to $\sim 19\%$ of the weight of protein. This weight remained nearly constant at $27^\circ C$ for 4 hours before the desorption was started. In order to avoid a sudden "blowoff" of the sample due to rapid reduction of pressure, a reducing capillary tube was inserted between the

sample and the pumps in such a manner that the vapor could be drawn through this capillary until a pressure of 10^{-3} mm was reached. Then the system was opened fully to the vacuum line. The loss of weight due to desorption was measured over a long period of time until a constant weight was reached under high vacuum. Curve 1 in Figure 1 gives the results for the first desorption run. The final horizontal portion of this curve indicates the complete dry state of a new weight of sample. The ordinate of the horizontal part of this curve shows the gain of original weight in [mg/100] of samples due to exchange after the first adsorption-desorption. Then followed a second adsorption of about 20% of D_2O . After reaching the approximate equilibrium of adsorption, the sample was desorbed for a long time as before, until the desorption curve remained horizontal. The protein sample again weighed more than at the end of the first run, and this increase is shown by Curve 2 in Figure 1. The same process (adsorption-desorption) was repeated several times, and the seven curves (1-7) of Figure 1 show the results obtained by using sample 2. The same procedure had

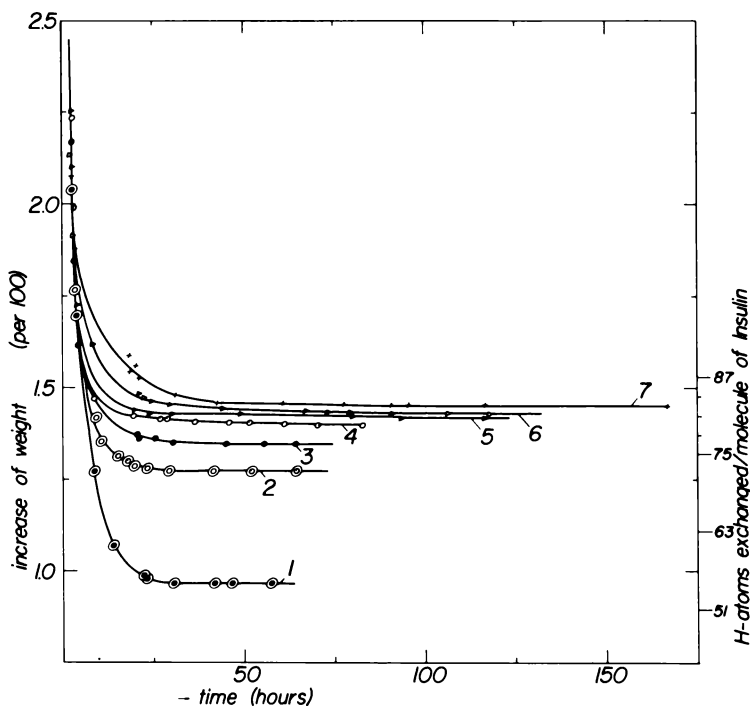


FIGURE 1. The complete desorption followed at 27° (after every adsorption) of D_2O from insulin, as a function of the time.

been followed in the study of sample 1; the results are similar but not diagrammed in Figure 1. All the values of the successive gains in weight due to exchange vs. the successive summation of adsorbed amounts of D_2O for each adsorption operation are presented in Figure 2 for two samples of insulin.

From Figure 2, it can be seen that the first adsorptions followed by complete desorptions on both samples of insulin cause the highest increase of original weight (70-80%) which may be characterized by slope O-O'. This may also indicate and H \rightarrow D exchange in peptide functional groups -CO-NH- as the easiest.

Further adsorptions-desorptions cause a slower increase in original weight (as well as in H \rightarrow D exchange). This is approximated by a slope C-D drawn to fit some of the experimental points as shown. This may indicate a group of labile hydrogens in functionalities of lower activity. Continuation of the adsorption-desorption of D_2O leads to a markedly slower but linear increase of original weight characterized by slope A-B up to a maximum of original weight increase, and of H \rightarrow D exchange reached. This slope may be associated with the activity of a third group of functionalities containing the labile hydrogens. "Activity" is used here only as a term to characterize the probable steps of exchange suggested.

