ABSTRACTS
of the
NORTH DAKOTA ACADEMY OF SCIENCE

Official State Academy
(Founded December, 1908)

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GRAND FORKS, NORTH DAKOTA

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PREFACE

At this time we are introducing a new format for the publication of Abstracts of Proceedings of the Academy. In this way it is possible to have the abstracts available at the time of the May meeting.

Abstracts are published in sequence according to the numbers assigned to the papers in the program. The sessions, titles and authors of papers have not been indexed, in order to expedite the publication and release of the abstracts. For your convenience you will find the paper numbers at the outer edge of the pages, in line with page numbers.

Full papers will be published in the Proceedings of the Academy. Abstracts will not appear in that issue.

F.A.J.

Grand Forks, North Dakota
April, 1968

PAPER NUMBER 0

Mathematical Model for Sight-In of a Telescopic Rifle Sight, D.W.Y. Cheng*, Department of Industrial Engineering, College of Engineering, North Dakota State University, Fargo, North Dakota.

Since rifle cartridges vary inherently, sighting of a telescopic rifle has long presented a problem. Today almost all rifle manufacturers sight-in their rifles at a set range by test fire only. The purpose of this paper is to develop a mathematical technique for this sight-in procedure. By using mathematical and operations research methods, the optimal sight-in condition is established by minimizing the total error. Three different models are presented: 1) A Deterministic Model, 2) A Constrained Model, and 3) A Stochastic Model.

PAPER NUMBER 1

EFFECT OF DIETARY RESTRICTION UPON DIELDRIN POISONING IN CHICKENS, J. H. Seifert*, K. L. Davison and J. L. Sell. USDA, ARS, Metabolism and Radiation Res. Lab., and Dept. of Animal Science, North Dakota State University, Fargo, North Dakota.

Forty-eight mature leghorn hens were allotted at random to four equal groups and fed diets containing 0, 20, 40 and 80 ppm dieldrin. The hens were penned individually, but because of pen design they were fed and watered in blocks of three. Biopsies of abdominal fat were taken initially and at biweekly intervals for a period of six weeks to measure dieldrin accumulation. Eleven of the twelve hens fed the diet containing 80 ppm dieldrin and five of the twelve hens fed the diet containing 40 ppm dieldrin had died by the sixth week. At this point, 1, 210 and 340 ppm of dieldrin were found accumulated in the fat of hens fed diets containing 0, 20 and 40 ppm dieldrin. Feed was withheld at the end of the sixth week, and the hens were observed more frequently to record behavior and time of death. The average number of days from beginning of feed restriction to death was 29, 17 and 13 for hens fed the diets containing 0, 20 and 40 ppm dieldrin, respectively. Carcasses were ground, freeze-dried and analyzed for dieldrin. With respect to these diets, the carcasses contained an average of 6, 10 and 15 percent fat at death, and the fat contained 5, 1286 and 1038 ppm dieldrin.

PAPER NUMBER 2

PROPERTIES OF LIPOXIDASE AND HYDROPEROXIDE FLAXSEED AND THEIR OCCURRENCE IN OTHER SEEDS, B. A. Vick* and D. C. Zimmerman. Dept. of Biochem., N. Dak.State Univ., Fargo, North Dakota.

Lipoxidase catalyzes the enzymatic oxidation of certain polyunsaturated fatty acids to their hydroperoxides. In some seed tissues the hydroperoxide is subsequently isomerized to a keto-hydroxy fatty acid by a hydroperoxide isomerase enzyme. Separation of the two enzymes from flaxseed extracts was accomplished by ammonium sulfate fractionation. Activity measurements during 10 days' germination showed that the total lipoxidase activity decreased about 90% and the hydroperoxide isomerase activity decreased about 65%. The cotyledons contained most of the lipoxidase activity while the hydroperoxide isomerase was most active in the hypocotyl. Flaxseed lipoxidase was inhibited by 2 X 10^{-4} M nordihydroguaiaretic acid and 10^{-3} M iodoacetamide. Hydroperoxide isomerase is destroyed by heating for 5½ min. at 55°. It is not inhibited by 10^{-3} M KCN, 10^{-4} M p-chloromercuribenzoic acid, 10^{-3} M EDTA, or 10^{-3} M iodoacetamide. In addition to flaxseed, hydroperoxide isomerase has been found in barley, soybean, wheat embryo, alfalfa, and mung beans.

PAPER NUMBER 3

METABOLISM OF DIELDRIN ^{14}C BY SHEEP, R. H. Hedde* and K. L. Davison. North Dakota State University, and USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota.

Two sheep were fed dieldrin for five weeks, then orally dosed with 180 μc-dieldrin-^{14}C in gelatin capsules. Carbon dioxide was collected for 24 hr. after dosing. Feces were collected at 24-hr. intervals for 72 hr. At the end of 72 hr., the sheep were killed, samples of various tissues collected, and the entire carcass ground and sampled. No radioactivity was detected in the carbon dioxide traps. Radioactivity in the urine represented 9% of dose in one sheep and 5% in the other, with peak excretion occurring about the 16th hr. Radioactivity excreted in the feces was similar to the levels found in the urine. Dieldrin metabolites were poorly extracted from urine with benzene, diethyl ether, butanol, acetonitrile, and ethyl acetate. Extraction was accomplished by acidifying the urine below pH 2.5 and extracting with ethyl acetate. Further cleanup was done by gel filtration on LH-20. This product was then concentrated and applied to activated silica gel for thin-layer chromatography. Chloroform:methanol (2:1) was used to develop the chromatograms. Scans of the radiochromatograms indicated two areas of approximately equal activity at $R_f = .3$ and .85. Dieldrin had an R_f of 1.00 in this system.

PAPER NUMBER 4

SORPTION STUDY OF H_2O AND D_2O VAPORS ON O-PHTHALIC AND SUCCINIC ACIDS, M. A. Kishta* and W. S. Hnojewyj. Col. of Chem. and Phys., NDSU, Fargo, N. Dak.

The adsorption isotherms for water vapor at 25° and 35° on lyophilized, completely vacuum-dried, o-phthalic and succinic acids have been established. The total amounts of water vapor adsorbed in monomolecular layer were evaluated by extrapolating the upper slopes of the sorption isotherms at the saturation pressures of water vapor. Their values in moles H_2O/moles acid are; 0.605 for o-phthalic and 0.711 for succinic acid. The differential heats of adsorptions, calculated by the Clausius-Clapeyron method, shows noticeably the higher values for o-phthalic acid, a difference of about 30 kcal/mole at very beginning of adsorption. As the amount of adsorbed water vapor reaches monolayer coverage, the differential heats of adsorption for both acids approach the value of the heat of condensation of water vapor for both acids. Preliminary data of hydrogen Deuterium exchange effect on these acids were obtained by means of successive adsorptions of D_2O vapor followed by complete desorptions. Supported by a North Dakota State University Research Grant.

PAPER NUMBER 5

FLORAL ABSCISSION IN TULIPA, M. K. T. Lam*. Dept. of Biol., Univ. of N. Dakota, Grand Forks, North Dakota.

Petal abscission in tulips was studied. Materials were preserved in 70% ethanol and embedded in paraplast. Sections were utilized for microchemical tests and for permanent mounts. Pectates were present in the cell walls and middle lamellae before abscission was evident. During the later stage, pectates changed to soluble pectin which led to the detachment of the cells in the abscission layer. In one group of flowers the petals did not abscise. The change to water soluble pectins had not occurred. Hence, prior to the abscission of tulip petals the cementing pectates undergo change to water soluble pectin; otherwise petals do not abscise.

PAPER NUMBER 6

TRAFFIC LIGHT CONTROL BY COMPUTER SIMULATION, Kent F. Matzek*. Dept. of Industrial Engineering, School of Engineering, North Dakota State University, Fargo, North Dakota.

The handling of a large volume of traffic through a single lane traffic light has been a serious problem to traffic planning officials. The need for a simple versatile system to determine the time cycle has long been recognized.

Data for this paper was collected by actual observation of the intersection studied. In the intersection under study, 90% of the traffic flow was in the North-South bound direction. With this unproportionate amount of traffic in these two directions, it was determined to develop a system to: a) reduce the queue length of North-South traffic; b) while yet obtaining the minimum waiting line for the East-West direction; c) and also to accomplish all of this with a realistic model. The result of this study was the design of a computer simulator based on a Poisson arrival time, with the capability to determine possible bottlenecks, and the correct time cycle for the North-South bound lanes. The final system allows for the complete simulation period of three hours. The program provides a minute by minute summary of cars passing through the intersection, queues, holdovers, and other necessary information. In verifying the results of this model with empirical data, only small differences were noted.

PAPER NUMBER 7

DRY MATTER LOSS OF BARLEY AND OATS STORED AT HIGH MOISTURE, D. O. Erickson, George Pratt and W. E. Dinusson. Dept. of An. Sci. and Ag. Eng., N.D.S.U., Fargo, N. Dak.

Organic materials stored at relatively high moisture levels undergo fermentation which may result in organic matter loss. However, harvesting grains at relatively high moisture levels (20-30%) has several agronomic economic advantages over conventional dry harvest methods. In order to prevent molds and spoilage, these grains must be stored in oxygen-free bins. Storage, processing, handling, and feeding have been studied at this station and reported. Five experiments with high-moisture barley and oats were run in conjunction with beef feeding trials to determine the extent of organic matter loss due to fermentation. Representative samples (2-5 kg) were put in cheese cloth bags and placed at random locations in the silo. Dry matter analysis of the samples both in and out were run by oven dry (105°C) and Brown Duval methods. Phosphorus (used as a non-fermentable indicator) was analyzed by the perchloric acid digestion method reported by Bolin and Lockhart (N. D. Academy Sci., 1960). Loss in dry matter ranged from 0 to 5.88% with an overall average of 2%. Analysis of variance indicated no significant dry matter loss in barley or oats stored under anaerobic conditions.

PAPER NUMBER 8

CALF VOLATILE FATTY ACID AND AMINO ACID DIGESTA CHARACTERISTICS, P. P. Williams, W. E. Dinusson and E. J. Thacker. USDA, ARS, Metabolism and Radiation Research Laboratory, and Department of Animal Science, North Dakota State University, Fargo, N. Dak.

Calves reared in isolation, with and without ruminal protozoa, were maintained on a pelleted ration consisting of alfalfa, grain, and wheat bran in a ratio of 2:1:0.2. Ruminal digesta samples were collected 1.5 hr after morning feedings and analyzed by gas chromatography for volatile fatty acid (VFA) concentrations. VFA values for calves with ciliated protozoa had a higher ratio of C_2/C_4, an increase in propionic acid, and less total VFA concentrations than calves free of ciliated protozoa. When ruminal digesta samples were fractionated into bacterial and protozoal components, they were analyzed by column chromatography for amino acid concentrations. Ruminal bacteria and protozoa of the genera Isotricha and Entodinium contained amino acids identified as alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and aspartic and glutamic acids.

PAPER NUMBER 9

LYSINE CONTENT OF WHEAT, RYE, AND TRITICALE VARIETIES GROWN IN NORTH DAKOTA, E. Villegas*, Jeng-yen Lin*, C. E. McDonald, and K. A. Gilles. Dept. Cereal Chem. and Tech., NDSU, Fargo, N.D.

The proteins of a number of varieties of cereals have been reported to be nutritionally most limiting in the amino acid lysine. To facilitate analysis of numerous cereal varieties for lysine a triplicate-sample method for lysine determination was developed on the automated amino acid analyzer (Cereal Chem. In press). This method was used to determine the lysine content in varieties of hard red spring wheat, durum wheat, and rye now grown in North Dakota. Also analyzed were lines of triticale (a synthetic cereal) grown last year in North Dakota. The mean lysine content of the protein of wheat, rye, and triticale varieties grown in North Dakota is similar to the over-all level that we have found for these cereals which were grown in various parts of the world. Supported in part by North Dakota Wheat Commission and Rockefeller Foundation.

PAPER NUMBER 10

RELATIONSHIP OF WHEAT PROTEIN DIETS TO PLASMA AMINO ACIDS, J. C. Crandall and F. A. Jacobs. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, North Dakota.

Weanling rats were fed two strains of raw wheat, "Chris" and "Red River 68" with and without supplementation. At selected intervals animals were sacrificed and amino acid pools were measured by column chromatography. The animals developed a state of malnutrition as evidenced by growth. In all instances, animals fed wheat showed changes in the plasma amino acid pattern, which was consistent with different mixtures but more accentuated where growth was stimulated by supplements. Supplementation of the diet with cod liver oil (CLO) accentuated the increase of threonine, serine, and alanine. Other elevated amino acids showed a return toward normal levels after CLO supplementation. Plasma amino acids which deviated under these dietary conditions seemed to increase as a group; namely, threonine, serine, proline, alanine, cystine, and sometimes tyrosine. The amino acid changes in these pools seem to be a reflection of metabolic function as well as the distribution of the amino acid as fed. Dietary stress such as protein malnutrition not only influences the amino acid pool distribution of the various tissues, but may well alter the metabolic functions. (Supported in part by NIH grant FR-5407-04).

PAPER NUMBER 11

LIQUID ANION EXCHANGE CHROMATOGRAPHY FOR URINARY CONJUGATES, J. D. Robbins, J. E. Bakke and V. J. Feil. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. Dak.

Procedures have been developed for the isolation and identification of sulfuric acid esters and glucuronide conjugates from urine. These conjugates were obtained from the urine of rats dosed with 4-benzothienyl N-methylcarbamate. A liquid anion-exchange column was developed which removed the conjugates from urine by non-ionic adsorption. The liquid anion-exchange column was prepared by coating Porapak Q with trioctylamine. Removal of the conjugates was done by elution with methanol, which removed the amine-metabolite salts. The conjugates were separated by chromatography on an AE cellulose column eluted with 0.1 M ammonium formate (pH 4). Further purification was accomplished by paper chromatography (Whatman No. 1 developed in acetic acid-isoamyl alcohol-water, 10:40:50). Final purification of the sulfuric acid ester was done by gel filtration on Sephadex LH-20. The glucuronide was chromatographed as the trimethylsilyl ether derivative. These procedures allow for the isolation and identification of sulfuric acid esters and glucuronide conjugates from urine.

PAPER NUMBER 12

EXPERIMENTS ON THE INCORPORATION OF ATP^{32} INTO VIRAL RNA, W. E. Cornatzer, Shuang-Shine Tsao* and Beverly B. Teter*. Dept. of Biochem., Sch. of Med., Univ. of N. Dak.

Experiments reporting the incorporation of ATP^{32} nucleotides into RNA polio virus material are reported. A complete system containing 6 μmoles of $MgCl_2$, 0.5 μmoles of ATP^{32} ($2x10^6$ c.p.m. per mole), 2 μmole each of GTP, CTP and UTP, 0.01 ml of purified poliomyelitus virus (10^{-7} $TCID^{50}$), and 1 ml of HeLa cell polymerase. The reaction was incubated 37° for 2 and 4 hours. The reaction stopped by additional 3N $HClO_4$. The acid insoluble RNA was washed three times in 0.1 N NaOH, reprecipitated with 0.5 N $HClO_4$, and radioactivity was determined. A two fold increase in radioactivity was observed in the RNA fraction after 4 hours. If HeLa cell nuclei were added to above flask, a three fold incorporation increase was observed. Additional flask containing the above system was incubated for 4 hours, centrifuged, chromatographed on Ecteola cellulose column and eluted with increasing concentration of phosphate buffer for purification of virus by the method of Hoyer (Science 127, 859, 1958). The radioactivity and optical density peaks (260 mμ absorbance) of the elutate are similar to non-radioactive virus. (Aided by a grant from United Health Foundation).

PAPER NUMBER 13

LIPASE IN THE ALIMENTARY TRACT AND HEMOLYMPH OF THE COCKROACH. P.P. Hipps*, D. R. Nelson and B. J. Cook*. Metab. & Rad. Res. Lab., USDA, and Dept. of Biochem., N.D. State Univ., Fargo, N.D.

Zone electrophoretic analysis on starch gel of the gastric cecae of the American roach has revealed the presence of seven major carboxylic esterases which are capable of hydrolyzing alpha-naphthyl acetate. Two of these enzymes were resistant to 10^{-4} M DDVP and showed slight activity towards beta-naphthyl laurate. A purification procedure was described for one of the resistant esterases.

The purified enzyme preparation from the gastric cecae was able to hydrolyze tripalmitin in addition to alpha-naphthyl acetate. This enzyme was inhibited 30% by 10^{-4} M DDVP. An enzyme was found in the hemolymph which can hydrolyze tripalmitin to free fatty acids.

PAPER NUMBER 14

FLOUR MILLING IN NORTH DAKOTA: AN EXERCISE IN HISTORICAL GEOGRAPHY. Frank E. Vyzralek. Valley City, N. Dak.

Subjecting the changing character of flour milling in N. Dak. to map analysis, four distinct geographic patterns and historic phases of the industry can be discerned. Until 1885, simple stone mills, buhr mills and New Process mills made up the technological equipment of the industry. The pattern of mill locations showed concentration in the Red River Valley. After 1885, most new mills used the full gradual reduction process and older units were soon converted. Concentration increased in the Valley, but mills also followed the spread of settlement westward. In addition, the 1890s marked a period of intense competition, consolidation and low market prices for flour. The period 1900-1920 marked the dominance of the merchant mill accompanied by the introduction of a practical self-contained mill to supply local markets. The locational pattern shows a balance over the state. After 1920, overproduction of flour, drought and financial stringency combined to drive marginal units out of business. The geographic pattern remained balanced for several decades, but the industry eventually focused on Minot and Grand Forks and today on Grand Forks alone. The N. Dak. trend of early expansion and subsequent contraction has generally paralleled that of the nationwide milling industry.

PAPER NUMBER 15

ENVIRONMENTAL, EXTRA - ENVIRONMENTAL AND PREFERENTIAL PERCEPTION IN GEOGRAPHY, Brian R. Goodey* (spon: John C. Hudson). Dept. of Geography, Univ. N. Dak., Grand Forks, North Dakota.

The purpose of this paper is to outline the present directions of geographical research into the nature of perception. Initially three types of geographical perception are identified and discussed, these are environmental perception, extra-environmental perception, and preferential perception.

Illustrations of research into the two latter types of perception are drawn from a U.N.D. survey which was carried out in 1967. A map, The North Dakotan's United States, which has resulted from this research is discussed.

In conclusion, initial North Dakotan results are compared with those obtained in other States and it is found that generally North Dakota student perception is similar to that of students in other States. Research supported in part by U.N.D. faculty research grant.

PAPER NUMBER 16

SOME CHARACTERISTICS OF THE DIFFUSION OF COMMUNITY INNOVATIONS, R.J. Eidem* (spon: J.C. Hudson). Dept. of Geog., Univ. N. Dak. Grand Forks, North Dakota.

The distributions of community-accepted phenomena in North Dakota were analyzed to determine if a single diffusion pattern existed. It was assumed that if innovations introduced at different times followed a single diffusion pattern, then a communication hierarchy would be spatially recognizable. Four community-adopted innovations were examined. It was seen that the S-curve of the normal frequency distribution best fit the diffusion traits of both hospitals and swimming pools. Nursing homes and fluoridation plants were observed in their early stages of diffusion. Their diffusion curves described the expected, i.e. a slow rate of acceptance in their early stage followed by a rapid rise in number of adopters. It was observed that for all four innovations the flow of influence from larger to smaller communities appeared stronger than any directional flow. This was the one diffusion characteristic of a spatial nature shared by all four innovations. The findings suggest that community-oriented innovations diffuse from higher-order to lower-order centers in the State. The wide range of observed spatial distributions indicates that further study is needed to determine a more clearly defined diffusion pattern.

PAPER NUMBER 17

PRECIPITATION VARIABILITY AND MIGRATION OF THE NORTH DAKOTA FARM POPULATION, 1930 TO 1960, J.C. Hudson. Dept. of Geography, Univ. N. Dak., Grand Forks, North Dakota.

The occurence of regular sufficient amounts of precipitation is a crucial factor in the agricultural occupance of an area. The movements of population to and from areas of high precipitation risk may be interpreted as human adjustments to uncertainties and hostilities of the environment. Net migration rates for age groups were correlated with various measures of precipitation to gain a better understanding of this relationship in North Dakota. The study covered the period between 1930 and 1960.

It was found that mean annual precipitation was significantly correlated with migration rates for the 1930's, but generally not for the 1940's or 1950's. The role of the droughts of the 1930's is most significant. Other measures of precipitation proved to be poor predictors of migration.

PAPER NUMBER 18

RECOGNITION OF GROUNDWATER MICROFLOW SYSTEMS IN ALLUVIAL-CHANNEL BED FORMS. S. S. Harrison and L. S. Clayton. Dept. of Geol., Wis. State Univ.-Oshkosh, Oshkosh, Wis. and Dept. of Geol., Univ. N. Dak., Grand Forks, N. D.

Injection of dye into bed forms shows that channel water seeps into the upstream side of ripples and dunes, flows in a concave-upward arc downstream, and then emerges from the sediment at the bed-form crests. The microflow is caused by pressure variations from the hydrostatic on the surface of the bed forms. Pressure is greatest in the low areas, or troughs, between the bed-form crests; lowest pressure occurs in the areas directly over the crests. This pressure distribution results from variations in stream velocity over the bed forms. Experiments show that induced upward-moving seepage following a hydraulic gradient of +0.8 reduces the angle of repose on the bed-form faces by 6 degrees in medium sand. Natural microflow systems should have a similar effect, although the reduction of the angle of repose is probably slight.

PAPER NUMBER 19

TWO GENERATIONS OF SPHENE IN THE TUNK LAKE GRANITE, SOUTHEASTERN MAINE, F. R. Karner. Dept. of Geology, Univ. N. Dak., Grand Forks, North Dakota.

Preliminary study of the accessory mineral sphene, CaTi $[SiO_4]$ (O,OH,F), in the Tunk Lake granite southeastern Maine, indicates that it has formed in two different environments of crystallization. In the outer part of the concentrically zoned granite body which is exposed over a circular area of 70 square miles, sphene occurs as euhedral crystals which appear to have formed early in the crystallization history of the rock. In the center of the body sphene occurs as irregular masses which, along with biotite, secondarily replace primary hornblende, suggesting an origin by late-stage crystallization. The two occurrences help to clarify the problem of early versus late accessory-mineral formation in igneous rocks.

PAPER NUMBER 20

COCCOLITHS FROM THE NIOBRARA FORMATION (UPPER CRETACEOUS) OF NORTHEASTERN NORTH DAKOTA, Rodney M. Feldmann and F. D. Holland, Jr., Dept. of Geology, Kent State Univ., Kent Ohio, and Dept. of Geology, Univ. of North Dakota, Grand Forks, North Dakota.

Abundant coccoliths, minute calcareous skeletal plates from flagellated protists, were recovered through a 120-foot section of calcareous clay shale of the Niobrara Formation from a well in Walsh County, North Dakota. A technique based on settling times rather than repeated centrifuging was developed for extraction of the nannofossils prior to examination and photography under phase contrast conditions at 1250X. Fifteen different forms assignable to eight genera in the Coccolithophoridae and four genera _incertae sedis_ were identified and illustrated. Crushed coccolithophorids are probably the source of the "white specks" used as markers in the Niobrara Formation of the northern Midcontinent. The abundance of coccoliths, but apparent lack of diversity, may indicate boreal waters in the North Dakota Cretaceous sea. This preliminary study, paleogeographic implications, and possibility of stratigraphic zonation as an aid to identifying shallow petroleum prospects suggest importance of search for other coccolith-bearing strata and detailed descriptions of faunas based on electron microscopy as well as on light microscope observation.

PAPER NUMBER 21

GENETICS WITHIN OUR GENERATION, M. L. Buchanan, Department of Animal Science, North Dakota State University, Fargo, North Dakota.

(Invitation Paper: Abstract Not Submitted)

PAPER NUMBER 22

A CHEMICAL APPROACH TO THE STUDY OF ARTHRITIS, G. A. Abbott, Professor Emeritus, Univ. of North Dakota, Grand Forks, N. Dak.

The element phosphorus plays an extremely vital role in all cell metabolism. Dietary data shows that serious imbalances in the intake and excretion of phosphorus are common, causing cumulative metabolic disturbances. The biochemical importance of phosphorus is summarized in NUTRITIONAL DATA. (1). The daily excretion of phosphorus in the urine of an adult is from 0.8 to 1.3 grams. Average, 1.1 grams. (2). Life processes depend on phosphorus. Deficiencies must therefore come from less vital tissues. This withdrawal,or "mining" of phosphorus from various parts of the body, is the basic fact upon which this chemical approach is based. Its effects are incidious and cumulative. The victim is unaware of it until pain is finally felt, in muscles and joints. Already damage has occured. Standard, balanced diets do not always provide necessary phosphorus any more than they do vitamins, amino acids, and minerals, now generally supplemented. This phosphorus deficiency hypothesis could and should be subjected to clinical tests made only by competent physicians working in close cooperation with expert biochemists and expert dieticians. They should determine the best forms of phosphorus for supplements, conditions for their assimilation, with due regard for possible harmful side effects.

PAPER NUMBER 23

MAGNESIUM DEFICIENCY IN THE LAYING HEN, Raja N. Hajj and J. L. Sell. Dept. of Animal Science, North Dakota State University, Fargo, North Dakota 58102.

The influence of levels of dietary magnesium (Mg) on the laying hen was studied in a series of experiments. Feeding a semi-purified basal diet containing 55 ppm of Mg resulted in a considerable decrease in Mg concentration in egg contents and blood serum within one week. Mg concentrations in liver, heart and bone from hens fed either the basal ration or a ration with 100 ppm added Mg were lower than that of hens fed 200 or 400 ppm added Mg. Calcium (Ca) concentration in tissues was not affected except in the case of the liver where an increase in Ca concentration was observed in hens fed the basal ration. Histological studies of the liver from hens fed the basal ration showed a considerable accumulation of fat globules in the cells. Oxidative phosphorylation, measured as P/O ratio of liver homogenates was lower for basal-fed hens than that of hens fed rations containing 100 or 400 ppm added Mg.

PAPER NUMBER 24

STUDIES ON THE NUCLEOTIDE INHIBITION OF THE GLUCOSE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES ASSOCIATED WITH THE YEAST ENZYME, Richard N. Horne, Wayne B. Anderson, and Robert C. Nordlie, Dept. Biochem., Univ. N. Dak., Grand Forks, N. Dak.

In these studies a dialyzed preparation of commercial yeast glucose-6-phosphate dehydrogenase (G-6-PDH) was used. This enzyme has been shown to catalyze the oxidation of both glucose-6-P and β-D-glucose. While nucleotide inhibition of G-6-PDH previously has been reported we have found that the glucose dehydrogenase (GDH) activity of this enzyme is similarly effected. The nucleotide inhibition is highly pH-dependent. ATP(2.5mM) was observed to inhibit both activities by 25-35% at pH 8.5, while at pH 7.0 an inhibition of 70-80% was noted. Kinetic studies show ATP inhibition to be of the competitive type relative to the substrates glucose-6-P or glucose with a K_i of $5.8\text{-}9.2 \times 10^{-4}$M. The inorganic anions HCO_3^-, $SO_4^=$ and $HPO_4^=$ previously have been reported to selectively activate GDH while they inhibit G-6-PDH (Anderson and Nordlie, Biochem. 7 (1968) in press). In the present study, HCO_3^- was observed to alleviate the ATP inhibition of GDH, while it accentuated the inhibition of G-6-PDH. ATP competitively inhibits the HCO_3^- stimulation of GDH yielding an apparent K_i of 1.1×10^{-3}M at pH 8.0. This nucleotide-inorganic anion interrelationship may play a role in the selective control of the two activities catalyzed by this multifunctional enzyme.

PAPER NUMBER 25

POTENTIATION BY DETERGENTS OF ATP INHIBITION OF LIVER MICROSOMAL GLUCOSE 6-PHOSPHATE PHOSPHOHYDROLASE. P. T. Johns and R. C. Nordlie. Dept. of Biochem., Univ. of North Dakota, Med. School, Grand Forks, N. Dak.

The effects of the detergents cetyltrimethylammonium bromide (cetrimide), lysolecithin, and palmityl-CoA on the inhibition by ATP of glucose 6-phosphate hydrolysis have been studied. Kinetic studies have shown that inhibition by ATP is competitive and that K_i(ATP) = 19 mM at pH 7.5 in the absence of detergent. When microsomes were exposed to detergents, inhibitor constants for ATP were decreased to the following values: 2.0 mM with 0.1% cetrimide, 6.2 mM with 0.4 mM lysolecithin, and 7.1 mM with 41 μM palmityl-CoA. It also was observed that the Michaelis constant was considerably reduced in the presence of detergents. However, at physiological concentrations of glucose 6-phosphate (0.13 mM) and ATP (5 mM) the presence of 0.4 mM lysolecithin increased inhibition by ATP from 21% to 43%. Similar increases in the extents of inhibitions were observed with cetrimide and palmityl-CoA. It was noted that K_i values decreased as detergent concentration increased. The observed potentiation of ATP inhibition suggests the possible physiological control of glucose 6-phosphate hydrolysis by natural detergents which potentiate ATP inhibition.

PAPER NUMBER 26

METABOLISM OF THE TRIAZINE HERBICIDES, SIMAZINE AND ATRAZINE, BY THE MILK GOAT, J. E. Bakke and J. D. Robbins. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. Dak.

The triazine herbicides, Simazine and Atrazine, are rapidly metabolized by the milk goat to yield, respectively, 14 and 21 urinary metabolites. The isolation and proof of structure of the organic soluble urinary metabolites will be reported along with excretion and milk and tissue residue data.

PAPER NUMBER 27

IN VIVO METABOLISM OF S-ADENOSYL-L-HOMOCYSTEINE BY THE RAT, J.A. Duerre and C.H. Miller. Dept. of Microbiol. Sch. of Med., Univ. of N. Dak., Grand Forks, North Dakota.

Experimental evidence presented by De La Haba and Cantoni (J. Biol. Chem., 234; 603, 1959) indicated that S-adenosylhomocysteine undergoes hydrolysis in mammals to adenosine and methionine or is degraded to α-ketobutyrate. After intravenous injection of S-adenosylhomocysteine-${}^{3}H$ (homocysteine labeled) into rats, less than 15% of the radioisotope was incorporated into protein methionine or excreted as α-ketobutyrate. The bulk of the isotope was found to be excreted in the urine as an unknown ketoacid. Studies using S-adenosyl-${}^{3}H$-L-homocysteine and S-adenosylhomocysteine-${}^{35}S$ revealed that both the sulfur and the adenosine moieties remained associated with the excretory product. These findings suggest that S-adenosylhomocysteine undergoes direct deamination to yield S-adenosyl-γ-thio-α-ketobutyrate. This compound was isolated and purified by chromatography and precipitation by phosphotungstic acid. It has an absorption maximum at 262 mμ, is ninhydrin-negative, 2,4-dinitrophenylhydrazine-positive, and reacts with orcinol. The compound reacts with nitroprusside in a manner characteristic of sulfoxides. Thus, it appears that this compound undergoes oxidation prior to excretion. Preliminary *in vitro* studies suggest the formation of this compound by liver extracts; however, the activity appears to be quite low. (NSF Support)

PAPER NUMBER 28

CAROTENOID PIGMENTS IN MUSTARD SEED, *BRASSICA JUNCEA*, R. H. Smith and H. J. Klosterman. Dept. of Biochem., N. Dak. State Univ., Fargo, N. Dak.

Mustard oil extracted from mature seed of commercial mustard, *Brassica juncea* showed an absorption spectrum that was characteristic of carotenoid pigments. The pigments were separated by Chromatography and characterized as β-carotene, *cis* and *trans* taraxanthin, and autoxanthin. In addition, two flavoxanthin-like pigments were noted in small amounts, but were not identified. The relative contributions of the components to the total yellow color is as follows: *cis* and *trans* taraxantin = 80%; β-carotene = 10%; miscellaneous minor constituents = 10%.

PAPER NUMBER 29

TRANS-AXIAL COORDINATION OF OXOVANADIUM(IV) IONS, R. G. Garvey, Dept. of Chemistry, North Dakota State Univ., Fargo, N. Dak.

The coordination of 4-substituted pyridine and quinoline 1-oxides trans to the vanadyl oxygen atom in several oxovanadium (IV) chelates has been investigated. Using an infrared procedure variations in absorption attributable to vanadium-vanadyl oxygen stretching modes ($\bar{\nu}_{V-O}$) were recorded for adduct formation with bis(2-4-pentanedionato)oxovanadium(IV), bis(1-phenyl-1, 3-butanedionato)oxovandium(IV), and bis(1, 1, 1-trifluoro-2, 4-pentanedionato)oxovanadium(IV). For the first two oxovanadium (IV) chelates, and in a variety of solvents, a linear correlation was established between the displacement of $\bar{\nu}_{V-O}$ upon adduct formation and a set of substituent parameters, σ_{pyno}, defined in terms of ligand pK_{BH^+} values. Bis(1, 1, 1-trifluoro-2, 4-pentanedionato)oxovanadium(IV) apparently forms adducts with the N-oxide ligands, but the complex was insufficiently soluble to adequately investigate the variations of $\bar{\nu}_{V-O}$.

PAPER NUMBER 30

SORPTION OF H_2O VAPOR AND HYDROGEN DEUTERIUM EXCHANGE EFFECT ON 4-CHLORO-3, 5-DIMETHYLPHENOL-FORMALDEHYDE HEPTAMER, W. S. Hnojewyj and R. H. Peterson. Col. of Chem. and Phys., NDSU, Fargo, N. Dak.

Adsorption and desorption isotherms of water vapor on 4-chloro-3, 5-dimethylphenol-formaldehyde heptamer are measured at 25 and 35°, which demonstrated some slopes in region of monomolecular layer sorption. The total monolayer sorption was approximated to 1.25 H_2O moles/mole of heptamer, for both adsorption and desorption. The isosteric heats at beginning of adsorption and at end of desorption, were found to be 17 and 30 kcal/mole respectively. These values decrease rapidly to the value of water condensation as amount sorbed approaches one mole. The results indicate the presence of only one active phenolic hydroxyl group per linear heptamer and may lead to a clarification of the structural and chemical properties of phenolic polymers. The H→D exchange as determined by successive adsorptions of D_2O or H_2O vapor followed by complete desorptions on the solid sample under vacuum at 10^{-7} mm demonstrate complete reversibility of $\sim$7 labile hydrogens of the hydroxyl groups of heptamer illustrating a precise method to check structure of phenol-formaldehyde polymers. Supported by a NDSU Research Grant.

PAPER NUMBER 31

PREPARATION OF THE 3-META-NITROPHENYL-5-METHYL-ISOXAZOLE-4-CARBOXYLIC ACID AND OF ITS ESTER, AMIDE, AND ANILIDE DERIVATIVES.

Franz H. Rathmann, North Dakota State University, Fargo, North Dakota, and Lui Jon Ravnaas*, Turtle Lake High School, Turtle Lake, North Dakota.

The condensation of the chlorooxime of meta-nitrobenzaldehyde, m.p. 122°C, with sodio-acetacetic ester yielded the ethyl ester of 3-(meta-nitrophenyl)-5-methyl-isoxazole-4-carboxylic acid (I), m.p. 92°C. Alkaline hydrolysis of (I) yielded the free acid (II), m.p. 192°, which with $SOCl_2$ gave the acid chloride (III), m.p. 78°C. Treatment of III with various alcohols, amines and hydrazines yielded the corresponding esters, amides and hydrazides: methyl ester, 105°; ethyl ester, 92°; amide, 197°; N-butyl amide 122°; anilide, 182°; hydrazide, 240°. Phenylene-1,4-bis-[3-(5-methyl-isoxazole-4-carboxylic acid ethyl ester)], prepared from the dichlorodioxime of terephthalaldehyde, gave white crystals, m.p. 160°C. Infra-red N.M.R. and mass-spectra were obtained on several of these substances.

PAPER NUMBER 32

MASS SPECTRA OF ISOXAZOLES. ELECTRON-IMPACT FISSION OF THE PARA-CHLORO-ANILIDE OF 3-PHENYL-5-METHYL-ISOXAZOLE-4-CARBOXYLIC ACID ($C_{17}H_{13}O_2N_2Cl$).

Franz H. Rathmann, John F. Watkins and Keith Klevegaard, Hormel Institute, University of Minnesota, and North Dakota State University, Fargo, North Dakota.

The mass spectra of the 3-phenyl-5-methyl-4-isoxazole-carboxylic acid para-chloro-anilide were obtained on a Hitachi-Perkin Elmer RMU-6D mass spectrometer using 10 to 70 eV impact electrons and various slit-widths, multiplier voltages and sensitivities. A strong group of peaks occurs at 312 to 316, corresponding to the various isotopes of Cl, C, and N of the molecular mass peak. Other especially strong peaks or combinations of peaks indicate loss of various atoms or groups of atoms: 277 (Cl; 235, 237 and 77 (C_6H_5); 186 and 126, 128 ($Cl\text{-}C_6H_4\text{-}NH$); 153, 155 and 158 ($Cl\text{-}C_6H_4N\text{-}C{=}O$); 144, 145 ($C_4H_2\text{-}C_3ONCO$); 63 ($C_5H_3$); 51($C_4H_3$); 43 (OCNH); and 109-113 (meta stable transition 312-316 to 186-7 by loss of $Cl\text{-}C_6H_4\text{-}NH$).

PAPER NUMBER 33

MUSSEL DISTRIBUTION AND ECOLOGY, FOREST RIVER, NORTH DAKOTA, A. M. Cvancara and J. M. Erickson, Dept. of Geol., Univ. N. Dak., Grand Forks, N. Dak.

Twenty-two stations were sampled for mussels on the Forest River, mainly during the early summers of 1965 and 1966. Six species, in approximate order of decreasing relative abundance, inhabit the river: Anodonta grandis, Lasmigona complanata, Lampsilis siliquoidea, Anodontoides ferussacianus, Lasmigona compressa, and Strophitus rugosus. S. rugosus was collected at only one station in the middle section of the river. Greatest concentrations of mussels occurred in the upper section of the river, from near the town of Forest River to near Fordville, where the maximum of 236 mussels was hand picked in one hour by two collectors in 1965. The Forest River is presumably barren of mussels from a few miles below Minto to its mouth. High chloride content and perhaps high turbidity inhibit the occurrence of mussels in its lower reaches. A high total chlorides value of 1230 ppm was measured in 1966. Of the three U. S. Red River Valley tributaries with high chlorides in their lower reaches (Park, Forest and Turtle; Cvancara, A. M., 1966, Proc. 1966 conf. environ. studies Lake Agassiz region, Winnipeg, p. 194), the Forest contains the most mussel species. Supported by UND (NSF) Faculty Res. Grant and N. Dak. Water Resources Res. Inst.

PAPER NUMBER 34

WETLAND VEGETATION OF THE TURTLE MOUNTAINS, NORTH DAKOTA. I. ANALYSIS OF THE FLORA. Robert L. Burgess and Dennis T. Disrud. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak.

In a study of fifty stands of wetland vegetation in the Turtle Mountains in north-central North Dakota, 143 species of plants were encountered. These represented 87 genera in 40 families. Analysis of the species by life form and various ecological characters, as well as phytogeographical affinities reveal interesting relationships with aquatic and sub-aquatic flora in general, with floristic element derivation with wetlands in neighboring regions, and with post-Pleistocene history of the upper midwest. Reasons for the present configuration of the wetland flora include reproductive and migrational attributes of the species, availability of habitat, and length of time since deglaciation. Supported in part by an NSF Traineeship (Grant No. GE - 7952).

PAPER NUMBER 35

AIRBORNE POLLEN ON THE NATIVE PRAIRIE. C. A. Willman* (spon: V. Facey). Dept. of Biol., Univ. N. Dak., Grand Forks, North Dakota.

The study consisted of a qualitative survey of airborne pollen on the native prairie. The study site is located T. 151 N., R. 52 W., Sec. 16, Oakville Township, Grand Forks Co., North Dakota, approximately 12 miles west of Grand Forks, North Dakota. Airborne pollen was obtained using the gravity slide method with a Durham Air Sampling Device from June 15 to August 15, 1967. Gramineae pollen predominated with Chenopodiaceae, Polygonaceae, Compositae and other pollen in lesser amounts.

PAPER NUMBER 36

FOODS OF MINK ON THE J. CLARK SALYER NATIONAL WILDLIFE REFUGE, NORTH DAKOTA, Wayne Cowan, Biol. Dept., Univ. N. Dak., Grand Forks, North Dakota.

This study was conducted to: 1) determine seasonal diets of mink, 2) establish the relationships between principal foods in the diet and prey population levels, and 3) ascertain possible feeding on waterfowl that had succumbed to botulism intoxication. A total of 310 scats collected during eight summer and fall seasons of an 11 year period were examined. The frequency of occurrence of muskrat and waterfowl remains in each year's scat samples was compared to annual populations of these species. Additional comparisons were made between utilization of waterfowl and estimated annual waterfowl losses to botulinus toxin. Meadow voles, muskrats, song birds, and waterfowl were the principal foods utilized. During periods of unavailability of any of these species, there was an apparent shift in diet toward other foods, including invertebrates and several species of small mammals. Mink predation had no apparent effect on muskrat population levels, but rather reflected availability of this species; occurrence of waterfowl remains in the scats was also in proportion to density of the prey populations. Evidence that mink fed on waterfowl suffering from the effects of botulism was inconclusive; however, there was some indication of use of infected waterfowl.

PAPER NUMBER 37

REPRODUCTION IN THE WHITE-TAILED JACK RABBIT IN NORTH DAKOTA.

Ted R. James and Robert W. Seabloom. Dept. of Biology, UND, Grand Forks, North Dakota.

Reproductive characteristics of the white-tailed jack rabbit (*Lepus townsendii campanius* Hollister) were determined from 836 animals collected between April, 1964 and December, 1965. A synchronous breeding pattern, with four well defined peaks of annual activity, was apparent. The breeding season was 148 days in length, and extended from late February through mid-July. A definite annual cycle of sperm production was found. The monthly weights of the testes and the epididymi corresponded to this cycle. The number of ova shed decreased steadily as the breeding season progressed. Females produced an average of 3.29 litters annually. Litter size ranged from one to nine, with an average of 4.6, and was effected by seasonal changes in prenatal mortality. Pre-implantation and intra-uterine loss was 21 per cent; and appeared to be more prevalent in older females.

PAPER NUMBER 38

NORTH DAKOTA FLEAS. I. *HYSTRICHOPSYLLA* FROM GRAND FORKS COUNTY.

O. R. Larson and S. A. Peterson*. Dept. of Biol., Univ. N. Dak., Grand Forks, N. Dak.

A perusal of the literature indicates that only 11 species of fleas have been reported from North Dakota. While examining local rodents in the fall of 1967, one female specimen of *Hystrichopsylla* (UND Parasit. Coll. No. 331) was removed from *Clethrionomys gapperi* collected near Arvilla. Earlier, a male flea of the same genus (UND Parasit. Coll. No. 202) was taken from a bumblebee nest at Inkster during the summer of 1963 by Dr. G. C. Wheeler. According to the revised genus (Holland, G. P., Canad. Entomol., 89: 303-324, 1957)both of our specimens are *H. dippiei dippiei* Roths. Fleas of the *dippiei* complex, mainly found in low numbers on rodents in the Rocky Mountain and western states, are notable in their large size (our ♂ = 4 mm, ♀ = 5.5 mm), and in the possession of two spermathecae by the female. The unusual presence of a flea in a bumblebee nest probably reflects the parasite's abandonment of a frightened, retreating rodent. The occurrence of *H. dippiei dippiei* in eastern North Dakota is the second report for the state, and appears to be the most easterly record in the continental USA.

PAPER NUMBER 43

THE RELATIONSHIP BETWEEN INTEGRAL GROSS AND INTEGRAL NET PHOTOSYNTHESIS VS THE RATIO OF INTEGRATED PHOTOSYNTHESIS-TO-INTEGRATED RESPIRATION, THE RATIOS OF P-TO-R AT 0, 1, 2, AND 3 m DEPTHS AND TRANSPARENCY IN SEVERSON LAKE, MINN. AND SPIRITWOOD LAKE, N. D.

G. W. Comita & E. G. Wolf, Dept. Zool., NDSU, Fargo, N.D.

In Severson Lake integral gross production correlates significantly with transparency and the ratio of integrated photosynthesis-to-integrated respiration. Integral gross photosynthesis is not significantly related to the ratio of P-to-R at 0, 1, 2 and 3 m, but a significant relationship was found for integral net production (except at 3 m) and transparency. In Spiritwood Lake significant relationships were found between integral gross production as well as integral net production vs transparency, P-to-R at 0, 1, 2 and 3 m levels and the ratio of integrated photosynthesis-to-integrated respiration. In both lakes the relationships are better with the ratio of integrated photosynthesis-to-integrated respiration. Supported by U. S. Dept. Int. Off. Water Res. P.L. 88-379, NSF Grants 1257, 4806, 16415, N. D. Game & Fish Dept., & N. D. Inst. Reg. Studies.

PAPER NUMBER 44

ELECTROPHORETIC ANALYSES OF MALE-STERILE AND MALE-FERTILE ANTHERS OF SUDANGRASS

Sultanul Alam and P. C. Sandal. Dept. of Agron., College of Agriculture, N. Dak. State Univ., Fargo, North Dakota.

Fresh anthers from each of two male-fertile and one male-sterile lines of sudangrass sampled at the pre-pollen stage were appropriately prepared to conduct electrophoretic analyses to measure total protein, basic protein, peroxidase activity, and cytochrome oxidase activity. Results of this study revealed a) 20 bands in male-fertile in contrast to nine in male-sterile anthers for total protein, b) eight bands in male-fertile and five bands in male-sterile anthers for basic protein, c) eight bands in male-fertile in contrast to seven bands in male-sterile anthers for peroxidase activity, and d) seven bands in male-fertile in contrast to two bands in male-sterile anthers for cytochrome oxidase activity. The consistently fewer number of chemical components observed in male-sterile anthers as measured by electrophoretic analyses suggests that heritable abnormal metabolic activity conditions male-sterility and subsequent pollen abortion in sudangrass.

PAPER NUMBER 45

ELECTROPHORETIC DIFFERENCES IN LEAF PROTEINS AMONG CLONES OF PERENNIAL SOWTHISTLE, S. Alam, A. R. Bell, J. D. Nalewaja and A. B. Schooler. Dept. of Agron., College of Agriculture, N. Dak. State Univ., Fargo, North Dakota.

Leaf proteins from 29 clones of perennial sowthistle, Sonchus arvensis L. were analyzed by disc gel electrophoresis in order to determine the relationship between the plant proteins and the reaction of the plants to specific herbicides. Proteins were extracted by grinding fresh young leaf tissue in potassium acetate buffer at pH 2.9. After electrophoresis, the gels were stained overnight in 0.5% aniline black in 7% acetic acid and destained electrically. The stained protein in polyacrylamide gel revealed six to eleven visible bands. The banding pattern was uniform within each clone at the same growth stage but varied among the different clones, and different growth stage within a clone. The plants with a greater number of visible protein bands appeared to be more susceptible to 2,4-D, dicamba and Tordon. Supported by USDA Grant ARA 12-14-100-9175(34).

PAPER NUMBER 46

KARYOTYPE ANALYSIS OF PEROMYSCUS MANICULATUS BAIRDII POPULATIONS IN NORTHEASTERN NORTH DAKOTA, R. A. Ahokas. Biol. Dept., Univ. of N. Dak., Grand Forks, N. Dak.

The karyotype of Peromyscus maniculatus bairdii inhabiting grassland and woodland habitats was investigated. Comparison of individual karyotypes was made to investigate the possibility of chromosomal polymorphism within the population. Cytological observations were made on bone marrow cells prepared by the blaze-drying technique, following intraperitoneal injection with .1 percent colchicine, from each of 25 animals. Analysis of the chromosomes revealed a 2n number of 48 with 4 pairs of acrocentrics, 6 pairs of large submetacentrics, 13 pairs of small metacentrics and submetacentrics, plus a large submetacentric X and a small submetacentric Y chromosome. It does not appear that chromosomal polymorphism exists within this population.

PAPER NUMBER 47

KARYOTYPE STUDY OF TWO SUBSPECIES OF ONDATRA ZIBETHICA.

G. W. Dewald. Biol. Dept., Univ. N. Dak., Grand Forks, N. Dak.

The purpose of this investigation was to determine karyotypes of *Ondatra zibethicus zibethicus* Linneaus and *Ondatra zibethicus cinamominus* Hollister. A standard karyotype is useful for basic information and may lead to speculations concerning chromosome evolution in the North American muskrat, *Ondatra zibethica*. The specimens were classified according to morphological characteristics and geographical ranges. Cytological observations were made on an average of 30 metaphase spreads for each of 12 specimens. The slides were prepared by the standard air-dried technique from the bone marrow of the animals following intraperitoneal injection with .05 percent colchicine solution. The preparations were stained with carbol fuchsin. Analysis of the karyotypes revealed 48 acrocentrics, 2 submetacentrics, 2 metacentrics and acrocentric X and Y chromosomes. The karyotypes from both subspecies were identical. This is the first study of karyotypes at the subspecific level; however, the results are similar to two records for the species. It would appear, therefore, that morphological variation in these subspecies is primarily genetic since no apparent variation at the cytological level was observed.

PAPER NUMBER 48

ANAEROBIC MICROBIAL CATABOLISM BIOASSAY METHOD FOR DI-N-BUTYL SUCCINATE,

P. P. Williams, M. C. Helgeson* and E. J. Thacker. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. Dak.

This is believed to be the first report of a pesticide biological assay method for anaerobic microorganisms. The major use of di-n-butyl succinate (Tabutrex) is as an insecticide/repellent for flies on farm animals and agricultural premises. Ingestion by ruminants of foodstuffs contaminated with this compound, in turn, would expose the insecticide to rumen microbes. When Tabutrex was incorporated as an emulsion in a rumen-microbe-seeded medium and incubated under CO_2, clear areas in the medium indicated breakdown of the compound. Gas chromatographic analysis has now confirmed one of the degradation products as succinic acid. Other data suggest that young calves possess large quantities of this nonspecific esterase, and that 2.8 to 15.7% of the microflora of older calves have this esterase. Rumen protozoa, *Isotricha* sp. do not produce this enzyme extracellularly.