From the amino acid sequence, together with the accepted structure of the insulin molecule as elucidated by Sanger *et al.* (5), and also as described by

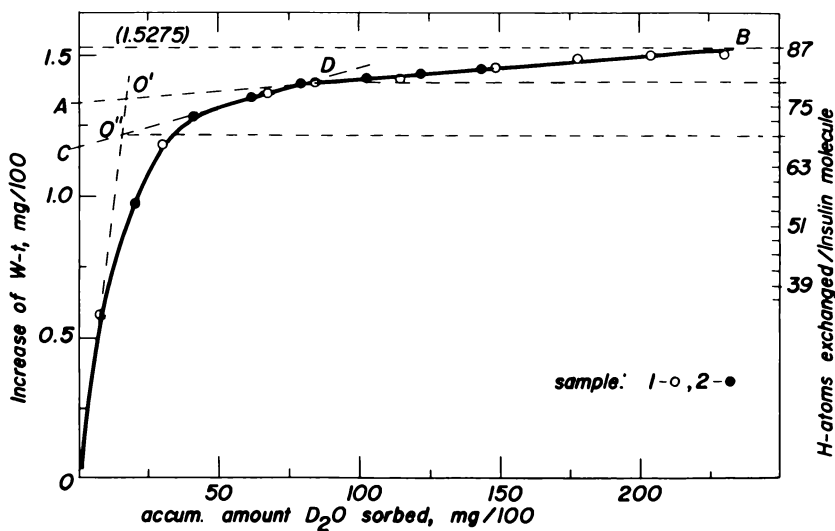


FIGURE 2. H \rightarrow D exchange effect, as increase of original weight of solid insulin (acid form), due to repeated sorptions of D_2O and followed by complete desorptions at 27° .

Thompson (6), the molecular weight of the smallest unit was calculated to be equal to 5733.

The amino acid composition of such a unit has functionality "sites" containing labile hydrogens. Their specificity and number as well as the number of labile hydrogens are presented in Table 1 (5,6).

The numbers of labile H-atoms in the insulin molecule-unit from Table 1 were used to calculate the expected gain of original weight. This calculated maximum is equal to 1.5275 mg/100, if all labile H-atoms are exchanged for D-atoms.

The gain of weight equivalent to the number of exchanged H-atoms is presented on the right ordinate of Figure 1 and Figure 2. These data compared well with the experimentally obtained results for two samples, which demonstrates that all 87 labile H-atoms are exchanged as a result of interaction with D₂O vapor. From the shape of the curve of Figure 2, the H→D exchange can be approximated to three stages of activity of the functional groups that may be characterized by the three slopes. It is clear that the number of activities H→D exchange could be expected to equal the number of different functionalities (even more if one considers the presence of primary and secondary H-atoms in the same functional groups). Therefore, one may expect as many slopes (with transitions in between) as number of activities.

However, since different activities usually have different functional dependences on temperature, it is logical to expect that some of the activities may be of equal or nearly equal values at certain temperatures. Such equality of activity values gives actually a linearity of H→D exchange (appearance of slopes less in number but larger). This is a suggested explanation for the appearance of three stages under our experimental condition for H→D exchange at 27°C.

From Figure 2, the first stage of exchange along the slope O-O' up to point O'' (a cross point with slope C-D), involved ~69 H-atoms; the second stage along the slope C-D (from point O'' to D, crossing point with a slope A-B) involved an exchange ~10 H-atoms; and the third stage, along the slope A-B, involved exchange ~8 H-atoms.

The question of identifying from which functional groups, and during which stage the H-atoms are exchanged, cannot be answered yet.

From previous experience dealing with similar solid adsorbents (1,2,3,4,7) however, it is certain that the labile H-atoms from peptide- and acidic - functionalities are easiest to exchange. The closest to them in exchangeability are the labile H-atoms, the first H from the primary amino, and from aromatically bound -OH functionalities. These, together with the above mentioned, most probably form the first stage of H→D exchange.

As to the other stages it can only be suggested that during the second stage of exchange the labile H-atoms may be from -OH, -NH, and even partly from amido-functionalities. This leaves for the third stage of H→D exchange the secondary labile H-atoms from the amino- and the amido-functionalities.

Further, the exchange is reversible, as the preliminary tests showed (not presented for insulin). Also the adsorptivity is not a necessary prerequisite for

TABLE 1. Active sites (functionalities) and "labile" H-atoms in a unit of the insulin molecule (5,6).

Number of	$\begin{array}{c} \text{O} \text{ H} \\ \parallel \\ \text{-C-N-} \end{array}$	R-OH	>OH	>NH	-NH ₂	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-C-OH} \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-C-N=} \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-C-NH}_2 \end{array}$	Σ
Active Sites	49	4	4	4	4	6	1	6	78
Labile H-atoms	49	4	4	4	8	6	—	12	87

exchangeability, as stressed elsewhere (3), but seems to occur also due to collisions between the surrounding D_2O vapor molecules with the functional groups containing labile hydrogens in the solid, demonstrating also its statistical nature.

CONCLUSION

This study presents evidence, similar to that shown for the case of hemoglobin (4), for the complete exchange of all labile hydrogens present in a solid enzyme by using the adsorption-desorption method. Eventually this method may be utilized in the clarification of the molecular structures of unknown compounds.

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