PAPER NUMBER 49

ANTIMICROBIAL ACTIVITIES OF LINATINE AND OTHER HYDRAZINO ACIDS. J. L. Ninnemann*, J. L. Parsons, and H. J. Klosterman. Depts. of Bact. and Biochem., N. Dak. State Univ., Fargo, North Dakota.
Continuing investigations with the vitamin B_6 antagonist (linatine) isolated from flaxseed and identified as 1-[(N-γ-*L*-glutamyl)amino]-*D*-proline by Klosterman et al. (1967), show it to be the only known naturally occurring representative of a new group of hydrazino acid antibiotics. The *D*- and *L*-isomers of both linatine and 1-aminoproline (demonstrated to be the active part of the molecule) were found to be effective against a wide variety of bacteria, particularly gram negative types. In most cases, 1-amino-*D*-proline proved to be the most active compound. A comparison of the antibiotic activities of linatine and the other aminoprolines with ten synthetic hydrazino acids again clearly demonstrated the greater effectiveness of 1-amino-*D*-proline. Physiological studies are currently underway to determine the site and mode of action of the antibiotic. Of the water soluble vitamins tested, vitamin B_6 was the only one capable of reversing aminoproline inhibition, indicating a specific blockage of some B_6 requiring enzyme(s). *Azotobacter chroococcum* inactivated 1-amino-*D*-proline after 24-28 hr. It is hypothesized this inactivation is accomplished through its conversion to *D*-proline. Support by NDSU Ag. Expt. Sta., and NIH (AI 08220-01).

PAPER NUMBER 50

THE EFFECT OF ANTIBIOTICS ON RUMINAL BACTERIA, Chia-li Wang*, B. B. Baldwin, and R. S. Fulghum. Dept. of Bact., N. Dak. State Univ., Fargo, North Dakota.

We have reported (*Appl. Microbiol.*, *16* (2):301, 1968) on antibiotic disc assays to show the patterns of resistance and susceptibility among autochthonous ruminal bacteria. There was some evidence that antibiotic resistant individuals occurred within susceptible species.

The present work reports on *in vitro* tube dilution inhibition studies of fifteen species of ruminal bacteria against nine antibiotics, using concentration of 0, 0.1, 1, 10, 100, and 200 µg of antibiotic activity per ml. of liquid culture medium. Among these nine antibiotics, ten species were resistant to kanamycin, nine to neomycin, nine to streptomycin, two to tylosin, two to bacitracin and one to penicillin G. All were susceptible to erythromycin, oxytetracycline, and chlortetracycline. Susceptibility to the various antibiotics in these species ranged from 0.1 to 200 µg of antibiotic per ml. of media. In general, it appears that susceptible pure cultures of ruminal bacteria are bacteriostatic but not bactericidal in response. Furthermore, the data suggests that mutation to antibiotic resistance occurs in some species.

PAPER NUMBER 51

DEVELOPMENT OF HETEROGENOUS POPULATIONS OF PURPLE-SULFUR BACTERIA IN SEWAGE LAGOONS, Harvey W. Holm* and John W. Vennes, Dept. of Microbiol. Sch. of Med. Univ. of N. Dak., Grand Forks, North Dakota.

Waste-contribution by potato-processing industry allows the annual emergence of purple-sulfur bacteria in the municipal sewage lagoon at Grafton, N. Dak. Dominant organisms in the lagoon during the purple-sulfur phase (ca. July) included the genera Thiocapsa, Thiopedia and Chromatium. Other microbial populations observed included the enterococci, which decreased throughout the summer; algae, which increased in August; and the sulfate reducers, coliform and total bacteria all of which were at maximal levels at the time of maximal purple-sulfur populations. Sulfied, sulfate and total carbohydrate levels were minimal during the month of July. The alkalinity was 900-1000 mg/1 and the pH was 8.2-8.4 during the purple-sulfur phase. No significant changes were noted in phosphates.The efficiency of the lagoon was not impaired by the purple-sulfur bacteria, since the BOD_5 dropped exponentially during the summer(s).

PAPER NUMBER 52

IMMUNOLOGICAL BEHAVIOR OF BOVINE CARBONIC ANHYDRASE ISOZYMES, Walter Kisiel* and George Graf* (Spon: H. J. Klosterman). Dept. of Biochem., North Dakota State Univ., Fargo, North Dakota.

Three electrophoretically pure components (BCA-I, BCA-II, and BCA-III) of bovine red cell carbonic anhydrase (Carbonate hydro-lyase, EC 4.2.1.1) were obtained by preparative disc electrophoresis, and assayed for carbonic anhydrase activity potentiometrically. BCA-II and BCA-III were used as antigens to elicit antibody production in rabbits. A total of 20 mg. of each component, emulsified in Freund's adjuvant, was injected intracutaneously over a period of three weeks and the antisera were tested by the Ouchterlony double-diffusion method. Anti-BCA-II and Anti-BCA-III showed positive precipitation patterns against all three electrophoretically pure components. The results indicate that, in contrast to the immunological behavior of human carbonic anhydrase, electrophoretically separable forms of bovine carbonic anhydrase are immunologically not distinguishable.

PAPER NUMBER 53

SINGLE-STAGE ADRENALECTOMY IN EWES, I.E. Berg and J.E. Tilton. North Dakota State University, Fargo, North Dakota.

Modifications of existing adrenalectomy techniques were undertaken to establish an efficient method of adrenal removal in large numbers of sheep. Fasted, prepared ewes were given an intravenous sedative. Anesthesia was completed by infiltration of a local anesthetic. Two incisions, one on each side, were used. The incisions were made parallel and 2 cm ventral to the transverse processes of the lumbar vertebrae. The incision was started 2 cm posterior to the last rib and continued 10-12 cm caudad. The peritoneal cavity was at no time invaded. The gland was freed from its attachments by digital manipulation. A curved forceps was placed across the attachment to the posterior vena cava with respect to the left adrenal or the renal vein associated with the right gland and the gland removed. The forceps was left in place until incision closure was nearly completed. Ligation was not attempted. Sixty-nine ewes were adrenalectomized. Six of these died as a direct result of surgery, two from internal hemorrhage and four from what was presumed to be surgical shock and/or anesthetic overdose. Following adrenalectomy, the ewes were maintained by daily injections of 20 mg. cortisol and 5 mg. deoxycorticosterone.

PAPER NUMBER 54

THE USE OF CELL CULTURES FROM DISPERSED EMBRYONIC HOUSE FLY (MUSCA DOMESTICA) TISSUES IN STUDIES OF DIFFERENTIATION, P.E.EIDE and M.M. JENRICH*. Met. and Rad. Lab. USDA Univ. Sta., Fargo,N.D.

Large populations of cells undergoing synchronized differentiation are needed:(1) to work out details and interacions of synthetic and other metabolic pathways of cells undergoing differentiation and (2) to look at the factors that account for the differences of behavior observed between cell and organ cultures. We have recently developed a method at this laboratory to obtain with relative ease clean, uniform, and actively growing dispersed cell cultures from embryonic house fly tissues. Under properly controlled conditions, these cells exhibit the ability to develop into "differentiated tissue." Dependent upon enviornmental conditions, the types of tissue most prevalent are either muscle or nerve or some combination of the two. Other types of tissue are also observed along with some "nondifferentiated" material. A study of the relationship of differentiation to mitosis was carried out using autoradiographic tracer techniques. Cultures were pulsed with H^3-thymidine at various intervals over a two-week period. Results show a high rate of DNA synthesis (mitotic activity) during the early stages of differentiation ans lessening to a point where no mitotic activity is observed.

PAPER NUMBER 55

EFFECTS OF SULFUR AND LIGNITE ON DAKOTA SOILS. Diane R. Nash, St. Mary's High School, New England, North Dakota.

Several years ago I noticed that soil near lignite deposits on our farm seemed to produce more vegetation than soils away from such deposits. Earlier research articles have correlated such increased productivity with the breakdown of lignite into humic acids. My interest in the problem led to a series of experiments which involved the conditioning of four types of Dakota soils with various combinations of lignite in the form of leonardite, sulfur, and microorganisms. For each soil type, the various combinations and controls were seeded with two different types of wheat grass. Melted snow was used to water the seeded plots. The results indicate that the amended soils were definitely more productive. The beneficial action of sulfur and lignite involves a lowering of the soil's pH, and a release of essential plant nutrients. The reclamation and increased productivity of alkaline soils is therefore possible through the use of sulfur and lignite.

PROCEEDINGS
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EDITOR

Francis A. Jacobs

Published jointly by the University of North Dakota, North Dakota State University, Minot State College, Jamestown College and Dickinson State College.

JULY, 1969

GRAND FORKS, NORTH DAKOTA

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PREFACE

Papers appearing in this issue of the Proceedings have appeared in Abstract form at the time of the Sixtieth Annual Meeting of the North Dakota Academy of Science, May 3 and 4, 1968, held at North Dakota State University, Fargo. The order of papers follows the sequence in which they were presented at the meeting, except for the student competition and invited papers. For the convenience of the reader there is an author index included in this issue.

Editorial changes have been kept minimal. It is assumed that the style of each paper follows one which is appropriate for its respective field. The content of the papers and conclusions drawn are the responsibility of the authors. It will be noted that the first paper is printed on page 29, to follow the Abstracts in page-sequence for the completion of Volume XXII of the Proceedings of the Academy.

F. A. Jacobs

Grand Forks, North Dakota

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Dennison Award Competition

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Invited Paper

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PROPERTIES OF LIPOXIDASE AND *HYDROPEROXIDE ISOMERASE* FROM FLAXSEED AND THEIR OCCURENCE IN OTHER SEEDS

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Second Place Winner
A. Rodger Denison Student Research Competition

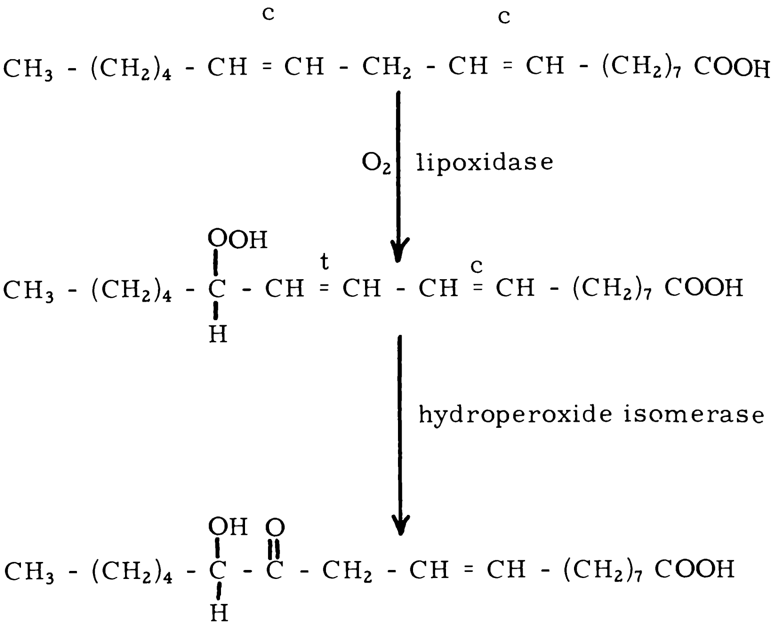
Lipoxidase catalyzes the enzymatic oxidation of certain polyunsaturated fatty acids to their hydroperoxides. In some seed tissues the hydroperoxide is subsequently isomerized to a ketohydroxy fatty acid by a hydroperoxide isomerase enzyme. Separation of the two enzymes from flaxseed extracts was accomplished by ammonium sulfate fractionation. Activity measurements during 10 days' germination showed that the total lipoxidase activity decreased about 90% and the hydroperoxide isomerase activity decreased about 65%. The cotyledons contained most of the lipoxidase activity while the hydroperoxide isomerase was most active in the hypocotyl. Flaxseed lipoxidase was inhibited by a 2 X 10^{-4} M nordihydroguaiaretic acid and 10^{-3} M iodoacetamide. Hydroperoxide isomerase is destroyed by heating for 5 1/2 min at 55°. It is not inhibited by 10^{-3} M KCN, 10^{-4} M *p*-chloromercuribenzoic acid, 10^{-3} M ethylenediaminetetraacetic acid (EDTA), or 10^{-3} M iodoacetamide. In addition to flaxseed, hydroperoxide isomerase has been found in barley, soybean, wheat embryo, alfalfa, and mung beans.

INTRODUCTION

For several years it has been known that the oxidation of certain unsaturated fatty acids to their hydroperoxides is catalyzed by the enzyme lipoxidase (3). This paper will deal with lipoxidase extracted from flaxseed *Linum usitatissimum* L., and certain other seeds and also the subsequent isomerization of the hydroperoxide to a ketohydroxy fatty acid by a second enzyme, hydroperoxide isomerase. The reactions for linoleic acid are:

EXPERIMENTAL

Assay. The reaction to form the hydroperoxide was followed by the increase in absorption at 234 mμ as the conjugated diene molecule is formed. The isomerization reaction was likewise followed by a decrease in absorption at 234 mμ as the conjugated system is disrupted. The presence of the keto-hydroxy fatty acid was checked periodically by its ability to reduce dichlorophenol indophenol. All of the assays inlcuded heat denaturation (10 min at 100°) to show that the observed reactions were enzymatic.



$$CH_3-(CH_2)_4-CH\overset{c}{=}CH-CH_2-CH\overset{c}{=}CH-(CH_2)_7\,COOH$$

$$\Big\downarrow O_2 \;\text{lipoxidase}$$

$$CH_3-(CH_2)_4-\underset{H}{\overset{OOH}{\overset{|}{\underset{|}{C}}}}-CH\overset{t}{=}CH-CH\overset{c}{=}CH-(CH_2)_7\,COOH$$

$$\Big\downarrow \text{hydroperoxide isomerase}$$

$$CH_3-(CH_2)_4-\underset{H}{\overset{OH}{\overset{|}{\underset{|}{C}}}}-\overset{O}{\overset{\|}{C}}-CH_2-CH=CH-(CH_2)_7\,COOH$$

The substrate consisted of a 1:1 ratio (by volume) of linoleic acid 8.05 x 10^{-3} M and Tween 20 (polyoxyethylene (20) sorbitan monolaurate) as an emulsifying agent in pH 9.0 borate buffer (2). Other substrates used were linolenic acid and Tween 20 in borate buffer, and linoleic or linolenic acid dissolved in absolute ethanol (1).

Flaxseed was germinated in moist paper toweling at 25° in the light. Extraction of the enzymes was accomplished by incubation of ground-up seeds or their acetone powders in 0.1 M phosphate buffer of pH 7.4 for one hour. The mixture was then centrifuged at 12,000 x g and the supernatant saved.

Separation of lipoxidase and hydroperoxide isomerase from flaxseed acetone powder extracts for independent assays was obtained by ammonium sulfate fractionation. The 42-50% fraction contained no lipoxidase activity but good hydroperoxide isomerase activity. Extracts containing lipoxidase activity but free from hydroperoxide isomerase activity were obtained by heading the crude extract for 5 min at 55°C.

RESULTS

The effect of flax seedling age on lipoxidase and hydroperoxide isomerase activities was determined by harvesting seedlings after 2, 4, 6, 8 and 10 days of germination. The results are shown in Table I.

TABLE I

LIPOXIDASE AND HYDROPEROXIDE ISOMERASE (HI) ACTIVITY DURING FLAXSEED GERMINATION

Age (days)	Lipoxidase Specific Activity (ΔA/min/mg)	HI Specific Activity (ΔA/min/mg)	Protein Concentration (mg/ml)	Total Protein mg	Lipoxidase Total Activity (ΔA/min)	HI Total Activity (ΔA/min)
2	0.606	-0.972	19.3	77.2	46.7	-75.0
4	0.269	-0.839	15.6	62.4	16.8	-52.4
6	1.80	-1.88	5.2	20.8	37.4	-39.1
8	0.672	-1.45	6.4	25.6	17.2	-37.1
10	0.224	-1.51	4.6	18.4	4.1	-27.8

Both enzymes decrease in total activity as the seedling becomes older. It is significant to note, however, that the total lipoxidase activity at 10 days decreases to less than one-tenth that of the two-day level, while total hydroperoxide isomerase activity decreases to only about one-third. Since these data reflect activity in the whole seedling, it was hard to tell what occurred in the individual tissues. In order to provide a picture of what happened, seven-day-old flax seedlings were separated into root, hypocotyl, and cotyledon and the enzyme activity in each section was determined. The results are shown in Table II.

TABLE II

DISTRIBUTION OF ENZYME ACTIVITIES IN SEVEN-DAY-OLD FLAX SEEDLING

Plant Section	Lipoxidase Specific Activity (ΔA/min/mg)	Hydroperoxide Isomerase Specific Activity (ΔA/min/mg)
Root	-	-0.115
Hypocotyl	-	-0.859
Cotyledon	0.070	-0.476

Lipoxidase activity appeared only in the cotyledon, whereas hydroperoxide isomerase activity appeared in all parts with a significantly higher specific activity in the hypocotyl. Although the total activity of the hydroperoxide isomerase enzyme decreases with the age of the plant, its high specific activity in the hypocotyl suggests that it was synthesized there.

Assays were run with several known enzyme inhibitors present. Flax lipoxidase activity was lost in the presence of 2 x 10^{-4} M nordihydroguiaiaretic acid, a known inhibitor of plant lipoxidases. It was not affected by 10^{-3} M KCN or 10^{-3} M (EDTA), an efficient metal complexing agent. These results are in agreement with the work on other lipoxidases, namely that nordihydroguaiaretic acid is a competitive inhibitor of lipoxidase and that lipoxidase does not require a metallic ion for activity. Flax lipoxidase activity was also inhibited by thiol-attacking 10^{-3} M iodoacetamide but unaffected by 10^{-4} M p-chloromercuribenzoic acid.

Hydroperoxide isomerase activity in flaxseed was unaffected by 10^{-3} M KCN, or 10^{-3} M EDTA. Both lipoxidase and hydroperoxide isomerase were denatured when the assay was conducted in 4 M urea.

The presence of hydroperoxide isomerase in a number of other seeds was determined. Ammonium sulfate fractionation of an extract from a soybean acetone powder revealed a small amount of hydroperoxide isomerase activity in the 30-40% fraction. An extract of barley seeds displayed good activity of both lipoxidase and the isomerase.

A wheat germ (wheat embryo) extract was prepared and assayed for enzyme activity. Both enzymes were present and could be separated by ammonium sulfate fractionation. The 30-40% fraction showed lipoxidase activity but no hydroperoxide isomerase activity. Thin layer chromatography revealed that the reaction products were the same as those from the flaxseed enzymes.

Alfalfa seeds were found to have good lipoxidase activity and a small amount of hydroperoxide isomerase; however, the two could not be separated by ammonium sulfate fractionation alone.

An extract of an acetone powder of mung beans gave good activity for both enzymes. A reasonably good separation was obtained by ammonium sulfate fractionation with the hydroperoxide isomerase enzyme in the 0-30% fraction and the lipoxidase enzyme in the 40-50% fraction.

There appeared to be no isomerase activity in jack beans and the results with green peas were inconclusive. The demonstration of the hydroperoxide isomerase enzyme in the six varied sources reported here indicates that the enzyme may be of general distribution. As such, it may play an important role in the oxidative metabolism of linoleic and linolenic acid. Future work will determine the exact nature of the reactions involving the keto-hydroxy fatty acids.

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FLORAL ABSCISSION IN *TULIPA*

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Abscission is a natural phenomenon leading to the detachment of leaves, fruits, flowers, stems and other plant organs. Investigation into the anatomy and physiology of the process in leaves had led to an extensive literature reviewed by Pfeiffer (1928) and Addicott and Lynch (1955). Paucity of information on floral abscission led to the present investigation on *Tulipa* which was selected mainly for its large turgid flowers, amenable to hand-sectioning during abscission.

METHODS

Flowers were collected at different stages of blooming. Preserved materials were stored in 70% ethanol; others were stored in the refrigerator for hand sectioning. The flower was trimmed until only a narrow region between receptacle and petals remained. They were dehydrated, embedded and sectioned at 10-15 μ. Sections were utilized for microchemical tests and for permanent mounts. Pectic substances were detected by ruthenium red staining, solubility tests and the ferric chloride hydroxylamine test (Jensen, 1962). Pectic acid was extracted with 2% ammonium hydroxide and insoluble pectates with 1% hydrochloric acid followed by 2% ammonium hydroxide. The presence of pectins was confirmed by extraction with hot water after detection by the ferric chloride hydroxylamine test. The duration of each treatment was one hour at 80°C. Starch, proteins and cellulose were tested with the standard IKI, eosin and IKI and sulphuric acid respectively.

RESULTS

When the flower was in the bud stage, the abscission zone was not differentiated. The latter is apparent after the flower has been blooming for a day or two. This zone of small, round cells began to differentiate at the base of the petal. The differentiation extended towards the receptacle and outwards to the periphery. When the abscission zone was fully developed, it consisted of four to twelve layers of cells. These cells are characteristically small, elongated with dense cytoplasm and contain a conspicuous nucleus, hence are characteristic of meristematic cells. Starch, proteins and cellulose are found in the cells throughout the abscission zone.

The presumptive abscission zone during the early blooming stage revealed the presence of pectic materials throughout the cell walls including the middle lamellae (Figure 1). Treatment with hot water led to considerable loss of pectic materials, presumably water soluble pectins. Subsequent treatment with 2% ammonium hydroxide was ineffective in removing the remainder, therefore the pectic materials at this stage in development are mainly pectates and water soluble pectins.

The abscission zone became distinguishable in older flowers, that is, those which had been opened for about seven days. Microchemical tests revealed that the pectic materials were present as pectates and pectins, with the latter predominanting at the time of abscission. Substantiation of the preceding was obtained through solubility tests.

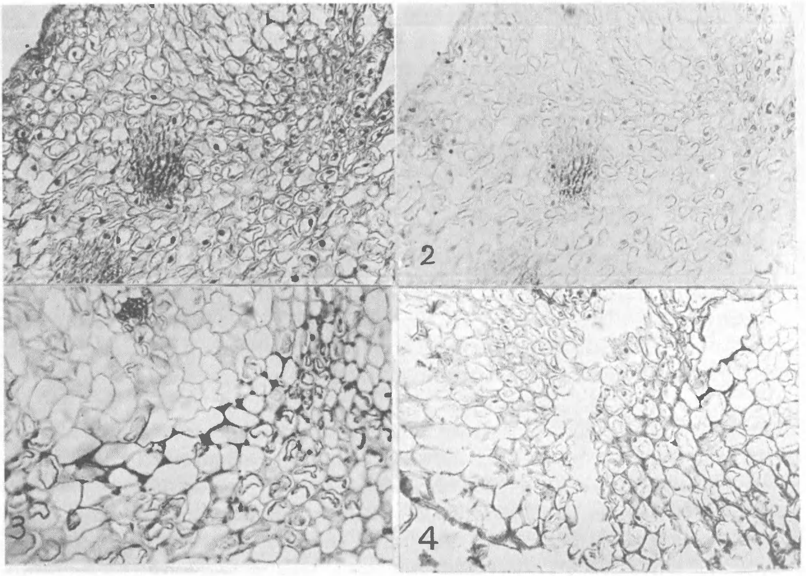

FIGURE 1—L. S. two-day old tulip flower; abscission zone in brackets at base of petal.
FIGURE 2—L. S. two-day old tulip flower after treatment with 1% hydrochloride acid. Pectic material removed. Same section photographed in Figure 1.
FIGURE 3—L. S. seven-day old tulip flower. Abscission zone prominent.
FIGURE 4—L. S. nine-day old tulip flower during natural abscission process.

A lesser amount of water soluble pectin was removed by the standard water treatment than was present in the younger blooming stage (Figure 3). Separation of the abscission zone cells followed the consecutive treatment with HCl and NH_4OH. Thus, the cells were cemented together by pectates and not pectic acid.

Later, prior to abscission, the zone undergoes further changes in the pectates to water soluble pectins leading to the separation of the cells in the abscission zone (Figure 4). No indication of changes in the cellulose was obtained, neither was there the formation of a lignified protective layer. Unlike the process in leaf abscission the pectic materials undergo a change to water soluble pectins without intermediary pectic acid.

In the abscission zone, no definite abscission layer was observed. Separation of the cells began in the cortical part of the zone and extended sideways. In some flowers, only one layer of cells broke apart. In others, several layers distorted, resulting in the detachment of the petal. No cell division accompanying abscission was observed. The exposed surface dried up and turned brown, functioning as a protective layer.

During this investigation attention was given to the early blooming Red Emperor variety. Many of its flowers retained petals which were faded and dry. When sections of these flowers were prepared and tested microchemically, it was found that the insoluble pectates were present in an apparently unchanged state, thus preventing abscission.

DISCUSSION

The process of abscission in the cells of the abscission zone may involve two phases of chemical alternations. The soluble pectins are converted to insoluble pectates during the early developmental stages. Later, with maturity, the insoluble pectates are methylated to form soluble pectins which lead to separation of cells in the affected area. Investigators have recognized four types of such separations: 1) the middle lamella dissolves while the primary wall of adjacent cells remains intact (Facey, 1950); 2) both middle lamella and primary wall dissolve leaving the secondary cellulose wall intact (Eames and MacDaniels, 1947); 3) entire cells of the abscission layer dissolve (Lloyd, 1916); 4) a mechanical breaking through abscission layer walls (Pfeiffer, 1928).

In *Tulipa* flowers, abscission is preceded by chemical changes in the middle lamellae and in the pectic materials incorporated in the cellulose walls. The calcium pectates of the middle lamellae were changed to a water soluble pectin thus leading to the separation of the cells within the abscission zone. There was no indication of an intermediate pectic acid such as has been found for leaf abscission (Facey, 1950). Neither was there any evidence forthcoming for cellulose breakdown. Hence this investigation reveals that floral abscission differs fundamentally from leaf abscission in the sequence of chemical changes, in that as far as can be determined, calcium pectate is converted to water soluble compounds without intermediate insoluble forms.

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EFFECT OF DIETARY RESTRICTION UPON DIELDRIN POISONING IN CHICKENS

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Chlorinated hydrocarbon insecticides are commonly found in our environment (1, 5). Because of their prevalence, they may be ingested with food and feedstuffs by man and domestic animals; and their accumulation in fat tissues of the body has been observed in many species of animals (12). It has been suggested that, during periods of fat mobilization, these insecticides deposited in body fat (10, 11) may be released into the blood and transported to the brain until a concentration is reached which may cause death of the animal. Mobilization of fat and subsequent release of insecticides has been suspected as one of the major causes of death in migrating birds (2). Therefore, it seems logical to determine what effect these insecticides which have accumulated in body tissues may exert on an animal during dietary restriction.

The following experiment was conducted to determine the effect of dietary restriction on survival of chickens that had been previously fed varying levels of dieldrin, a chlorinated hydrocarbon insecticide.

MATERIALS AND METHODS

Forty-eight leghorn hens were selected at random from a common pen and placed in individual cages. But, because of the facilities, they were fed *ad libitum* and watered in units of three. These three-hen units were then randomly assigned to four experimental groups of four units each. The four groups were fed rations containing the following levels of dieldrin: (1) control—"O" ppm; (2) 20 ppm; (3) 40 ppm; and (4) 80 ppm. These rations were fed for a period of six weeks, at the end of which time all feed was removed.

The control ration was a commercial laying mash containing 16% protein. Technical grade dieldrin, which is 85% 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene, or HEOD, was dissolved in corn oil and premixed into a small quantity of the control ration. Sufficient quantities of this premix were then mixed into additional laying mash to give the levels of dieldrin desired in rations 2, 3 ,and 4. These rations were analyzed before feeding to make certain that they contained the correct amount of HEOD. Hereafter, HEOD will be referred to as dieldrin.

Feed consumption was measured weekly by difference in weight of the feed containers. Consumption per hen per day was then calculated.

The hens were weighed and sampled for abdominal fat at biweekly intervals through the sixth week. The fat biopsies were performed as follows: The feathers were removed from the abdomen and the abdomen cleaned with an aqueous solution of germicide; about 0.5 ml of 2% procaine hydrochloride was injected subcutaneously along the midline; an incision about 2 cm in length and about 3 cm below the anal opening was made; 0.5 to 1 g of fat lining the abdominal cavity was removed using forceps and scissors and using care not to damage the intestines and surrounding tissue; 0.1 ml of an antibiotic containing penicillin and streptomycin was injected within the abdominal cavity; the wound was closed with stainless steel clips, and an ointment containing Polymyxin B, Bactracin, and Neomycin was smeared over the closed wound. The samples of fat were placed in labeled 1-oz bottles, sealed, and refrigerated until analyzed.

As the hens died, the date was recorded, and the hens were weighed, scalded, and plucked. The entire carcass was then ground and mixed, and a 75- to 100-g sample was saved. These samples, and the fat samples obtained by biopsy, were then freeze dried on a Virtis Freeze Dryer preparatory to dieldrin analysis.

Dieldrin was extracted from the feed and tissues by refluxing them for 24 hr with petroleum ether in a soxhlet extractor, and the extracts were diluted to a constant volume. A 1- to 25-ml aliquot of extract from the rations was evaporated to dryness in a test tube with a gentle stream of nitrogen. Two milliliters of dehydrohalogenation reagent (3) was added, and the tubes were heated in a water bath for 15 min at 75 to 80°C. After cooling, 1 to 5 ml hexane was added, the dieldrin partitioned into the hexane upper phase by shaking, and 1μl of the hexane layer was injected into the gas chromatograph.

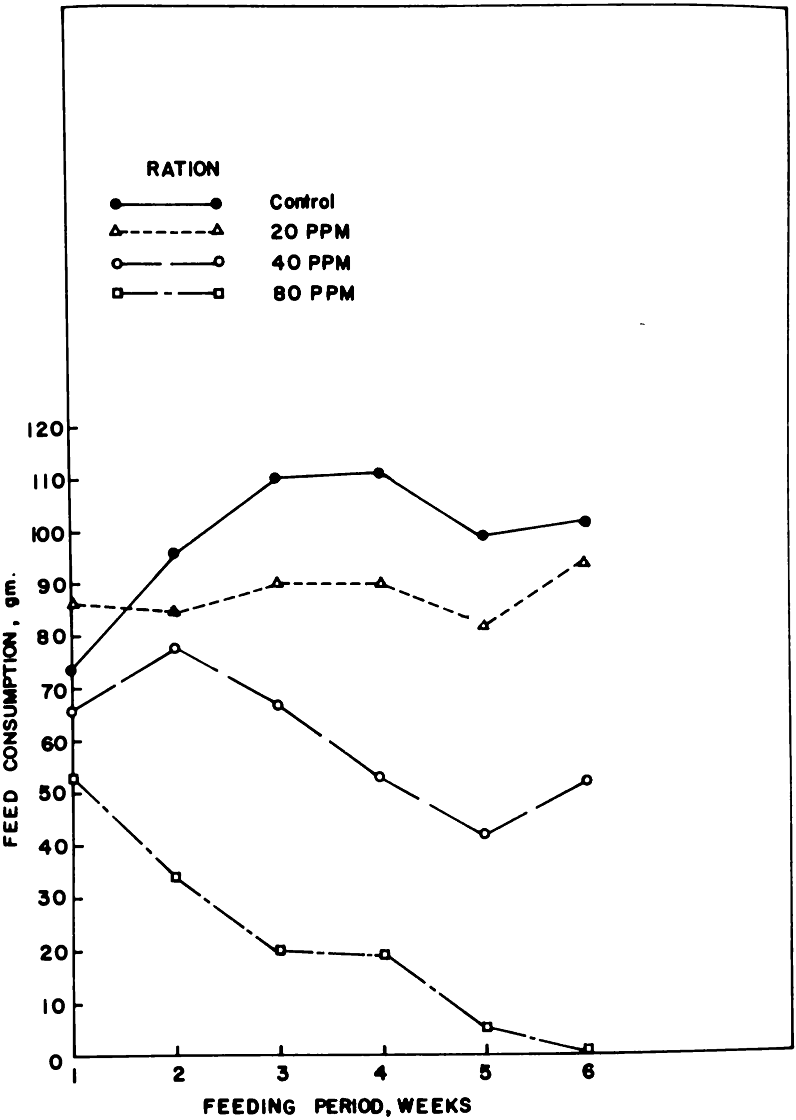
The extracts from the carcass and fat samples were cleaned up by the Florisil method of Johnson (7), with slight modification. Dieldrin -^{14}C was added to the extract before cleanup, and the radioactivity in the effluent from Florisil was measured so that loss of dieldrin during cleanup would be detected. The effluent from the Florisil column was diluted to 250 ml, a suitable volume taken and evaporated under nitrogen, the residue redissolved in hexane, and 1 μl of the hexane solution injected into the gas chromatograph. Suitable dilutions were used to keep the unknowns within the range of detection.

Following extraction and cleanup, dieldrin was measured by electron capture gas-liquid chromatography. The chromatograph column was 6 ft long x 3 mm I. D., contained 5% DC-200 silicone oil on 60 to 80 mesh Chromasorb W, and was operated at 210°C with a gas flow of 40 ml/min. Appropriate standards of dieldrin in hexane were injected in duplicate intermittently between unknown samples and peak heights were measured. From 10 to 500 picograms of dieldrin could be detected with this system.

The percentage fat in the carcasses and fat tissues was determined by loss in weight during extraction.

RESULTS AND DISCUSSION

The average feed consumption per hen during weeks one and two was quite low, ranging from 36 to 86 g per bird per week (Figure 1). Two factors probably contributed to this low consumption: 1) the hen had been moved into the cages from floor pens the day the experiment started and had not adapted to the new environment; 2) biopsies were performed on every hen the first day of the experiment. Feed consumption increased in the control group during the first three weeks, and increased slightly in the group fed 20 ppm dieldrin over the 6-week experimental period. Hens fed rations containing 40 or 80 ppm dieldrin, however, ate much less than those fed 0 or 20 ppm dieldrin. The difference in feed consumption is reflected in the biweekly changes in the body weights of the experimental groups (Figure 2). Hens fed the control ration, or the ration containing 20 ppm of dieldrin, lost little body weight over the 6-week feeding period. In contrast, the hens fed 40 or 80 ppm lost considerable weight over this period of feeding. The amount of weight loss was directly related to the feed consumption and inversely related to the level of dieldrin in the feed. Eleven of the twelve hens fed the diet containing 80 ppm dieldrin and five of the twelve hens fed the diet containing 40 ppm dieldrin had died by the sixth week. Only the weights



FIFURE 1—Average daily feed consumption per hen.

of hens surviving at the end of each time period during the feeding phase of the experiment are plotted in this figure.

It can also be seen from Figure 2 that the weight of the hens at death was directly related to the level of dieldrin fed. Here again,

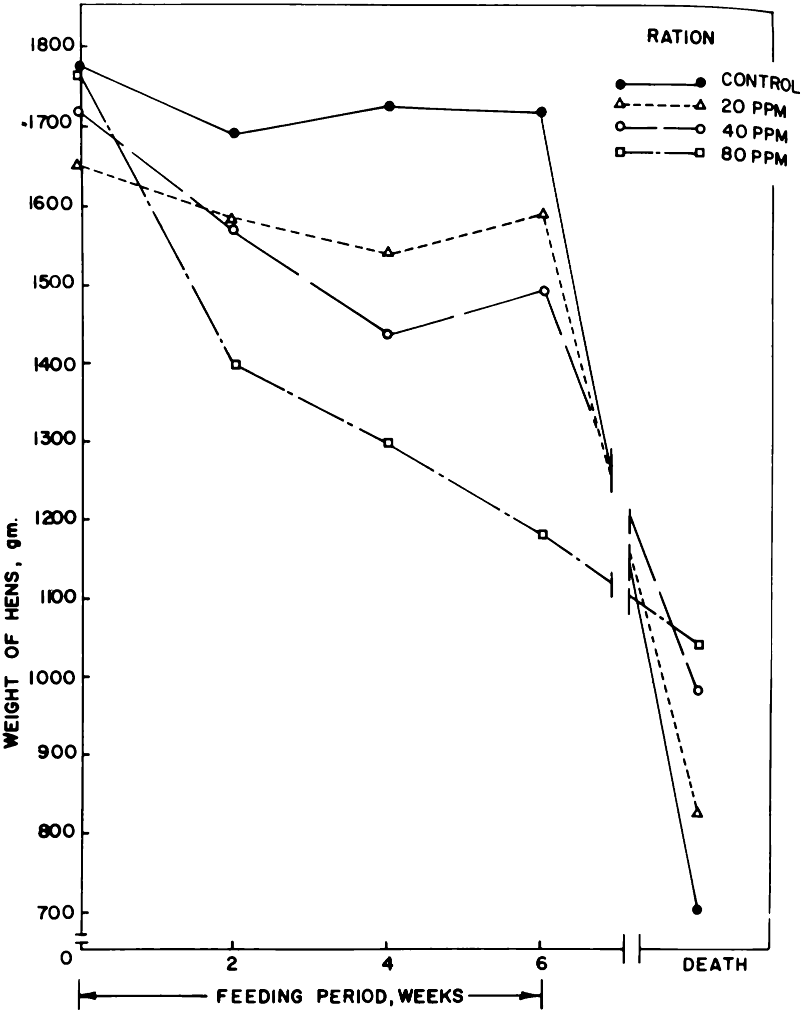


FIGURE 2—Average weight of hens fed various levels of dieldrin.

hens that died before the sixth week were not included in the average weight plotted. The average weight of all hens at death was 700, 870, 950, and 990 g for those fed the rations containing 0, 20, 40, and 80 ppm dieldrin, respectively. Comparing these values with their respective values on the graph, it can be seen that the same direct relationship holds with respect to the level of dieldrin fed, and that hens fed the two higher levels of dieldrin were voluntarily starving during the feeding period.

Upon autopsy, carcasses from all the control hens looked the same. The body tissues and organs were visibly free of fat. The gizzards were void of food material and shrunken; and, in some cases, they contained bile. Carcasses of hens fed dieldrin were more variable. Occasionally, small amounts of fat were visible and the gizzards contained some feed.

The survival of the hens is shown in Figure 3. Obviously, survival was inversely related to the level of dieldrin in the ration. But survival was also directly related to the rate of feed consumption. At this point we are beginning to see that the toxicity of the insecticide, its effect on feed consumption, and the effects of starvation are confounded. Data in Figure 4 and Table I show this confounding more clearly.

The concentration of dieldrin in the fat samples and in the fat extracted at death is presented in Figure 4. Dieldrin accumulated rapidly in the fat and accumulated in proportion to the level fed. The concentration of dieldrin in the fat was increased by starvation. At the end of starvation, the concentration of dieldrin in the extractable fat from the carcasses of hens fed the rations containing 20 ppm dieldrin was higher than that in the fat of hens fed the rations containing either 40 ppm or 80 ppm dieldrin. The results as discussed here are tabulated in Table I.

TABLE I

AVERAGE FAT AND DIELDRIN CONTENT OF STARVED HENS

Dieldrin fed, ppm	0	20	40	80
Survivors to sixth week	12	11	7	1
Survival f. pt. of starvation, da.	28.6	17.0	12.6	19.0
Fat in freeze-dried carcasses, %	6.3	10.3	15.1	14.2
Dieldrin, freeze-dried c., ppm	0.3	124.5	157.7	139.1
Dieldrin in extractable fat,[a] ppm	-	1286	1038	1009
Total dieldrin per carcass, mg	-	31.8	51.1	43.8

[a] A range of 0.3 to 5.6 ppm dieldrin was observed in samples from control hens. Later work disclosed that the control samples were contaminated during freeze drying.

During the second week of the experiment, it became apparent that the ration containing 80 ppm dieldrin was toxic. The toxicity of the ration containing 40 ppm dieldrin appeared during the fourth week. We knew that these levels of dieldrin were quite high, but we did not expect toxicity and deaths in this short a feeding period.

We had anticipated that the levels of dieldrin in the rations fed in this experiment would give a dieldrin intake of about 0, 1, 2, and 4 mg/kg body weight of the hens. In other studies at the Metabolism and Radiation Research Laboratory, sheep have been fed dieldrin at a level of 2 mg/kg body weight for periods of 32 weeks. This observation provided the basis for selecting the levels of dieldrin that were fed to the chickens. Also, no problems of feed consumption were encountered with the sheep, so the reduced feed consumption of the chickens was not anticipated.

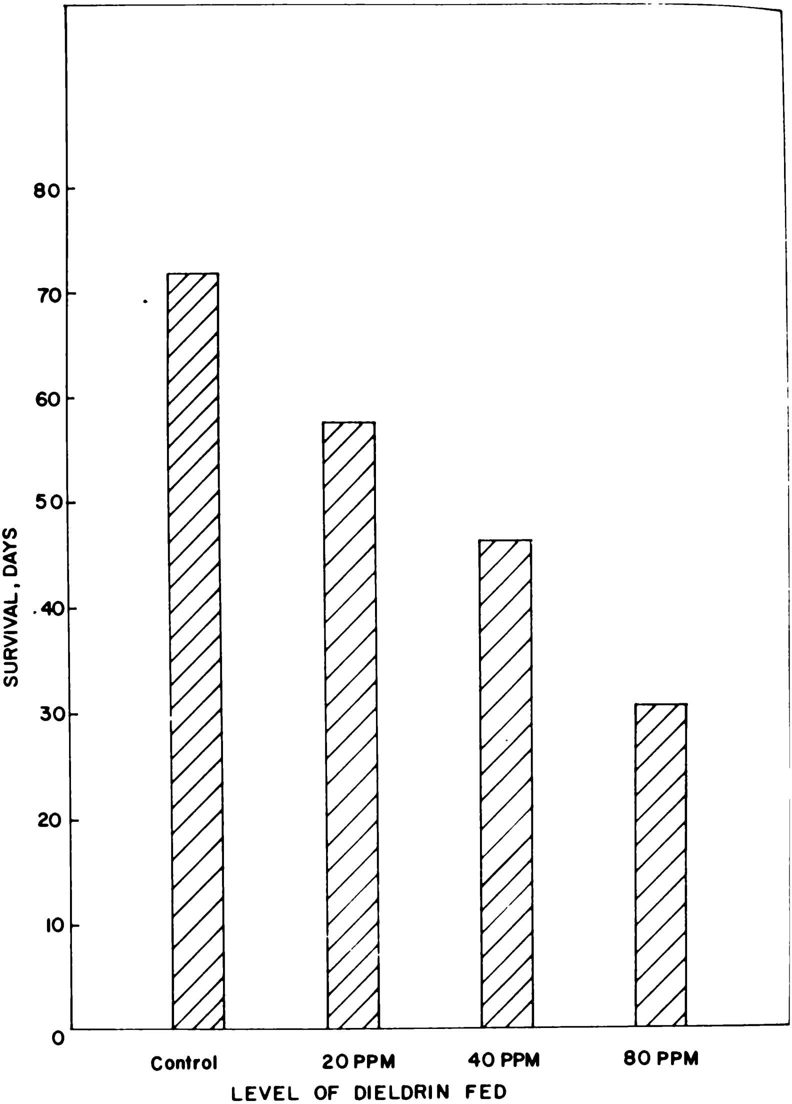


FIGURE 3—Average length of survival of hens fed dieldrin.

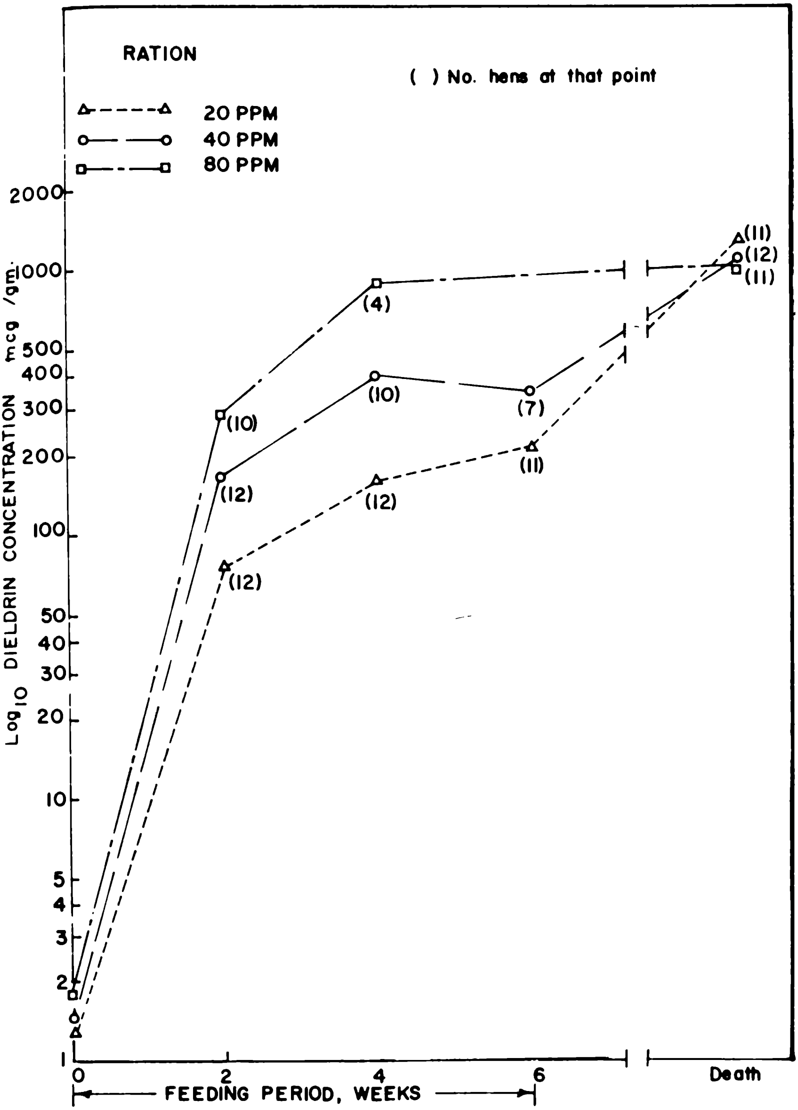


FIGURE 4—Dieldrin concentration in chicken fat.

The objective of this experiment was to determine the effects of insecticides stored in body fat upon survival of hens during diet restriction. But, because the hens were already being stressed by reduced feed consumption and by the toxicity of the dieldrin during the feeding phase, the observations made during the period of starvation cannot be clearly interpreted.

These results suggest that in further experimentation lower levels of dieldrin should be fed and that steps should be taken to equilize feed consumption. It is also suggested that the hens be given time to adjust to the cage environment before beginning the experiment, and that longer intervals of time elapse between taking biopsies for fat.

SUMMARY

Forty-eight leghorn hens were fed rations containing 0, 20, 40, and 80 ppm dieldrin for a period of 6 weeks, and then starved to death. During the feeding period, feed consumption, changes in body weight, and survival were inversely related to the concentration of dieldrin in the ration, while accumulation of dieldrin in the body fat was directly related to level of dieldrin in the ration. During the starvation period, the concentration of dieldrin in body fat increased. Body weight and percent fat in the carcasses at death were directly related to level of dieldrin fed, but the low rate of feed consumption and the toxicity of the high levels of dieldrin during the feeding phase make these data difficult to interpret. A followup study for determining toxic effects of insecticides stored in body fat during starvation is recommended, and some improvements in experimental technique are suggested.

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SORPTION STUDY OF H_2O AND D_2O VAPORS ON *O*-PHTHALIC AND SUCCINIC ACIDS[1]

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The adsorption isotherms for water vapor at 25° and 35° on lyiphilized, completely vacuum-dried, o-phthalic and succinic acirs have been established. The total amounts of water wapor adsorbed in the monomolecular layer were evaluated by extrapolating the upper slopes of the sorption isotherms at the saturation pressures of water vapor. Their values, in moles H_2O/moles acid are; 0.610 for o-phthalic and 0.715 for succinic acid.

The differential heats of adsorptions calculated by the Clausius-Clapeyron method, show noticeably higher values for o-phthalic acid. The difference was about 30 kcal/mole at very beginning of adsorption. As the amount of adsorbed water vapor increases toward monolayer coverage, the differential heats of adsorption for both acids approach the value of the heat of condensation of water vapor. Preliminary data of $H \leftrightharpoons D$ exchange effect on thise acids were obtained by means of successive adsorption of D_2O vapor followed by complete desorptions.

INTRODUCTION

Interactions of solids with vapors, gases, yield valuable information concerning the physical and chemical properties, leading to determination of molecular structures. Such information is important in many branches of science, as it is indicated in the number of publications.

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In our laboratory, many similar investigations have been made on synthetic (1,2) and natural (3,4,5) solids after developing their surface area by a new approach using high vacuum technique. However, the solids used were of complex or polymeric structures. The desired goal here was to use a similar technique to study certain properties like sorptivity and $H \rightleftarrows D$ exchange effect on solid monomers whose structures, physical and chemical properties, are known. So, o-phthalic and succinic acids were chosen as the model substances, which may be used to investigate the influence of the nature of molecular backbone (aromatic and aliphatic) on the sorption behavior.

EXPERIMENTAL

a. *Apparatus and Procedure.* The combined McBain gravimetric micro-balance with a high-vacuum system was utilized and its manipulation was similar to those described earlier (3). The apparatus provides a sensitivity of better than 1.5 X 10^{-5} g. Degassing of the samples were followed as described under (3).

The procedure for adsorption included an introduction of increments of H_2O and D_2O into the sample area, which was at a constant temperature, and then the measurements of pressure and the amounts of adsorption on the sample were recorded until the equilibrium was reached. The data at each equilibrium gave one point of the isotherm, that is the plot of amount sorbed versus equilibrium pressure.

b. *Materials.*

1. O-phthalic acid was on an analytical grade from Mallinkrodt Chemical Works, St. Louis, Missouri.

The sample was lyophilozed to develop a high surface area. The process involved freezing of a dilute solution into a Pyrex vessel and then the removal of water solvent by sublimation under vacuum. The freezing was accomplished while spinning the flask, which was 1/3 full, in a bath of dry-ice and acetone, then the immediate attachment of the flask to the vacuum line prevents melting. When the sublimation of water was nearly complete, the resulting material remained in a highly voluminous state. The material was used for further desorption in high vacuum and determination of the dry-weight of particular species for sorption (of 200 to 250 mg, dry weight).

2. *Succinic Acid* was of an analytical grade from the same source. The species for investigation were prepared by similar method as described in 1.

3. *Deuterium Oxide* used was of 99 to 99.8 per cent purity, from K & K Laboratories, Inc., Painview, New York.

4. *Water,* triple-distilled, was used as a solvent, and for sorption it was again redistilled in the vacuum system.

RESULTS AND DISCUSSION

a. *Sorption isotherms of H_2O vapor on lyophilized* o-*phthalic acid at 25 and 35°.*

1. *Sorption at 25°.* Curve 1, Figure 1 (and Figure 2), show the re-

producible adsorption which starts at a fair speed on the most active sites. When these sites are saturated, the isotherm goes into a slope of slower adsorption, indicating saturation of less active sites. Further adsorption produces a new slope which starts at about 12.6 mm equilibrium pressure and goes up to the saturation pressure of H_2O at 25° ($\simeq$ 23.76 mm). It is very difficult experimentally to continue measurements at an equilibrium pressure of a magnitude close to the saturation point.

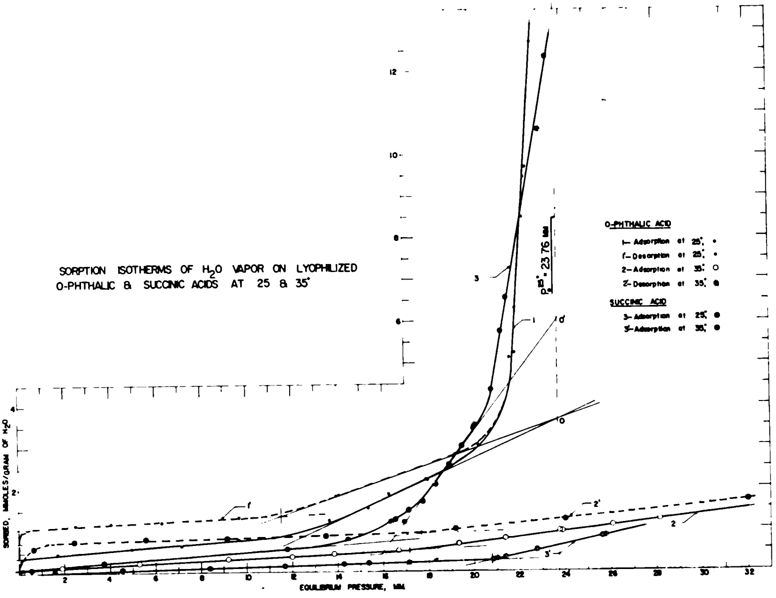

FIGURE 1—Sorption isotherms of H_2O vapor on lyophilized o-phthalic and succinic acids at 25 and 35°.

In this run, however, it is evident that the considerable length of the final slope justifies the approximation of the total monolayer sorption value up to saturation pressure with a good precision. The total monolayer sorption, which is considered to be as summation of particular sorptions on different kinds of active sites (functional groups), is found to be 0.610 moles H_2O/mole acid. In this adsorption isotherm two definite slopes and the initial curvature are present. The slopes indicate that there are two different kinds of sites—functional groups involved in an interaction within the molecules of H_2O. The presence of the curvature in the initial adsorption suggests that the initial adsorption may be caused by sites of some higher and probably closely related activities, which may be distinguished on a sample at lower equilibrium pressure.

A desorption isotherm at this temperature is represented by curve 1^1, Figure 1, which contains two slopes and a curvature similar to the curve of adsorption. Its upper slope, approximated up to the saturation pressure, has the same value of the total monolayer sorption. The desorption isotherm, however, demonstrates higher values of amounts sorbed (held) on the sample below the saturation pressure, which can be characterized as a hysteresis. A hysteresis in desorption on natural substances is a general, but not definitely explained, phenomenon (3) in which the activation energy seems the main factor.

2. *Sorption at 35°*, curve 2, Figure 1, represents a reproducible adsorption isotherm. At this temperature a decrease of time required for equilibration has been noticed. Curve 2 has two slopes and an initial curvature. These slopes are below those obtained at 25°.

Curve 2^1, Figure 1, is the desorption isotherm which again can be approximated to two slopes and a curvature at low pressure. Furthermore, an extension of the upper slopes of curve 2 and 2^1 to the pressure of saturation (not shown in Figure 1) meet at the value lower than the total monolayer sorption approximated at 25°.

Such a discrepancy should be clarified in further investigation, however, it is evident that at this temperature some of active sites may become not accessible for sorption (higher degree of hydrogen bonding formed).

b. *Adsorption isotherms of H_2O vapor on lyophilized succinic acid at 25 and 35°.*

1. *Adsorption at 25°*. Curve 3, Figure 1-2, represents the adsorption isotherm of H_2O vapor on succinic acid at 25°, which has a similar slope as that of o-phthalic acid. The equilibrium time for this isotherm was shorter, and the amounts adsorbed along the first slope were lower than those of o-phthalic acid. However, the upper part of isotherm demonstrated a higher adsorptivity.

The extension of the final slope to the pressure of saturation gives a value of total monolayer sorption equal to 0.715 moles/mole acid, which is remarkably higher than that of o-phthalic acid.

2. *Adsorption at 35°*. It is represented by curve 3, Figure 1, and has the same general characteristics as those mentioned above. It should be noted here again that an extension of the upper slope to the pressure of saturation (not shown in Figure 1) meets a value higher than that of o-phthalic acid at 35°

c. *Comparison of sorption data on two acids.*

For more precise comparison of the two acids, the amounts adsorbed expressed in mole H_2O/mole acid for the adsorption isotherms at 25° are shown in Figure 2. The values of total monolayer sorptions indicated by the extension of their respective upper slopes to the saturation pressures were, 0.610 for the o-phthalic and 0.715 for the succinic acid. These values of adsorption are considered to be pro-

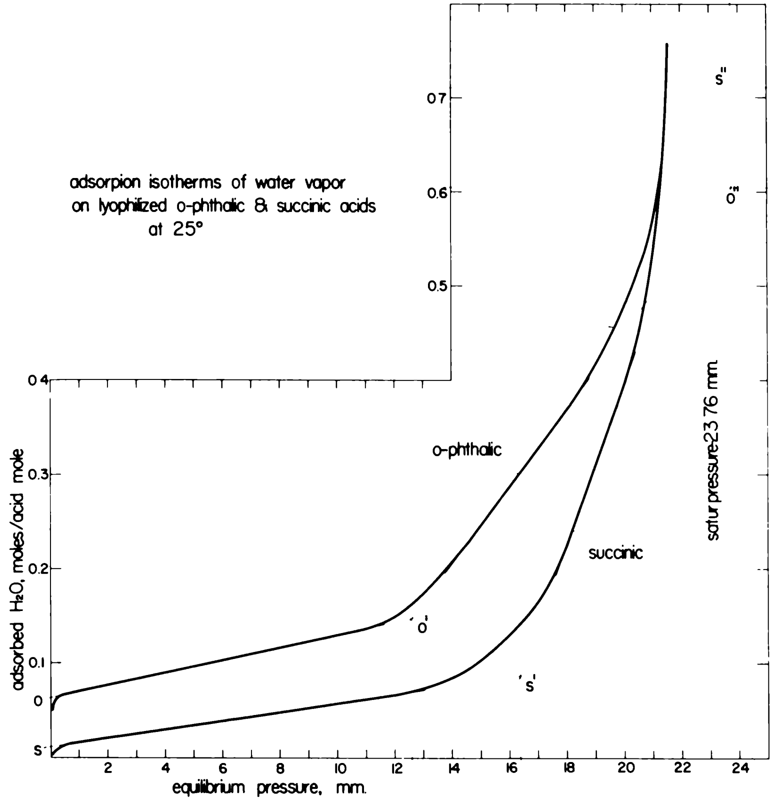

FIGURE 2—Adsorption isotherms of water vapor on lyophilized *o*-phthalic and succinic acids at 25°.

portional to the chemical activity of the acids. They, also, may be expressed as being proportional to the surface areas which may be calculated by assuming effective cross section of H_2O molecule equal $\simeq 30\text{Å}^2$(6); so that calculated surface areas will be about 660 m^2/gm and 1080 m^2/g for *o*-phthalic and succinic acids respectively.

The appearance of two slopes in sorption isotherms is justified by the presence of two carboxylic groups, which may be of different activities in the solids, or have their activities changing after a certain amount of sorption. In either case, changes in structure may take place (reopening-breaking of the weaker hydrogen bonds). However, if every carboxylic group present represents one active site, it could be expected that the amounts of adsorption in a monolayer is up to 2 H_2O mole/mole acid. Values obtained experimentally for both acids were far below those expected. Because the surface areas of the samples were developed to the highest degree permitted experimentally, it can be concluded that parts of the functional carboxylic groups were involved in crystalline-hydrogen bonding and not accessible for sorption. Furthermore, the difference in total monolayer of adsorption indicates the influence of the nature of the acid, namely, aliphatic or aromatic backbone of their molecules.

d. *Differential heats of water sorption on* o-*phthalic acid at 25 and 35°.* The isothermic data was used to calculate the differential heats of adsorption and desorption by the use of Clausius-Clapeyron method. These heat values, $-\Delta H$ in Kcal/mole, were then plotted against the amounts of sorbed H_2O, expressed in mole H_2O/mole acid. The resulting curves are shown in Figure 3 (dashed parts of less precision). From this plot it can be seen that the maximum value of the heats of sorption are in the same range, about 90 Kcal/mole. However, the differential heats of desorption are shifted to the greater amount of sorption. This shift is apparently caused by hysteresis in desorption.

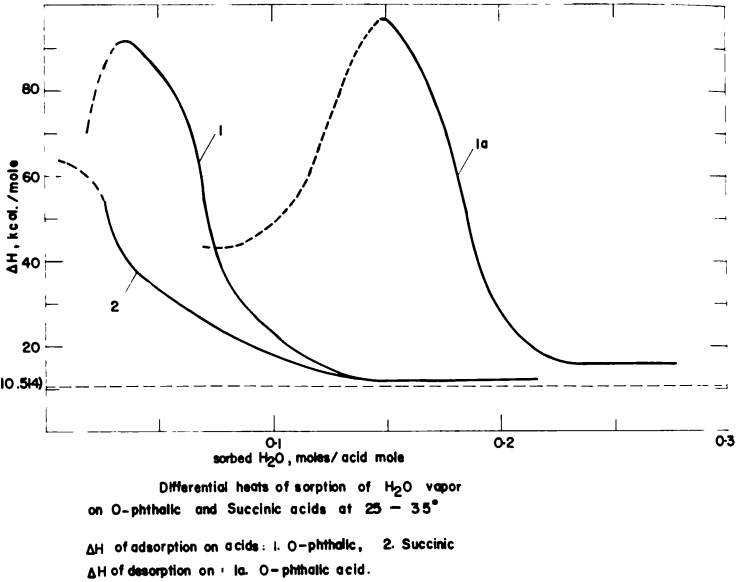

FIGURE 3—Differential heats of sorption of H_2O vapor on o-phthalic and succinic acid at 25-35°.

A relatively high values of sorption heat indicates a strong bond and explains the rapid initial equilibrium during sorptions. The further sorption is characterized by the heat of water condensation.

If the hysteresis of desorption is dependent on the activation energy, as is suggested (3), therefore, the activation energy may be evaluated from the following relation: $\Delta H_{des} - \Delta H_{ads} = Kq$, where K is a proportionality factor and q-activation energy.

e. *Differential heats of water adsorption on succinic acid at 25 and 35°.* It is shown in Figure 3. Comparing ΔH of water adsorption on both acids, we notice that o-phthalic acid has higher values along the whole process. The difference of their maxima is about 30 Kcal/mole. As the amount of adsorbed water vapor reached ~ 0.15 moles/mole acid, ΔH of adsorption for both acids approach the value of the heat of condensation of water vapor.

From the preceding data of sorption isotherms, as well as the differential heats of sorption, it was evident that the acid of aromatic nature, (o-phthalic) was characterized by stronger bonding. But its total amount adsorbed was about 85% compared to that of the acid of aliphatic nature (succinic).

f. *Hydrogen⇆Deuterium exchange on succinic acid at 35°.* Upon completing the isotherms for H_2O, the same sample was used to adsorb D_2O in order to exchange labile hydrogen, present in -COOH groups of acid, for deuterium. This was achieved during the adsorption-saturation of sample with 5 to 7% D_2O of weight. To accelerate adsorption-saturation, the temperature of sample was lowered. Afterwards the temperature was raised to 35° and D_2O and eventually HOD was completely desorbed at 10^{-7} mm of pressure during 24 hours. An increase of weight due to exchange, according to the general eq-n $(HOOC\text{-}(CH_2)_2\text{-}COOH + n\ D_2O \rightarrow DOOC\text{-}(CH_2)_2\text{-}COOH + (n\text{-}1)\ D_2O + DOH)$, was recorded. This partially deuterated sample was again exposed to D_2O and desorbed as before. The succinic acid showed a further gain in weight. It should be mentioned that during the complete desorption it was observed the sublimation of samples equal to 0.006%/hour, which was taken into account for every new run. Four runs of adsorption-desorption were taken. Figure 4 shows these results, the ordinate giving the percentage gain in weight of succinic acid as the result of successive adsorptions followed by complete desorptions. The abscissa gives the cumulative amounts of D_2O that were adsorbed during each operation. The maximum gain in weight, for the fourth run, due to exchange, is equal to 1.244% recalculated on the original weight of the succinic acid. This weight gain accounts for an exchange of about 73% of the hydrogens that are theoretically exchangeable.

From the shape of curve it can be concluded that complete exchange of labile hydrogen is possible, as it was also established in case of o-phthalic acid by preliminary tests (not presented yet). Since there was a desire to check reversibility of the exchange, the further H⇆D exchange was discontinued. This deuterated sample was then exposed to H_2O vapor which replaces the adsorbed D_2O. This is followed by complete desorption by a similar procedure. The two points of the reversible exchange of D⇆H justifies the suggestion

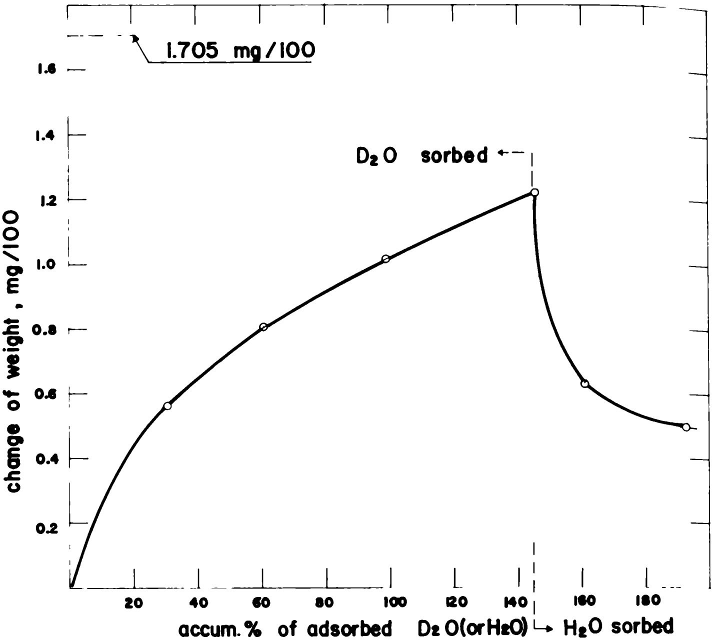

FIGURE 4—Changes of weight of succinic acid due to Hydrogen Deuterium exchanges occuring during successive adsorptions, followed by complete desorptions at 35°.

that the process is reversible as it can be seen from the last part in Figure 4.

During the complete desorption, besides the sublimation at very low pressure may be expected, at least partly, some formation of anhydride of the acid which might have falsified the actual change of weight due to $H \leftrightarrows D$ exchange effect. However chemical analysis of samples of succinic acid desorbed during 12 to 48 hours at 10^{-7} mm of pressure did not show any such occurence. Some formation of anhydride form of *o*-phthalic acid, however, has been noticed.

From our date of sorption isotherms and $H \leftrightarrows D$ exchange, it can be definitely concluded that the adsorption of H_2O occurred basically on the sites -HCOH, which practically not involved in hydrogen bonds, as it was also stated for synthetic polymer (2). The $H \leftrightarrows D$ exchange, however, take place in sites -carboxylic groups, which are "free" as well as involved in hydrogen bonds.

CONCLUSION

Interactions of solids, *o*-phthalic and succinic acids of highly developed surface areas occurred in stages. The activity of the beginning stage of adsorption was higher for o-phthalic acid than that of succinic acid. But in the final stage, the activity of succinic acid exceeded that of o-phthalic acid by ~15%. The monomolecular amounts of adsorption expressed in mole H_2O/mole acid were 0.715 and 0.610 for succinic and o-phthalic acids respectively. These values of adsorption compared to the theoretical one comprise 35% and 30%.

The differential heats in the sorption region of 0.05 to 0.11 mole H_2O/mole acid, were about 60 and 90 Kcal/mole for succinic and o-phthalic acids respectively. These values indicate strong bonding of the acids. As adsorption increases beyond the above mentioned range, the differential heats approach the heat of condensation of water. $H \leftrightarrows D$ exchange, on succinic acid, occurred very rapidly in the range of 35% of the theoretically calculated amount of labile hydrogen, which seems free (not bonded). Further exchange is slower and practically linear, indicating that the hydrogens are involved in bonds.

ACKNOWLEDGEMENT

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GENETICS — IN THE SPAN OF OUR GENERATION

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Invitation Paper

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Lancelot Hogben in Science for the Citizen (1938) said, "Whether we chose to call it pure or applied, the story of science is not something apart from the common life of mankind. What we call pure science only thrives when the contemporary social structure is capable of making full use of its teaching, furnishing it with new problems for solution and equipping it with new instruments for solving them. Without printing there would have been little demand for spectacles."

The history of the science of Genetics can be used as a striking example of the interdependence of the development of the scientist and acceptance and use of that knowledge by the layman.

On March 26, 1900, the Dutch botanist Hugo deVries published a paper under the title, "SurLoi de Disjonction des Hybrids" in which he stated "specific characters—are composed of separate units. — In the hybrid the character of one of the parents is visible or dominant while the antagonistic character is latent or recessive."

On April 27, 1900 Professor Correns, a German botanist, presented a paper entitled, "Gregor Mendel's Rule on the Behavior of the Progenies of Hybrids."

In June, 1900 the Austrian botanist, Von Tschermak, published his paper, "On Artificial Crossing in *Pisum sativum.*"

Correns and Tschermak acknowledged having read the published work of Gregor Mendel, while deVries used Mendel's terms "dominant" and "recessive."

Thus, within the life-span of some here in the audience was the science we know today as "Genetics" proclaimed and acknowledged by some of the leading botanists of the world. These basic facts are so readily accepted by the student of today that it is quite easy to assume this is ancient knowledge. Few of our students can realize that a generation ago our knowledge of astronomy far exceeded the knowledge of heredity.

The magnitude of the discovery of variation within a species following an exact mathematical formula can only be appreciated by a look at the scientific climate prevailing at that time.

The climate was well stated in 1900 by Oscar Hertwig in giving a summary of the history of biology during the past century. According to Eric Nordenskjold in his "History of Biology," Hertwig "sharply criticized physiology, which, in his view, had created a brilliant experimental technique and had discovered a number of facts concerning the chemical and physical processes of organisms, but, on the other hand, had neglected all other vital phenomena. The results was that a whole category of the most important physiological problems — namely, fertilization and embryonic development — had fallen entirely into the hands of the morphologists and been dealt with by anatomists, zoologists, and botanists. While the professional physiologists had thus reverted to 'an empty mechanism' and imagined that the explanation of life was only a chemico-physical problem, botany was the first to free itself from this narrowness of view."

This was the time of Darwinism and the belief in the immutability of the species; of pangenesis and belief in inheritance of acquired characters.

While botanists rediscovered the basic laws of heredity and discovered Mendel's paper of 1865 outlining the laws of segregation and independent assortment in 1902, Cuenot broke new ground with his paper on the inheritance of coat color in the mouse, and it was demonstrated that these laws applied to both plant and animal kingdoms.

While W. E. Castle of Harvard University first investigated the effects of inbreeding and outcrossing, using Drosophila, it was Thomas Hunt Morgan who used the fruit fly to unite cytological observations and genetic variation and laid the ground work for an understanding of the mechanistic scheme so long accepted and taught in beginning courses in genetics. Knowledge of the linear arrangement of the particles of inheritance associated with the 4 chromosomes of the fruit fly lead to an understanding of the modifications of the law of indenpendent assortment, known as linkage, and the sister phenomonen of crossing over.

The work of Bateson and Punnett with the chicken by 1908 had demonstrated the effects of more than one pair of genes upon a single character—the rose, single and pea comb of fowl, giving us modifications of the simple 3:1 and 9:3:3:1 ratios of the mathematician.

In order to show the impact of the science of genetics on animal agriculture, I must outline for you the history of the breeds of livestock that have played and still play an important part in stabilizing and maintaining the quality of our commercial livestock.

Livestock improvement predates written history of civilization. Selection for desirable traits must have followed immediately the domestication of selected animals. The traits selected for depended upon the needs of man. The swiftest horse to enable early man to escape his enimies, the swifter and stronger dogs to pursue and capture the primitive man's food, the more docile and tractable beasts of burden and species to be used for milk, meat or fiber.

The Arabian breed of horses today are descended from the selected horses of the rulers of Arabia at the time history began to be written. The breeds of working dogs, herders such as the Border Collie, and hounds for pursulit likewise originated before written history.

The major breeds of cattle and sheep were established in the British Isles during the 1700's. Robert Bakewell, born in 1725, in England is credited with laying the foundation of present day pure breeding methods. Bakewell was the first to have definite goals for selection, the first to test prospective sires and cull on the basis of evaluation of his progeny. He was the first to close his herds to outside blood and practice inbreeding. It was Bakewell's apprentices who laid the foundation for the Shorthorn breed in 1783, and the Hereford breed in 1742.

The Coats Herd Book, founded in 1822, initiated the permanent record of ancestry for the Shorthorn breed. The Hereford Herd Book was first published in 1846—30 years after the first Herefords were imported into America—and the Angus records date from 1851.

Thus it can be seen that the foundation of our pure breeds preceded even the first discoveries of Gregor Mendel. It is of interest to me that the methods of Bakewell and the founders of the other breeds that have persisted to the present day are the methods that would have been dictated had they been familiar with the basic principles of genetics.

Perhaps other men with other methods failed in their attempts, but I doubt if it is chance that those men who adopted methods that have stood the test of time were the ones who made a lasting contribution.

The immediate impact of the discovery of a workable system to explain heredity upon the livestock breeders of that day was negligible. During the period from 1900-1920, the animal breeder's closest approaches to genetic research were trials designed to demonstrate the benefits to be derived by continued use of purebred sires upon the nondescript herds of that day. These herds, as far as the beef industry was concerned, for example, were largely descended from cattle left by the early Spanish explorers and the ships' cow that brought the settlers to our eastern shore.

By 1920 a new direction began to be apparent. Sewall Wright, employed by the United State Department of Agriculture, published the first o fhis series of articles on "Systems of Mating"

"Biometric Relations Between Parent and Offspring
The Effects of Inbreeding
Assortative Mating Based on Somatic Resemblances
The Effects of Selection"

and his paper on "Correlation and Causation."

I believe students of the present day could well ponder a statement of Sewall Wright's in the first paragraph of his series.

> "Investigations on the mechanism of heredity have progressed to such a point that there need be little hesitation in accepting the recent statement of East and Jones (1919), that 'Mendelian heredity has proved to be the heredity of sexual reproduction; the heredity of sexual reproduction is Mendelian'. It has also become clear, however, that most cases of inheritance are far from exhibiting the simplicity which Mendel was fortunate enough to find in certain variations of the pea."

By 1930 Sewall Wright had moved to the Zoology Department of the University of Chicago and published his paper on "Evoluation in Mendelian Populations."

Sewall Wright pioneered in applying the principles of biometry and statistics to variation in form and function of both continuous and discontinuous sorts. He should be credited with laying the foundation for population genetics. He also in his 1930 paper placed the divergent views of the Darwinian evolutionists and qualitative geneticists in logical order.

Brilliant as was the work of Sewall Wright, he was not in close contact with either the successful breeder of purebred livestock or the students of animal husbandry in the nation's land grant colleges.

The first textbook entirely devoted to the application of current genetic theory to animal breeding was "Animal Breeding Plans," published in 1937 by Dr. Jay Lush of Iowa State College. Dr. Lush was an Iowa native, graduated from Kansas State College in 1916 and with a Ph.D. from the Genetics Department of the University of Wisconsin in 1922. He became a devoted student of Sewall Wright's work and has been the recognized leader of the application of population genetics to animal improvement for almost 40 years. Dr. Lush and his students analyzed the structure of many of our breeds, developed the use of progeny tests and selection indices and contributed to the development of performance testing programs aimed at improvement of economic traits, while other less practical animal geneticists were still concerned with the inheritance of color pattern and other traits of minor importance.

These developments gave rise to the establishment of cooperative breeding experiments involving the various state experiment stations and the U.S. Department of Agriculture such as the Regional Swine Breeding Laboratory in the 1930's; the Regional Beef Cattle Breeding Project—NC-1 in the 1940's; and the Regional Sheep Breeding Project—NC-50 in the 1950's.

The theories developed were wonderful. The cooperation with purebreeders was negligible. Few new ideas are ever accepted until they are needed and needed urgently. The fact that certain lethals and highly undesirable traits were inherited in a simple mendelian fashion served to make affected breeders aware of the science of genetics. Some of these abnormalities are:

Hairlessness in cattle
Cleft palate in swine
Black wool in sheep
Red animals, not recordable in the Angus and Holstein breeds
Hydrocephalus or "waterhead" in cattle and swine
Hernia in all species
Cryptorchidism

The list of significant hereditary abnormalities includes some 50 in cattle, 20 in sheep, and 30 in swine.

But the real awakening came in the late 1940's when a specific type of dwarfism became so prevalent in Hereford and Angus cattle as to endanger the survival of the breeds. The advice of Dr. Jay Lush was sought, and when the explanation of simple genetics was irrefutably demonstrated, the breed associations adopted pedigree barriers and advised their members to eliminate lines of breeding carrying the offending genes. The breeders and their associations accepted genetics and began to apply the principles to their daily tasks.

Since that time all major breed associations have adopted in some form the performance testing programs as outlined by the population geneticists and we now have similar programs in about 40 of the states. These programs make available comparable performance data on specific lines of breeding and individuals.

Progress within the last 20 years has been just short of phenomenal. Rate of growth in swine has increased sufficiently to make possible 225 pounds weights in 5 months rather than 9-12. Backfat thickness has been reduced from 3-4 inches to 1.0-1.25 inches. In cattle, where weights of 950 at 18 months were common, we now have 1,000 lb. steers in 12 months. In 1963 at our own Valley City Winter Show, the majority of the steers entered in the carcass show had in excess of 1.5 inches of fat, while at the 1968 show only one of the entries had more than 0.7 inches although all but one graded choice or higher. Since 1955 the number of laying hens in North Dakota has decreased by 50 per cent while the number of eggs laid per hen has more than doubled. In 1948 it was estimated that 8-10 per cent of beef calves born in North Dakota showed signs of snorter drawfism. Today the problem is of minor importance.

Many times the advice of scientists to the breeders may not be sound. It has long been known that undesirable recessive genes occur with relatively low frequencies in many of our pure breeds. The population geneticists have theorized that if the frequency of one of these genes is one in one hundred (0.01), then the occurrence of the homozygote showing the condition is expected to be one in ten thousand. Their statement has been that these traits should be considered genetic trash of no economic significance to the breed as a whole. They should be ignored.

While this sounds reasonable, it reveals the scientists' lack of understanding of practical breeders' management practices. The typical breeder within a short span of years will replace his entire herd and move on to a new bull. If the herd bull carries an undesirable gene that is new to his herd he may unknowingly retain a group of replacement females, one-half of which carry the undesirable gene. If his next bull likewise carries the same gene he can expect to have one calf in eight showing the undesirable trait (Table I).

If he continues to ignore this occurrence the situation might be slightly worse in the next generation.

The population geneticist not only ignored the fact that the breeder retained replacement heifers from whatever bull he was using, but also is quite lekely to select a new herd sire from the same line of breeding as previously used. This tendency is contributed to by geographical isolation, friendship with another breeder, reaction to advertising claims or show yard winnings. While most breeds at one time had a relatively broad genetic base, some inbreeding is essential from the closed herdbook policy. It can be proven there is a

TABLE I

Carrier Bull	Clean Cow Herd	
Nn	NN	
	Next Generation of Cows	
	Carrier Cows	Clean Cows
	Nn	NN
Second Carrier Bull	Nn	NN
Nn	Nn	NN
1	50%	50%
Abnormal	Carriers	Clean
12.5	25	
nn	Nn	12.5
	25	25.0
	Expected Ratio	
12.5%	50%	37.5%

tendency to concentrate the blood of a few sires as a result of a small number of breeders showing at major shows and advertising and merchandising their cattle well.

The high incidence of snorter dwarfism can be attributed to carrier bulls being used in the two leading herds, as far as merchandising is concerned, in the Hereford breed and the one top herd of Angus.

The so-called genetic trash may not have a high incidence within the total breed but can be disastrous to an individual breeder. Examples of some anomalies currently causing problems are:

> Hydrocephalus, reported to be a simple recessive, currently being diagnosed in two of our major breeds.
>
> Cleft palate, reported to be a simple recessive and long known in swine. One Hereford sire known to have sired cleft palates less than 10 years ago had sons and grandsons heading 90 per cent of herds of one of the major breeds.
>
> Miniatures—calves that reach a weight of 380 pounds at two years of age is possibly a simple recessive and apparently carried by the most popular sire of a major breed.
>
> Cryptorchidism—reported as a simple recessive—is a costly condition in swine, as the meat from a cryptorchid pig tastes like that of a boar.

A review of the literature such as contained in Bogart's book lists a surprising number of these anomalies as being due to a simple autosomal recessive. Why, with the many gene pairs involved in the production of a normal animal and the many types of gene interactions known, should these anomalies all be due to simple autosomal recessives and each be independent of all others?

Could it be that reaserch workers, as well as practical breeders, are, at times, guilty of over-simplification?

A careful study of the methods used in arriving at these uniform conclusions reveals a close similarity in approach. Each worker first noted the occurrence of an anomaly within a group of closely related animals. He then gathered a group of animals that had produced this specific anomaly and mated them. When the anomaly occurred in a frequency approaching the 3 to 1 ratio he usually stated that the condition could be attributed to the action of a single autosomal recessive gene. The reader reads his statement to be "is due to," not "could be."

How many other gene pairs may have been similar in this selected group of animals? In few cases were the investigators concerned with more than one anomaly at a time. For example, in 1950 most workers asumed that dwarfism was due to a simple recessive until Hazel of Iowa mater two types of dwarfs to prove the point. When the progeny of snorter dwarf mated to long head dwarfs proved to be normal in size but capable of producing either type, depending upon the type of mate, it had to be concluded that two different autosomes could produce similar results.

In recent years North Dakota herds have been found that within the span of a few years produced snorter dwarfs, hydrocephalus, cleft palates and miniatures. Was one man lucky enough to get all these anomalies at one time, or are they related? If hydrocephalus and cleft palates are independent and each occur in one-eighth of the matings, we should expect only one calf in 64 to show both anomalies. Our observations indicate that the two occur more frequently together than separate. I share the conclusion of Gregory (1967) that the assumption that these are independent is not consistent with the data.

In 1947 Hart and Guilbert of the University of California published the results of 10 years of study of a group of abnormalities which they called Acorn Calves. They concluded the condition was not hereditary and that no known nutritional deficiency or toxin could be found to be the sepecific cause. The production of one normal calf from mating of two affected animals indicated to them the condition was not due to a simple recessive.

Their observations were during periods of severde nutritional shortages in oak forest areas.

Similar anomalies have been observed in Idaho and Utah by Binns; he attributes the condition to ingestion of lupins native to that area during the 10th week of pregnancy.

We here at North Dakota State University have observed similar "crooked calves" being produced in several herds throughout the state. We have no lupins and few oaks. We have a herd of approximately 50 cows to calve later this summer after mating to affected bulls. We are quite anxious to see them. We feel an anomaly might be hereditary even if not due to simple autosomal recessive.

During these past 20 years new breeds have arisen in beef cattle, swine, and sheep tailored to fit today's conditions and developed by using sound genetic principles.

It would seem that genetics has become a useful tool in the battle to supply food to the hungry. All progress should not be, however, attributed to genetics, as knowledge in the fields of nutrition and management has also contributed heavily. Perhaps acceptance of one type of scientific knowledge has increased the awareness and use of all types of scientific knowledge by the nation's husbandmen.

The science of genetics has not stood still while our animal breeders caught up. From the time the early cytologists demonstrated that the hereditary material was carried in a linear fashion on the chromosomes of the cell nucleus many persons have speculated on the nature of the gene, and the mode of the gene's operation.

In 1944 Avery, MacLeod and McMarty announced the results of 10 years work. The DNA fraction of the cell nucleus appeared to be the active portion. In 1953 Watson and Crick presented an acceptable theory on the structure of DNA. Later work with viruses and bacteriophages have made possible an understanding of how mutations of the DNA molecule can take place and that there mutations can be related to observable changes in characters.

Today the speculation is on ways of controlling the DNA mechanism and in effect directing mutations. A recent news report proposes to sample amniotic fluid to discern the occurrence of genetic deformities from chemical tests and eliminate them in the foetal stage, thus practice eugenics without limiting the activity of the individual.

Experimental improvement programs are now underway at several state experiment stations, including North Dakota, involving selection, inbreeding, and irradiation in attempts to accumulate small, favorable mutations for gradual improvement of economic traits. Success of these experiments is predicated on the theory that single changes in the DNA chain or side chains may have minor effects but a series of them accumulated might be beneficial. Most of such work has been started with highly selected stock that has apparently plateaued, indicating exhaustion of genetic variability.

It has been my intent in this brief summary to relate developments in the pure science and the acceptance and use of this science by the practical animal breeders during the time from the turn of the century.

The history of these developments points out to me the fallacy of attempting to designate certain types of investigations as basic or pure science and other as applied. What was basic in 1900 became applied in 1920. Without the basic research of today, progress tomorrow would be quite limited.

I hope I have shown that within the span of our generation a basic science has been developed, partially applied and new fields or branches discovered for future study.

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LYSINE CONTENT OF WHEAT, RYE, AND TRITICALE VARIETIES GROWN IN NORTH DAKOTA[1]

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Cereals, the principal source of plant protein, supply 60% of the total world protein supply (1). As the world's need for food increases it is anticipated more and more of the needed protein will have to be supplied by plant proteins. Unfortunately, most cereal proteins are nutritionally deficient in several amino acids required by man. Lysine is the most limiting of the amino acids deficient in cereal protein (2, 3, 4, 5).

In 1964 Mertz *et al.* (6) discovered that corn seed protein is nutritionally improved by a mutant gene, the opaque-2 gene, which causes an increase in the lysine content of the protein. Genetic-biochemical research has been stimulated by this discovery to find other food seeds that contain "high lysine" genes. These could then be used as breeding stock for developing high lysine commercially acceptable varieties.

In our study the lysine content was investigated in several classes of wheat, rye, and triticale grown in North Dakota. Varieties of hard red spring wheat, sawfly resistant spring wheat, durum wheat and

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rye that are now grown in North Dakota were analyzed. Also analyzed were some varieties of triticale being tested as a new potential cereal crop.

METHODS

Sample preparation. Samples were finely ground with a Micro-Wiley mill to pass a 60 mesh sieve or with a Udy cyclone-hammer mill to pass a 0.024 inch screen. Moisture contents were determined by the 130° C air oven method (7).

Lysine analysis. Samples of about 30 mg were hydrolyzed with 3 ml 6 *N* hydrochloric acid in hydrolysis tubes fitted with a 28/15 ball joint and glass stopcock. The samples were frozen in dry-ice-alcohol bath, sealed under vacuum, and heated 22 hours in an air oven at 110° C. ± 2°. The hydrolyzates were filtered on 2 ml. fritted disc funnels into 25 ml. filtering flasks and the humin of each sample washed twice with 1 ml. aliquots of de-ionized distilled water. The hydrolyzates were evaporated to dryness over sodium hydroxide pellets in a desiccator under vacuum.

Dried hydrolyzates dissolved in 4 ml pH 2.2 sample buffer were analyzed for lysine on a Beckman model 120 B amino acid analyzer by a triplicate-sample method for lysine that was developed in our laboratory (8). Steps in this method are first the application of 3 samples of hydrolyzates to a 9 cm height column of PA-35 spherical ion-exchange resin. Then buffer is pumped through the column for a short time between addition of samples 1 and 2, and 2 and 3 to insure sufficient separation of lysine peaks on developing the chromatogram. The chromatogram is developed at 40° C. with pH 5.10 citrate buffer of 0.20 *M* sodium concentration. Lysine is eluted from the samples as 3 peaks respectively from the 3 samples applied. The column is regenerated with 0.2 *N* NaOH and then equilibrated for the next triplicate analysis. This method of lysine analysis is about twice as fast as the conventional one where the basic amino acids, as a group, are eluted from a short column with pH 5.28 buffer (0.35*M* $Na+$) at 55° C. (9).

Protein analysis. Nitrogen was determined by either the micro-Kjeldahl or macro-Kjeldahl method (10), and protein was calculated by using the factor 5.70 for wheat and triticale and 6.25 for rye.

Lysine and protein content is expressed on a dry weight basis.

RESULTS AND DISCUSSION

Hard red spring wheat. Lysine content of hard red spring varieties is given in Table I. This class of wheat has a very high quality for baking bread. Lysine in the protein varied from 2.49% for Selkirk grown at Minot to 2.86% for Manitou from Fargo. Mean lysine for all these spring wheat samples was 2.67% at a high average protein level of 19.1%.

Protein content of wheat is important because an adequate quantity of protein is needed in the diet and our studies (11) as well as that of other workers (12, 13, 14) indicate there is a significant in-

TABLE I

LYSINE CONTENT OF PROTEIN OF HARD RED SPRING WHEAT

	Fargo		Minot	
Variety	Protein	Lysine In Protein	Protein	Lysine In Protein
	(%)	(%)	(%)	(%)
Selkirk	17.5	2.62	19.3	2.49
Justin	18.4	2.81	21.9	2.62
Chris	18.8	2.70	19.4	2.67
Manitou	18.2	2.86	19.5	2.58
Crim	18.4	2.74	19.3	2.59

verse relationship between lysine content in the protein and protein content of the grain. We also have found the relationship to exist for rye and triticale (11).

Sawfly resistant spring wheat. In some areas of North Dakota a spring wheat resistant to sawfly must be grown. Table II gives lysine values found for varieties of sawfly resistant spring wheat. In general lysine in the protein of these varieties was a little higher than in the other hard red spring wheat varieties (Table I). Lysine in the protein varied from 2.68% in Chinook grown at Minot to 3.13 in Rescue also from Minot. Mean lysine content of sawfly resistant samples was 2.93% at 17.4% average protein level.

TABLE II

LYSINE CONTENT OF PROTEIN OF SPRING WHEATS RESISTANT TO SAWFLY

	Minot		Fargo	
Variety	Protein	Lysine In Protein	Protein	Lysine In Protein
	(%)	(%)	(%)	(%)
Fortuna	16.9	2.75	-	-
Rescue	19.4	3.13	17.3	3.07
Sawtana	16.8	3.09	16.2	2.87
Chinook	18.1	2.68	-	-

Durum wheat. This class of wheat is used for the production of high quality pasta products. Lysine values in the protein of most of the varieties now grown in orth Dakota are given in Table III. The highest level was 2.69% in Mindum and the lowest 2.02% in Leeds, both grown at Edgeley. Average lysine content of the durum samples was 2.42% at a high mean protein level of 20.0%. The lysine level of durum protein was found to be only slightly lower than in hard red spring wheat.

TABLE III

LYSINE CONTENT OF PROTEIN OF DURUM WHEAT

	Edgely		Fargo		Minot	
Variety	Protein	Lysine In Protein	Protein	Lysine In Protein	Protein	Lysine In Protein
	(%)	(%)	(%)	(%)	(%)	(%)
Mindum	19.2	2.69	16.1	2.48	21.4	2.26
Wells	22.4	2.26	18.6	2.50	20.2	2.37
Lakota	22.0	2.29	18.9	2.46	18.7	2.66
Stewart 63	23.0	2.36	18.1	2.60	19.5	2.45
Leeds	23.2	2.02	18.6	2.66	21.7	2.23

Rye. This cereal generally is grown in North Dakota for feed use. The samples were obtained from the Seed Stock Project at North Dakota State University. For comparison, results are included on two of the same varieties, Antelope and Caribou from Rumania. Lysine content of 3 rye varieties widely grown in North Dakota is given in Table IV.

TABLE IV

LYSINE CONTENT OF PROTEIN OF RYE

	North Dakota		Rumania	
Variety	Protein	Lysine In Protein	Protein	Lysine In Protein
	(%)	(%)	(%)	(%)
Antelope	12.0	3.69	13.3	3.33
Caribou	13.6	3.75	14.5	3.21
Frontier	8.5	4.68	-	-

Lysine in rye protein was found to be higher than in wheat. The average protein level of the rye seeds was much lower than wheat, and the inverse relationship of lysine versus protein content accounts for part of the higher lysine content found in rye. However, previous analysis in our laboratory on 125 rye varieties from various parts of the world clearly indicated rye protein contains more lysine than wheat (11).

Triticale. The varieties of triticale are a synthetic hybrid of durum wheat and rye. This cereal is reported to give high yields of high protein grain (15). Although this cereal is relatively poor in bread baking quality it may have potential in other food or feed use. Results of analysis on some varieties tested in North Dakota in 1967 are given in Table V.

TABLE V

LYSINE CONTENT OF PROTEIN OF TRITICALE

	Minot		Langdon	
Variety	Protein	Lysine In Protein	Protein	Lysine In Protein
	(%)	(%)	(%)	(%)
6403	22.3	3.14	-	-
6432-3	21.2	3.41	18.7	3.33
6433-6	21.7	3.09	18.5	3.34
6437-6	-	-	17.7	3.27
6443	20.7	3.24	-	-
6443-7	-	-	18.1	3.46
6456-3	20.0	3.35	17.4	2.99

Lysine content of the triticale protein varied from 2.99% in variety 6456-3 to 3.46% in variety 6443-7, both grown at Langdon. Mean lysine content was 3.26% at a high average protein level of 19.6%. The lysine content is higher than in wheat protein. This is probably due to the rye parent of this hybrid.

Comparison of lysine at 15.7% *protein.* Accurate comparison of lysine content between varieties that differ in protein content is not possible because of the significant inverse change in lysine to protein content of the grain. An attempt to compare values at an equal protein level is illustrated by the data of Table VI.

TABLE VI

ESTIMATED LYSINE CONTENT AT 15.7 PER CENT PROTEIN

	Lysine in Protein	
Cereal	North Dakota	Over-All
	(%)	(%)
Hard Red Spring Wheat	2.79	2.73
Spring Wheat, Sawfly Resistant	3.00	-
Durum Wheat	2.69	2.75
Rye	3.75	3.30
Triticale	3.51	3.36

To calculate the "over-all" lysine content (Table VI) at 15.7% protein level, the regression equations we found previously for lysine versus protein content were used. The samples analyzed for the data used to calculate these regression equations were hard red spring and durum varieties grown in both Mexico and North Dakota, rye varieties grown at various parts of the world, and triticale varieties grown in Mexico. The mean lysine contents in the protein of each of the cereal grown in North Dakota were recalculated to give the estimated amount that should have been observed at a 15.7% protein level. This was done by adding or subtracting from the mean contents the product of the slope of the appropriate regression equation for each cereal times the difference in protein from a 15.7% level. For sawfly resistant wheat, the slope of the equation for hard red spring wheat was used.

The estimated lysine content at 15.7% protein level is near the "over-all" values calculated for hard red spring wheat, durum wheat, and triticale. Lysine in rye protein was a little higher than the "over-all" value. We have not previously investigated sawfly resistant spring wheat, but in comparison to hard red spring wheat these data indicate that they are a little higher in lysine.

Comparison of the lysine content in the protein of the different classes of wheat and different cereals grown in North Dakota indicates rye and tritcale protein is the highest in lysine, sawfly resistant wheat next to the highest, and durum and hard red spring wheat the lowest. Except for sawfly resistant wheats, which were not studied previously, these results agree with the over-all results found for the hard red spring and durum wheat grown in Mexico and North Dakota, triticale grown in Mexico, and rye grown in various parts of the world (11).

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EXPERIMENTS ON THE INCORPORATION OF ATP^{32} INTO VIRAL RNA*

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An increase in nucleolar RNA turnover with tritiated H^3-uridine and H^3-cytidine has been demonstrated by Levy (1) in HeLa Cells after one hour of infection with polio virus. He was able to detect after 2 hours of infection, specific fluorescent antibody in the cytoplasm of the HeLa cell which continued to increase for 5 hours after

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infection. Salzman *et al.* (2) have shown that free nucleotides of the HeLa cell are the major source of viral RNA and there was no significant direct utilization of the cellular RNA for viral synthesis. Infection of HeLa cells by polio virus results in the apperance of a cytoplasmic RNA polymerase (3). The polymerase activity did not appear until after 2 hours of infection and rose to maximum at 4-6 hours of infection. An enzyme system from rat liver (4) or bacteria (5) has been shown to catalyze the incorporation of C^{14}-ribonucleoside triphosphates into ribonucleic acid. This communication reports the preliminary results of experiments which attempts to measure the incorporation of ATP^{32} nucleotides into RNA polio virus, using the experimental procedures similar to that of Weiss and Nakamoto (5) for RNA synthesis.

METHODS

HeLa cells were grown in 1,000-ml culture bottles in a stationary state at 36° C. After a period of 5 days, the growth medium (6) was

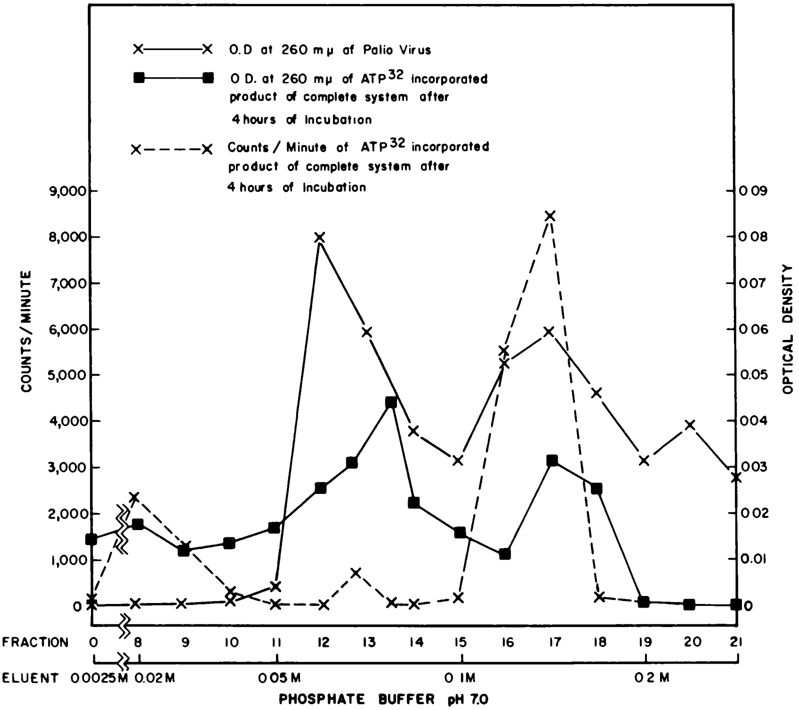

FIGURE 1 — Chromatographs of ATP^{32} incorporated product on ECTEOLA cellulose column. Counts per minute and optical densities at 260 mμ of phosphate buffer (0.0025 – 0.2 M) pH 7.0 eluents. Optical densities at 260 mμ of phosphate buffer eluents of rechromatographed purified polio virus from ECTEOLA cellulose.

removed from the 5 bottles and cells were rinsed once with 0.05 M phosphate buffer pH 7.0. The cells were collected in phosphate buffer 7.0 and centrifuged at 1,400 x g for 10 minutes to precipitate the cells. The precipitated cells were resuspended in 5 ml of phosphate buffer and homogenized in the cold for 1 minute in a Potter-Elvehjem homogenizer with Teflon pestle. The homogenate fraction was separated into cellular fractions by the method of Hogeboom *et al.* (7) and Griffiths and Pace (8). The homogenate was centrifuged in the cold for 700 x g for 15 minutes to remove nuclei. The nuclear pellet was rehomogenized in phosphate buffer pH 7.0 and centrifuged; the supernatants combined. The nuclear pellet was resuspended in 1.5 ml phosphate buffer and used in experiments as nuclear source. Mitochondria and microsomes were separated from the combined supernatants by centrifugation at 25,000 x g for 20 minutes and discarded. The supernatant was collected and used for the RNA polymerase. Penman *et al.* (9) have separated a cytoplasmic RNA polymerase in this fraction.

The incorporation of ATP^{32} into RNA polio virus was carried out similar to that reported by Weiss and Nakamoto *et al.* (5). The complete incubation system contained 6 μmoles of $MgCl_2$, 0.5 μmoles of ATP^{32} (International Chemical and Nuclear Corporation), 2 μmoles of GTP, CTP, and UTP (Pabst Laboratories), 0.01 ml of purified poliomyelitis virus (10^{-7} $TCID^{50}$) from ECTEOLA cellulose column by the method of Hoyer *et al.* (10), ($TCID^{50}$ = the dilution of virus just sufficient to kill 50 per cent of the 5-day old cultures in the test), and 1 ml of polymerase enzyme preparation. An additional flask contained the above mentioned items with the omission of either RNA polymerase. A third flask contained the complete system plus 0.1 ml of HeLa cell nuclei. The mixtures were incubated at 37° for 2 hours, 1-ml sample removed for radioactivity analysis, and the remainder was incubated for 4 hours. A sample was taken for bacterial culture which on examination was negative. The reaction was stopped by the addition of 0.3 ml of 3 N $HClO_3$ and the precipitate collected by centrifugation for 2,500 x g for 10 minutes. The acid insoluble residue was washed three times by solution in 0.1 N NaOH and reprecipitation with 0.5 N $HClO_4$ (5). The final residue was suspended in 3 ml of 0.02 N NaOH, and 1 ml was dried for radioactivity analysis in steel planchet determined with an ultra-thin window, gas flow geiger tube (Tracerlab TGC-14) and correcting for decay.

An additional flask containing the complete system plus HeLa cell nuclei as mentioned above, was incubated for 4 hours, immediately centrifuged at 100,000 x g for 90 minutes, and the pellet collected. The pellet was resuspended in 0.0025 M phosphate buffer (pH 7.0) and chromatographed on ECTEOLA cellulose column and eluted with increasing concentrations of phosphate buffer (0.0025 M to 0.2 M) pH 7.0 by the method of Hoyer *et al.* (10). Hoyer *et al.* (10) used this method to purify the poliomyelitis virus. Fractions of 3-4 ml were collected. The optical densities at 260 mμ and radioactivity were determined on each fraction. An additional ECTEOLA cellulose column was prepared on which was placed purified poliomyelitic virus, suspended in 0.0025 M phosphate buffer. The virus was eluted with increasing concentrations of phosphate buffer, 3-4.0 ml fraction collected and optical densities at 260 mμ determined .

RESULTS

Table I shows the data of incorporation of ATP^{32} nucleotide into RNA polio virus. The omission of RNA polymerase gave the lowest incorporation value with no difference between the 2- and 4-hour interval on incubation. There was an increase incorporation at the end of 4 hours of incubation.

TABLE I

COFACTORS REQUIREMENT OF ATP^{32} INCORPORATION

The complete system contains 6 μmoles of $MgCl_2$, 0.5 μmoles of ATP^{32} (2 x 10^6 c.p.m. per μmole), 2 μmoles each of GTP, CTP, and UTP, 0.01 ml of poliomyelitis virus (10^{-7} $TCID^{50}$), and 1 ml of polymerase preparation. The reaction was incubated at 37° and 1-ml sample was removed at the end of 2 hours and remained incubated for 4 hours. The reaction was stopped by the addition of 0.3 ml of 3 N $HClO_4$, and the precipitate collected by centrifugation. The acid insoluble residue was washed three times by solution in 0.10 N NaOH and reprecipitation with 0.5 N $HClO_4$. The final residue was suspended in 3 ml of 0.02 N NaOH, and 1 ml was dried for counting.

Additions	Counts/minute Incubation time: 2 hours	4 hours
Complete	574	844
Omit polymerase	180	224
Complete + nuclei*	1784	2174

*0.1 ml of HeLa cell nuclei

A 2-3 fold increase of incorporation of ATP^{32} into viral RNA in 2 hours of incubation was observed in the complete system plus nuclei as compared to the complete system. There was a further increase of incorporation of ATP^{32} into the viral RNA in the complete system plus nuclei in 4 hours of incubation. This increase would suggest the appearance of viral RNA polymerase activity. Baltimore *et al.* (3) have shown that viral polymerase activity did not appear until after 2 hours of infection and rose to maximum at 4-6 hours of infection. The complete system containing the nuclei gave the greatest incorporation of ATP^{32} into RNA virus. This observation is in agreement with at of Baltimore's (11) who has shown the polio virus induces a viral RNA polymerase.

As further evidence that this was RNA viral incorporation, an additional flask, after 4 hours of incubation containing the complete system plus nuclei, was immediately centrifuged at 100,000 x g for 90 minutes for collection of pellet. The pellet was resuspended in 0.0025 M phosphate buffer (pH 7.0) and chromatographed on ECTEOLA cellulose by the method of Hoyer *et al.* (10) for the separation of virus material. Eluents were collected and radioactivity and optical densities determined as described above. Hoyer *et al.* (10) have used this method for the purification of poliomyelitis virus. The results of this experiment are shown in Figure 1. The ATP^{32} incorporated material eluted from the cellulose column agrees with the 260 mμ absorbing material. The rechromatographed purified poliomyelitis virus eluted from the cellulose column has similar 260 mμ absorbance. These preliminary results would suggest that ATP^{32} is incorporated into RNA virus. This aspect of the problem is being further investigated.

SUMMARY

A study attempting to measure the incorporation of ATP^{32} into RNA polio virus is reported. The material synthesized when purified from ECTEOLA cellulose has similar optical density as untagged poliomyelitis virus.

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ENVIRONMENTAL, EXTRA-ENVIRONMENTAL AND PREFERENTIAL PERCEPTION IN GEOGRAPHY*

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Located in a *terra incognita* between the activities of geographers, psychologists and planners, is the study of an important aspect of man's relationship to his environment. Although the distribution of man's activities on earth has long provided the core of geographical research, the examination of geographical perception has only recently received much attention.

INTRODUCTION

It is the purpose of the present paper to discuss briefly three distinct, though related, types of perceptual research in geography and to provide some evidence of research by referring to recent surveys taken from a student sample at the University of North Dakota.

Before proceeding to a brief discussion of the three types of perceptual study, we must first recognize what "is too often forgotten that geographical studies are not descriptions of the real world, but rather perceptions passed through the double filter of the author's mind and his available tools of argument and representation" (Curry, 1962, p. 21).

The geographer, or the historian for that matter, who presents his analytical description of an area such as the Red River Valley, is not describing what there *is* in such an area, but rather what he is *aware* of in that area. No two men are aware of exactly the same patterns of distribution, and hence, no two descriptions are identical.

In the 'sixties, there has been developed a substantial body of geographical literature on perception, on man's awareness of his surroundings. Early literature borrowed heavily from psychological and sociological studies, but more recently geographers appear to be developing their own lines of study. We can group such studies of perception into three broad categories.

ENVIRONMENTAL PERCEPTION

By far the largest number of papers have been concerned with *environmental perception*, that is, man's awareness through one, or a combination of his senses, of the features of his immediate environment. As examples of such work, we may note Gould's (1965) examination of the environmental perception exhibited by wheat producers on the slopes of Mt. Kilimanjaro in Tanzania. Lucas (1964) has sur-

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veyed the visitor's perception of "wilderness" areas in the Quetico-Superior border area of Minnesota and Ontario, and Burton and Kates (1964) have discussed the nature of hazard perception in resource management, a matter which has also received attention from Saarinen (1966).

Lowenthal (1961, 1967a, 1967b) has examined aspects of environmental perception in the United States and has indicated the importance of such research in the resolution of contemporary planning problems. Perhaps the best known study of environmental perception in the urban context is that by Lynch (1960). The low level of environmental awareness discovered in the studies by Lowenthal and Lynch has led to pleas for an improved education towards American landscape consciousness by Mattern (1966). In his recent paper, "The American Scene," Lowenthal (1968, p. 88) further illustrates the fact that Americans often fail to be aware of their surroundings. He concludes,

> . . . Let us try to look around wherever we are—not necessarily with close attention to form or detail, but fleetingly, musingly, dreamily, provacatively, any way, just as long as we see something. For "without vision the people perish."

If we can obtain a better understanding of the development of environmental perception, we may be able to direct attention towards greater environmental appreciation and care.

EXTRA-ENVIRONMENTAL PERCEPTION

Students of environmental perception have usually taken a rather small area as their universe, their primary interest being with man's direct experience. There is also the perception of an "invisible landscape," an area outside the immediate environment. Stea (1967, p. 27) describes the "invisible landscape" as an area, which through part of a person's habitat, is unavailable for his use, or at any rate, is not used. Although Steaa uses it at the immediate level, there is no reason why we should not expect the concept of "invisible environment" to include those areas of the world which man perceives but of which he has no direct experience.

To the North Dakotan, this may include Spain, India, Vietnam and the majority of the other 120 odd states in the world. If questioned, he will admit to having only a rudimentary perception of places which he may have read about but has not visited. His perception, or awareness, will not include many details of these areas. Parts of the world's "invisible environment" may be practically unknown to him. With regard to a remote area such as Cambodia, he may be tempted to remark, as did Sir Winston Churchill (Time, 1966) that, "I have lived seventy-eight years without hearing of bloody places like Cambodia. They have never worried me and I haven't worried them."

But modern communications have "made the world a village" and we cannot afford to relegate any areas to such a position of obscurity. Until recently, little has been done to discover the nature of student perception of areas with which they have had no immediate contact, aside from asking students to insert names on the map. In 1966, Gould reported on a number of surveys which were carried out in order to determine the "mental maps" of student groups, and it is upon his work that our pilot studies of North Dakota students have been based.

PREFERENTIAL PERCEPTION

A third, and practically untouched aspect of the geographical study of perception remains to be noted, and this is what might be termed *preferential perception.* Preferential perception refers to man's preferences for movement towards particular places. Some elements of preferential perception are included within environmental perception but in our study, two questions sought to discover North Dakota student preferences for residence in other American States and for tourist trips to European states. The ultimate objective of such studies in preferential perception is to discover the reasons for goal selection.

In 1967 a North Dakota survey, the full report of which will be found in Goodey (1968), was carried out with two groups of students, each of about two hundred members. The majority of the surveys were experimental in nature and require adjustment before further use is made of them. It should also be pointed out that introductory geography students were used as surveyors; student reports indicate that such participation in survey research was of value to these beginning geographers.

In the present context three aspects of the survey will be reported upon. One group of two hundred students was asked to name correctly each State on a blank map of the United States. This was, of course, a common test to determine student knowledge of the States. When mapped, the results showed a rapid decline in student knowledge of the States in all directions from North Dakota. Only the Dakotas, California and Florida were correctly identified by all. Identifications were much more accurate west of the Mississippi than to the east of it; major areas of error were the Eastern Seaboard States and the South. The historical core of the United States suffered most and one cannot help but believe that the traditional North Dakota rejection of the East has somehow been successful.

An attempt to construct a map of the average student's view of the United States is shown in Figure 1. In this cartogram (Cole, 1967) all States are located in correct relationship to each other but the shape of most is distorted. Each State is drawn proportionately to the percentage of the group failing to recognize it. The smaller the State proportionally to its true area, the poorer the sample identification—only the Dakotas, California and Florida are the correct size. Vermont and New Hampshire have been reduced by over fifty per cent!

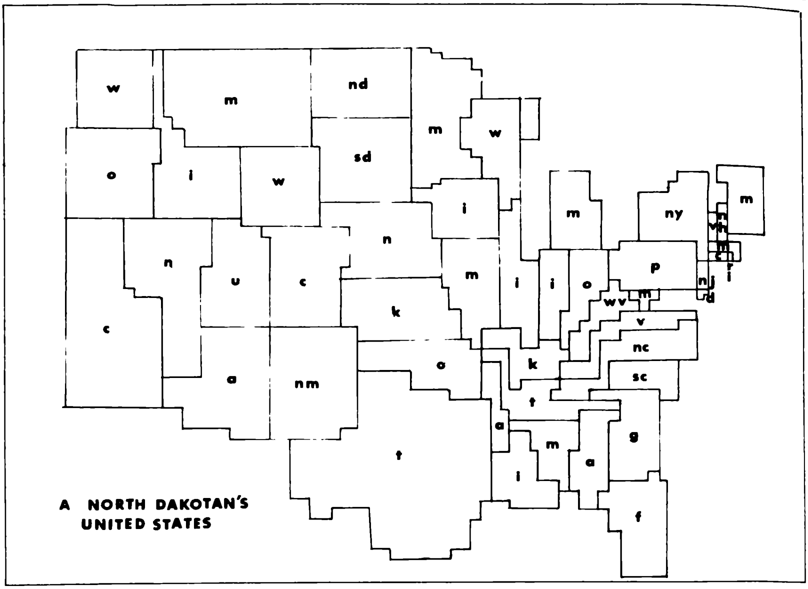

FIGURE 1—A North Dakotan's United States. A cartogram constructed according to data derived from a student perception survey carried out at the University of North Dakota, Summer 1967.

From a survey of State's visited by the sample it appears, that students tend to have visited those places which they can identify, or more likely, tend to identify those places which they have visited. The Deep South and the Eastern Seaboard have been visited by between only ten and twenty per cent of the sample and these areas were lowest on the recognition map. From this, and further circumstantial evidence, we learn that North Dakota students, and probably many others in the United States, have a very narrow perception of the "invisible environment" that lies outside their own State. Indeed such perception is often not detailed enough to include the correct distance and directions relative to the observer's position.

A further section of our 1967 Pilot study asked participants to rank the States of the United States according to their preferences for purposes of future residence. The results revealed that the areas of greatest attraction were the Western Coastal States and Colorado. Then, according to our generalized results, came North Dakota, Minnesota, Wisconsin, and Florida. The impression gained from these data was that young people perceived the West most favorably. This, converted into the "Go West Young Man" philosophy, is supported with reference to recent migration figures analyzed by Adams (1964).

Stea (1965) is amongst those who suggest that man, like the lower orders, places a high value on territory: in line with this idea it might be suggested that the North Dakotan has a formal territorial base in his own State but sees justifiable, if informal, extensions of this territory into the States of Washington and California, and more recently, Colorado.

This research in preferential perception was modeled after that of Gould (1966) and it is rewarding to compare our North Dakota results with those which Gould derived from student groups in California, Minnesota, Alabama and Pennsylvania.

On comparing our map with Gould's, it is evident that North Dakotans agree in many respects with their fellows in other States. Like the Californians, Pennsylvanians and Minnesotans they reject the South and have little interest in Utah, preferring the West Coast and Colorado. Californians and Pennsylvanians were more willing to give the Eastern Seaboard States a chance than were North Dakotans or (predictably) Alabamians.

In most respects the preferential perception maps of North Dakotan and Minnesotan students were very similar, but unfortunately the Minnesotan view of North Dakota was rather low, whilst North Dakotans perceived their eastern neighbor as being rather attractive.

In these initial surveys, we only scratched the surface of several interesting aspects of the perception of North Dakota students. We are left with a number of important questions to answer in the future. By searching for answers to these questions it is hoped that we can aid in the development of teaching methods which will encourage the sharpening of perceptive skills and thus allow more information to be made available for student's preferential perception and participation in an ever-shrinking world.

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SOME CHARACTERISTICS OF THE DIFFUSION OF COMMUNITY INNOVATIONS

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The concept of diffusion derives from the fact that ideas are not adopted by all people at the same time. As with individuals, groups must be aware of an innovation before it can be adopted. This idea of group awareness is used here to examine the spread of innovations from community to community throughout North Dakota.

Several questions immediately arise. Does information spread along a front from east to west? Do ideas flow from a central community in a wave that moves outward at an even pace in all directions? Or does information reach all communities at about the same time, regardless of community size or location?

To determine whether or not a single diffusion pattern exists, it was assumed that if innovations introduced at different times followed a single pattern, then it would be possible to recognize a communication structure in North Dakota. Four community-oriented innovations were examined. Hospitals, the earliest to be introduced in the state, were followed by the more recent innovations—swimming pools, nursing homes, and fluoridation plants, in that order. The locations of communities adopting the different innovations were mapped at intervals so that the spatial patterns and the diffusion curves could be plotted.

Several investigators have commented upon the characteristics of phenomena in the process of diffusion. According to Hagerstrand, diffusion is a function of communication, *i.e.* "one cannot adopt an innovation which is not one's own invention unless one has first seen it, heard of it, or read about it." In addition to communication, Hagerstrand suggests that a time-lag is normal between the time of first information and the time of adoption. What happens during the time-lag, says Hagerstrand, is just as important although less easy to assess since it is primarily an unseen mental process (2).

Empirical studies show that although innovations are radically different in nature, their spread exhibits a number of recurring traits (2):

> When the number of adopters—individuals, firms, cities, as the case may be—is measured over time an *S*-shaped curve normally appears. This curve shows a slow take-off stage of varying length, an intermediate stage of more rapid development and a final stage of declining growth which

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asymptotically seems to approach a ceiling. Different innovations run through this process with very different speed; also various degrees of irregularity are noted.

The spatial distribution of adopters seems linked with the three parts of the growth curve. At the beginning of the diffusion process when there are few adopters, those few are often clustered together, *i.e.* expansion most noticeably occurs close to existing innovations than at points of greater distance. This phenomenon of contagion, known as the "neighborhood effect," is seen in both early and late stages of diffusion.

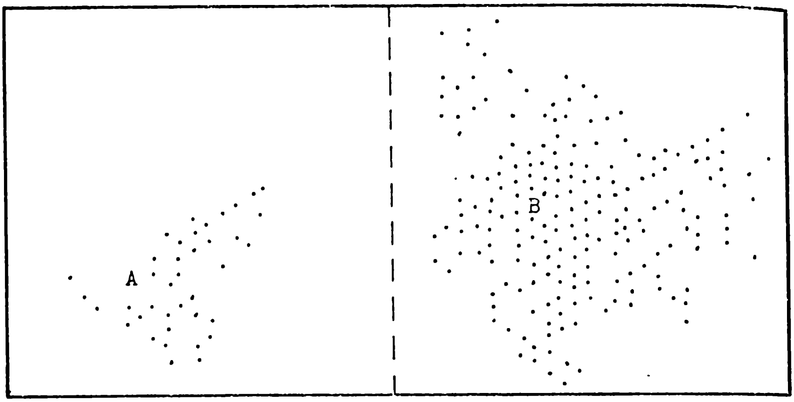

FIGURE 1—Early and later stages of diffusion (simulated) from points of origin *A* and *B*, respectively.

Another concept of information flow suggests that ideas are transmitted from higher to lower order centers. This "filtering-down" process is described by Hagerstrand (2):

> . . . it is important to note that innovations which emanate from the same center but start spreading at different points in time still tend to propagate along similar 'roads' and in the same spatial pattern, without of course repetition in details. One gets the impression that information and influences travel in a system of communication with a rather stable spatial configuration. The number of links between areas and places seem to remain very much the same over time even when the acting individuals change.

Should either the "innovation wave" or "filtering-down" communication systems exist in fact, the four innovations should exhibit similar diffusion patterns. If that is the case then an interurban communication structure should be evident as seen through spread of the community-oriented innovations originating at different times and of a rather dissimilar nature.

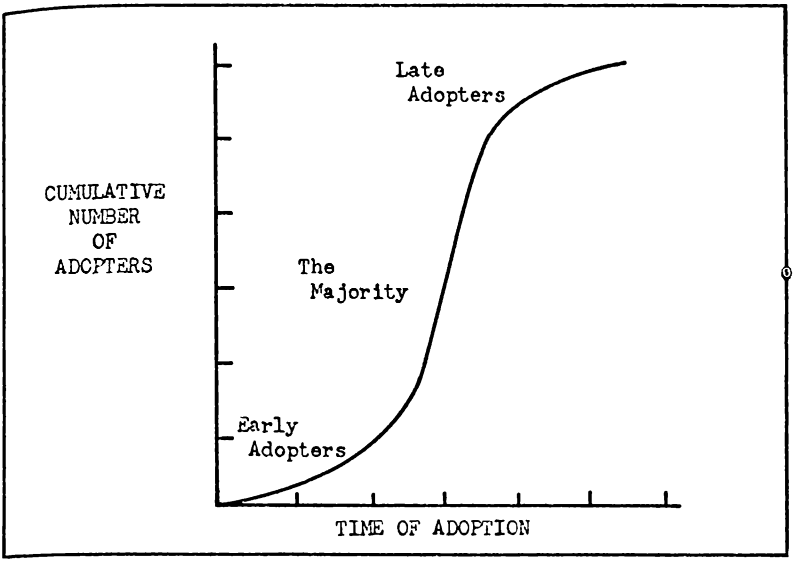

FIGURE 2—Idealized *S*-form of the logistic diffusion curve.

Pemberton has suggested that diffusion of culture traits occurs at a rate best described by the cumulative curve of normal frequency distribution. With studies including adoption of postage stamps by countries and state adoption of limits upon municipal taxation rates, he found that in each instance the diffusion is characterized by an *S*-form. Pemberton states that the normal frequency distribution would affect virtually all innovations (3):

> According to the order of simple chance, combinations of preponderant influences for or against adoption occur relatively few times; and there are, accordingly, relatively few instances of conspicuously early or conspicuously late adoptions. The most probable time of trait acceptance is the middle or average time and the probabilities of frequencies in other time periods follow the normal distribution.

For each of the four innovations examined here, a curve illustrates the diffusion of that trait. The two earlier innovations, hospitals and swimming pools, show the most clearly developed *S*-curves; in fact, two *S*-curves can be distinguished for both hospitals and pools. This double curve can be correlated with economic and political conditions affecting North Dakota and the United States. Despite the effects of economic depression and war upon the diffusion of the two innovations, the spread of the traits resumes following removal of external influences.

The diffusion curves for nursing homes and fluoridation plants

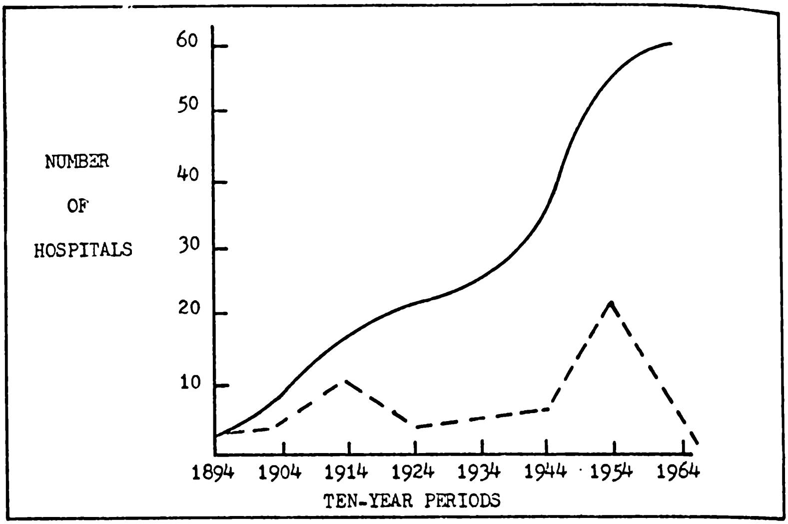

FIGURE 3—Diffusion curve and rate-of-increase of community hospitals in North Dakota.

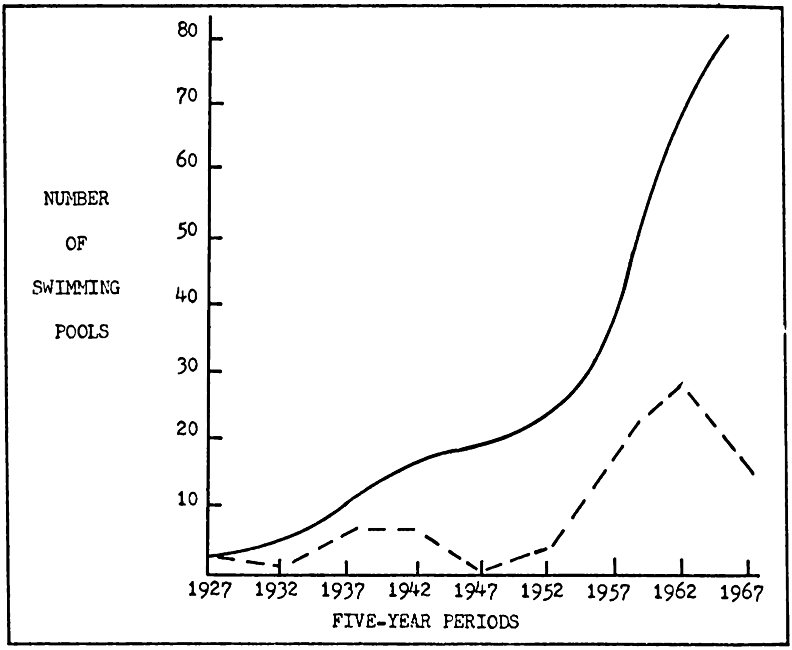

FIGURE 4—Diffusion curve and rate-of-increase of municipal swimming pools in North Dakota.

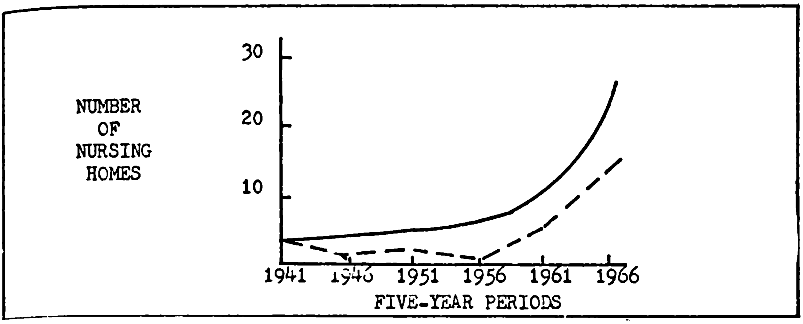

FIGURE 5—Diffusion curve and rate of increase of nursing homes in North Dakota.

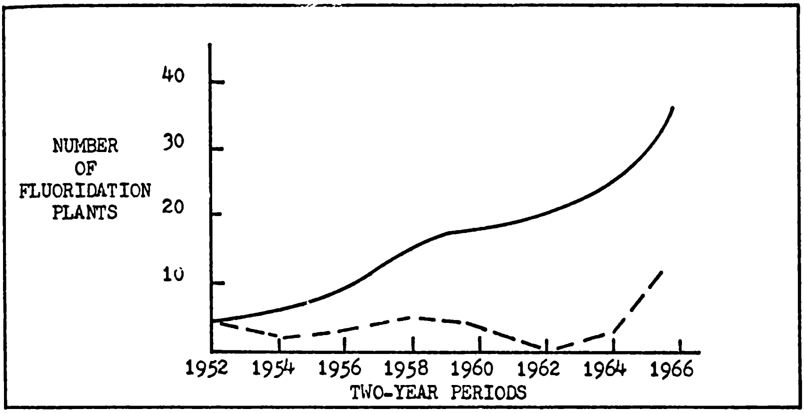

FIGURE 6—Diffusion curve and rate-of-increase of municipal fluoridation in North Dakota.

have not developed the definite *S*-form shown by the two other innovations. This follows Pemberton's observation that the fit is not so close in series involving small numbers of units. For both of the more recent innovations, however, the curves indicate that relatively few communities were adopters in the first years following the introduction of the traits. The curves then show a definite rise but no indication of "leveling" to complete the *S*-curve.

It may appear that hospitals and swimming pools are nearing the point of saturation, that point at which almost all who are going to adopt a practice have done so. Nursing homes and fluoridation plants, recently introduced and still spreading rapidly among the state's communities, may not reach the point of saturation until after hospitals and swimming pools. However, Bohlen has stated that "when several new practices are compared, a wide variation in time is found between the time of awareness and of adoption. Also, within a given practice there is a great variation in time-lag from awareness to adoption which seems to be related to certain personal and social characteristics of the adopters" (1).

Therefore, it appears possible that, depending on the innovation, adoption of recently introduced innovations could reach the point of saturation sooner than those ideas of earlier origin.

From an analysis of the various diffusion curves it is apparent that four different community innovations, introduced at different times into North Dakota, have diffused in rather similar forms. Most pronounced of the similarities is the diffusion from higher-order to lower-order centers, although in the case of fluoridation the introduction was to a lower-order community. Table I shows the rank of adoption for each of the innovations by the ten largest communities.

TABLE I

RANK OF ADOPTION OF FOUR INNOVATIONS BY THE TEN LARGEST COMMUNITIES IN NORTH DAKOTA

City	Hospitals	Pools	Homes	Fluoridation
Fargo	3	15	1	2
Grand Forks	2	16	7	13
Minot	11	1	3	8
Bismarck	1	2	2	7
Jamestown	14	11	10	9
Williston	16	4	4	6
Mandan	52	18	—	4
Dickinson	12	3	5	3
Valley City	19	17	—	11
Devils Lake	5	65	11	21

In fifty-five per cent of the possible instances, the ten largest communities were among the first ten adopters of each innovation. Further, in only five instances were the ten largest communities not ranked among the first twenty communities to adopt.

The two most recent innovations demonstrate even greater tendency for early acceptance among the larger centers; seven of ten nursing homes and fluoridation systems were adopted first among the ten largest communities in the state. This high figure compares with only four of ten hospitals and swimming pools first adopted among the same communities. It appears, then, that there is a definite flow-of-influence through the urban structure. Generally speaking, adoption of community innovations begins among the larger communities and then spreads to smaller communities. Considering the four innovations used in this report, there is no evidence that indicates a reversal of this flow, *i.e.* lower-order to higher-order centers.

The shape of each innovation's diffusion curve relates directly to the diffusion from higher-order to lower-order centers. After the larger centers have adopted the innovations, the rate of growth (reflected by the curves of diffusion) noticeably declines. The curves begin climbing again after a period of relatively few adoptions. That slack period is longest for the most expensive innovations—hospitals and swimming pools. Fluoridation plants also exhibit a slack period, but it is much shorter.

The diffusion curve for nursing homes does not indicate the slack period observed for the other innovations. It may be, however, that nursing homes, still in the earlier stages of the diffusion process, have yet to reach the slack period.

Diffusion curves for each innovation differ but little from one part of the state to the next. The state was divided into four areas of equal size from east to west and the diffusion curves plotted for each area. The slack period is reflected in the curves for both hospitals and swimming pools even when the state is considered section by section.

The flow of influence from larger to smaller communities appears stronger than any directional flow. The state was again divided into four areas of equal size from eact to west and the percentage of adopters in each area during each time period plotted. Only hospitals exhibited any tendency toward east-west diffusion, probably the result of the state's settlement pattern. The other innovations, more recently introduced, only show the dominance of the eastern one-quarter of the state in percentage of total adopters. This situation, however, is attributed to the greater number of potential adopters in the east compared to the sections further west.

SUMMARY

The distributions of community-oriented phenomena in North Dakota were analyzed to determine if a single diffusion pattern existed. It was assumed that if innovations introduced at different times followed a single diffusion pattern, then a communication hierarchy would be spatially recognizable. Four community-adopted innovations were examined. It was seen that the *S*-curve of the normal frequency distribution best fit the diffusion traits of both hospitals and swimming pools. Nursing homes and fluoridation plants ,were observed in their early stages of diffusion. Their diffusion curves described the expected, *i.e.* a slow rate of acceptance in their early stage followed by a rapid rise in number of adopters. The two older innovations appeared to be near the point of saturation; the two newer innovations are yet in the stage of rapid diffusion.

The adoption of both swimming pools and fluoridation plants by second-order centers was seen to be rather more common than adoption of either hospitals and nursing homes by those centers. Among other reasons, swimming pools and fluoridation plants are more economical to adopt by the smaller communities.

It was also observed that for all innovations the flow of influence from larger to smaller communities appeared stronger than any directional flow. This was the one diffusion characteristic of a spatial nature shared by all four innovations.

The findings of this report suggest that community-oriented innovations diffuse from higher-order to lower-order centers in the state. The wide range of observed spatial distributions indicates that further study is needed to determine a more clearly defined diffusion pattern.

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PRECIPITATION VARIABILITY AND MIGRATION OF THE NORTH DAKOTA FARM POPULATION, 1930-1960

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I. *Introduction*

The relationship between man and the land is an old problem in geography. Given a population engaged in agriculture, the occurrence of precipitation in regular, sufficient amounts is of crucial importance in this man-land relationship.

If man's occupance of his environment depends on the precipitation resource base, his occupance of the environment will cease, *ceteris paribus,* if precipitation does not occur in regular, sufficient quantities to permit him to carry on his normal occupation. This retreat will be expressed in the form of outmigration to a more favorable environment, or through occupational mobility by which the agriculturalist will turn to an alternative occupation either at the same site, or in an entirely different location. In terms of the Census definition of the rural farm population, there will be subtractions from that group no matter which alternative is selected. Clearly, not all men in the same environment perceive their environment in the same way. Because of the importance of other economic and social factors, precipitation will not produce an equal impact on all occupants of an environment. While some will be forced to abandon their position and seek a new one, still others will remain in their old environment. Hence, outmigration from an environment is almost never total, but at the same time, it almost always occurs.

Is it possible within the framework of areal association, to explain the relationship between these two dynamic phenomena, precipitation and migration, as they both vary through space and over time? The approach in this paper will be one of partial explanation through areal association. No attempt will be made to construct a model, or series of models that will accurately predict all off-farm migration, for this would obviously require the inclusion of several non-physical variables. The attempt here is to seek a better understanding of this particular man-land relationship, by discovering *how much* of the spatial variation in off-farm migration can be explained by precipitation, within a limited study area for various periods of time.

II. *The literature*

The problem of retreat of farm people from unfavorable precipitation conditions has been examined in several studies. In the descriptive manner, Bowman (1931), Hicks (1931), Webb (1931), Kollmorgen (1935), Kraenzel (1955) and others have outlined the major patterns of alternating advance and retreat of the farm population in the Great Plains during wet and dry years. The influence of prices of the factors of production and commodity prices are also highly involved and cannot really be separated from physical factors if an accurate assessment of causes of migration is to be made.

More recently, two studies of the Great Plains utilizing the concept of areal association have appeared in the literature, (Wright, 1959) and Robinson *et al.* (1961). Both of these studies found the coefficient of simple correlation between rural farm population density and precipitation to be a strong, positive one. These two studies operationalized precipitation conditions as being mean annual precipitation for 1940 to 1949 and rural farm population density as the number of rural farm residents per square mile in 1950. Robinson found $r=0.78$ and Wright, with a slightly different study area but still called the Great Plains, found $r=0.756$. The high correlation found here is measuring two factors. One, that farmers retreat from low-level precipitation conditions, and two, that they do not originally settle in areas of low precipitation.

In addition, Wright found that the coefficient of correlation between these two factors increased from $r=0.550$ in 1930, to $r=0.687$ in 1940 and then to $r=0.756$ in 1950. He states that this ". . . indicates that rural farm population has tended to adjust spatially to the precipitation conditions through the years".[1] If such a hypothesis is acceptable, then the real factor involved is the outmigration from areas

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[1] Wright, op. cit., p. 17.

of lower precipitation over and above that from those having more abundant precipitation since, the size of the counties has not changed and natural decrease (a surplus of deaths over births) did not occur in the Great Plains, which is the only other factor that could lower density.

III. *Hypotheses*

A. It is therefore hypothesized that a better measure of adjustment to precipitation resources would be outmigration rates from the rural farm population. This removes the effect of avoidance by settlers of areas of low precipitation in the original settlement. On the basis of the work cited above, it is hypothesized that outmigration rates will vary according to precipitation conditions.

B. It is possible that the lowering of farm population density has been caused largely by the outmigration of rural farm persons who are not directly affected by precipitation conditions. That is, it may be due to the well-known exodus of farm youths just finishing their schooling who leave the farm to seek education and employment in non-farm areas. This phenomenon operates fairly independently of precipitation conditions in the United States (see Bowles, 1956, pp. 1-14). It is therefore hypothesized that the highest correlation between precipitation and outmigration rates should be in the middle age groups, which contain the largest proportion of farm owners and operators. It is this group who will respond most directly to the effects of precipitation, and not older farmers who leave the farm population on retirement, or the younger groups who leave to seek opportunities elsewhere.

IV.. *The Universe, Spatial and Temporal*

The study area is confined to the 53 counties of North Dakota, for three 10-year periods, 1930-1940, 1940-1950, 1950-1960. While North Dakota is not claimed to representative of the Great Plains, it does present many of the problems outlined here (See Anderson, 1952, Cleland, and Hay, 1939).

V. *The Variables*

A. Precipitation.

The precipitation variables are operationalized here so as to reflect conditions that would affect man's occupance of the land and are of the nature demanded by a physical resource investigation.

(1) *Mean annual precipitation*—Since farmers in North Dakota have been accustomed to dry years along with wet ones, average annual precipitation for 10-year periods (1930-1940, 1940-1950, 1950-1959) between censuses, corresponding to effective crop years, are though to be the best, single measure of precipitation conditions. Farmers do not leave after a single drought year, but retreat after a series of droughts. A ten-year period is also convenient for Census purposes.

(2) *Precipitation standard deviation*—This variable, for the same time units as (1) reflects how well or how poorly the mean describes precipitation conditions. Extreme variablity between years can be more important than a low absolute level. Five drought years and 5 wet years could have the same mean as 10 moderate years, but the two would have much different standard deviations.

(3) *Coefficient of Variation*—Since drought is the crucial factor and cannot easily be forecasted, the amount of agricultural instability could be directly proportional to the standard deviation (σ) and inversely proportional to the decade long average $\frac{(1)}{\bar{\bar{X}}}$, or $\frac{\sigma}{\bar{\bar{X}}}$.

That is, the importance of variation is diminished by the effect of the overall level. Large values of the index would indicate extreme environmental instability.

(4) *Number of years having less than 14" total precipitation*—Since the standard deviation often increases with the size of the mean, these two variables (1) and (2) and their combination (3) do not take into account that a high standard deviation may indicate variability around a high mean annual precipitation amount. A threshold size for precipitation seems to exist, around 14", below which wheat yields fall more rapidly. On inspection of the data, this figure was somewhat arbitrarily arrived at, but in consideration of the threshold idea. The difference between 12" and 16", for example ,seems to be more critical than the difference between 16" and 20", although the range is the same.

B. Migration variables.

The total migration from the farm population was not used here, since it simply represents a cruder measure of who, in fact, is migrating. Old persons, including widows and retiring farmers are thought not be be directly involved in this man-land relationship, nor are small children under 5 years of age, who migrate only with their parents. For this reason, age-groups on both ends of the age-structure were removed. Four age-groups were used for the 1930-1940 period, 5 were used for 1940-1950 and six, 1950-1960. This was done so that it would be possible to trace the migration experience of various cohorts in the farm population over time. For the three periods, between 71.4% (1930's) and 96.8% (1950's) of the total migration was included. Of the 795 age-specific net migration rates that were computed, 99.4% were negative, indicating a net outmigration.

The census survival ratio method were used here to estimate net migration (see Hamilton and also Bowles, 1956). This involves computing survival rates from census to census for cohorts of a base population (the U.S. total population was used) and applying these to the specific age groups, as:

Where k= the ratio of survivors between decennial censuses (X,X-10) for the United States, in the ith cohort and P= population:

$$k = \frac{P^{i}_{X}}{P^{i}_{X-10}}$$

then the net migration rate for the ith age-group in the jth county in North Dakota is Mij,

$$Mij = \frac{p^{i}_{x} - (k)\, p^{i}_{x-10}}{p^{i}_{x-10}}.$$

This assumes:

1. That survival ratios for rural farm residents in North Dakota are the same as those for the U.S. population.
2. That the U.S. population is not significantly altered by international migration.

This type of ratio is called a forward survival ratio and was used here because of the preponderance of net outmigration.

VI. *Analysis*

A correlation analysis between appropriate variables revealed a rather low amount of explanation for some, and an insignificant correlation among others. The highest correlations were for the 1930's when precipitation conditions were poorest, as might be expected. However, only mean annual precipitation was significantly correlated with all age-groups, at the 5% level. Considering the framework within which this work is couched, it appears that precipitation is a significant factor areally associated with off-farm migration, but only for a decade marked by several severe droughts. For the 1940's and 1950's when precipitation was more abundant, no correlations exist that are more than two points above the 5% level. Also, the largest amount of explained variation is found in the group age 35-44 in 1940. This group is no doubt made up largely of farmers, which substantiates the second hypothesis.

That the other three precipitation variables do not show a significant correlation with migration rates leads to the hypothesis that precipitation conditions which they measure are not significant in man's perception of his environment.

Through regression analysis, the residual, or unexplained variation reveals where, in fact, the relationship does not hold. Since mean annual precipitation for the 1930's was the only variable having a significant correlation, it was the only one used in the regression. The areas that had more outmigration than expected (under-predicted) were counties mainly in southern and western North Dakota that had precipitation below the state average, whereas the Red River valley during the 1930's, had less migration than predicted.

TABLE I

PEARSON PRODUCT MOMENT COEFFICIENTS OF CORRELATION FOR PRECIPITATION AND MIGRATION VARIABLES

A. Precipitation variables (independent)

X1	mean annual precipitation
X2	precipitation standard deviation
X3	coefficient of variation
X4	number of years having less than 14" precipitation

B. Migration variables (dependent)

Migration rates according to age at beginning of decade

Y1	55-64
Y2	45-54
Y3	35-44
Y4	25-34
Y5	15-24
Y6	5-14

Correlation Matrix for 1930's

	Y3	Y4	Y5	Y6
X1	0.294*	0.459*	0.376*	0.336*
X2	0.260	0.152	0.170	0.025
X3	0.058	-0.159	-0.067	-0.200
X4	0.024	-0.117	-0.115	0.001

Correlation Matrix for 1940's

	Y2	Y3	Y4	Y5	Y6
X1	0.274*	0.256	0.045	-0.026	01.36
X2	0.226	0.143	0.066	0.028	0.060
X3	0.170	0.017	0.029	0.051	0.0040
X4	-0.115	-0.191	-0.018	1104	-0.076

Correlation Matrix for 1950's

	Y1	Y2	Y3	Y4	Y5	Y6
X1	0.258	0.140	0.283*	0.056	0.046	-0.037
X2	0.026	0.014	-0.035	-0.159	0.021	-0.169
X3	-0.062	-0.146	0.182	-0.168	-0.002	-0.251
X4	-0.151	-0.151	-0.225	-0.011	0.104	1013

* indicates significant at 5% level for 51 degrees of fredom.

Its precipitation was above the state average. Such patterns indicate the importantce of economic and social conditions, although such considerations are beyond the scope of the present study.

VII. *Conclusions*

Man's agricultural occupance of the land depends on the occurence of sufficient, regular amounts of precipitation. His retreat from unfavorable precipitation conditions can be expressed in terms of migration and analyzed using the concept of areal association. In North Dakota between 1930 and 1960, some expressions of this precipitation are significantly correlated with the outmigration of cohorts affected by precipitation. This relationship only holds for the 1930's, however, when several severe droughts occurred. Under relatively "normal" conditions, such as those prevailing during the 1940's and 1950's, variations in precipitation cannot be said to be a factor that is significantly correlated with off-farm migration in North Dakota. Mean annual precipitation is the only operational definition of precipitation conditions found to be significantly areally associated with migration rates.

DATA

Precipitation data were taken from the U.S. Department of Commerce Weather Bureau, *Climatic Summary of the United States, Supplement for 1931 Through 1952.* Data for 1930 were collected from U.S. Department of Agriculture Weather Bureau, 1932 Supplement No. *North Dakota,* Section 34. Data for 1952-1959 was collected from U.S. Weather Bureau *Annual Reports.*

Population data were taken from the U.S. Census of Population, 1930, 1940, 1950, and 1960.

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A NOTE ON THE RECOGNITION, CAUSE, AND POSSIBLE GEOLOGIC IMPORTANCE OF GROUNDWATER MICROFLOW SYSTEMS IN ALLUVIAL-CHANNEL BED-FORMS

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Groundwater microflow systems in natural bed-forms were noted during experiments on sediment transport in laboratory flumes. Ripple bed-forms consisting of medium sand were injected with dye as shown in Figure 1-A. Insipient movement of the dye through the sand was noticed after an elapsed time of 5 minutes (Figure 1-B). Dye movement was greatest beneath the gently sloping upstream side of the bed-forms. Figure 1-C shows the path of the dye 10 minutes after injection. By this time is was also apparent that dye just downstream from the crest of each bed-form was moving straight upward. Thirty minutes after injection, the dye had completed its course through the bed-forms and had emerged at the surface of the stream bed (Figure 1-D).

It was obvious from the dye movement that groundwater moves spontaneously through natural bed-forms. Water from the stream channel enters the upstream side of the bed-forms, flows in a concave-upward arc downstream, and emerges at the crest of the bed-forms. Water which enters the stream bed less than roughly ½ wavelength in front of the bed-form crest flows in an arc upstream and emerges at the nearest crest.

The microflow is caused by pressure variations from the hydrostatic on the surface of the bed-forms (Figure 2). The pressure on the bed-form surface is greatest in the low areas, or troughs, between the crests; lowest pressures occur in the areas above the crests (Raudkivi, 1967, p. 205). This pressure distribution is caused by variations in stream velocity over the bed-forms. With a uniformly sloping (flat) water surface, such as occurs over ripples, the velocity is great-

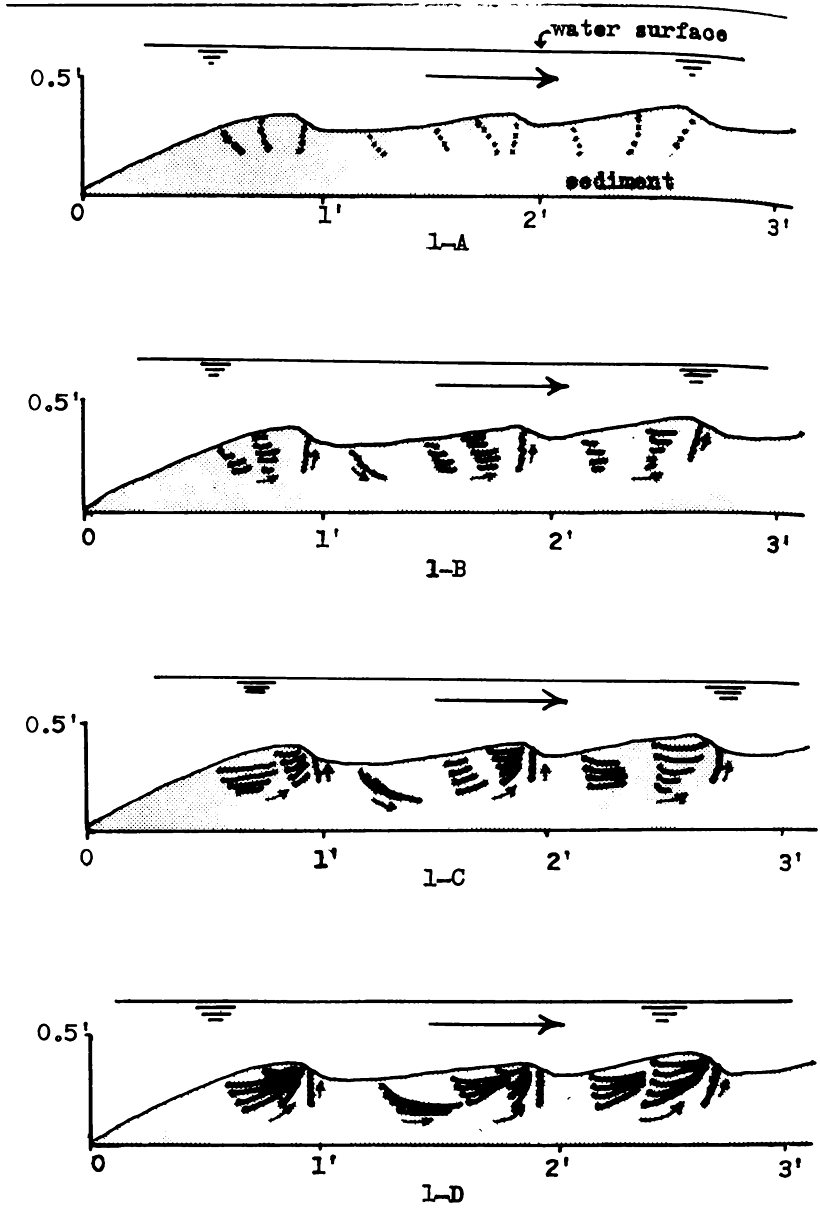

FIGURE 1—Movement of dye through natural bed-forms in a laboratory flume. 1-A. Dye at time of injection into bed-forms. 1-B. Dye movement 5 minutes after injection. 1-C. Dye movement 10 minutes after injection. 1-D. Dye movement 30 minutes after injection.

est over the crests of the bed-forms, where the decreased depth results in a reduction in the cross-sectional area of flow. Conversely, stream velocity is least in the trough areas where the cross-sectional area is greatest. With a slightly higher flow regime, which produces dunes, the wate rsurface is out of phase with the bed-forms, thereby further increasing the velocity variations. It is known from the Bernoulli and energy equations that an increase in stream velocity results in an increase in kenetic energy and a corresponding decrease in hydrostatic pressure. Thus, regions of accelerating, converging flow correspond to areas of decreasing pressure. Regions of decelerating, diverging flow correspond to areas of increasing pressure. These relationships, as applied to bed-forms, are shown in Figure 2.

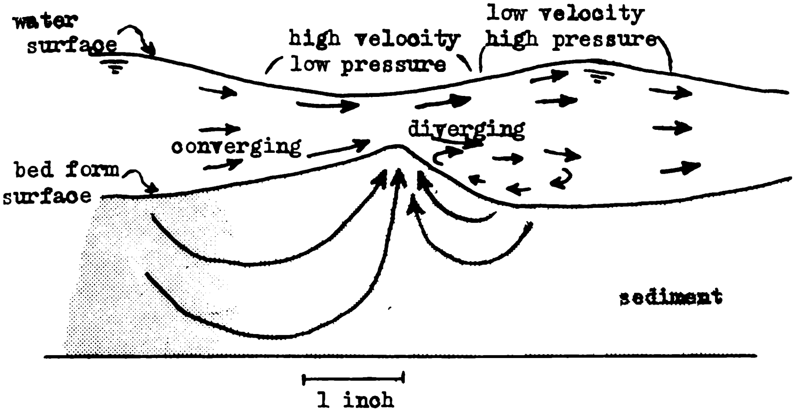

FIGURE 2—Pressure and stream-flow velocity distribution over bed-forms and their relationship to groundwater microflow systems.

The importance, if any, of the pressence of groundwater microflow systems in natural bed-forms is not known. Experiments show, however, that induced upward-moving groundwater seepage reduces the angle of repose on the lee face of bed-forms and thus decreases bed roughness and form-drag flow resistance. The angle of repose of the sediment used in these experiments is about 33 degrees without seepage. Induced upward seepage following a seepage gradient of +0.8 reduces the angle of the repose to about 27 degrees. Natural microflow systems should have a similar effect, although the reduction of the angle of repose and bed roughness is probably slight.

Turbid water seeping into the bed-forms may also introduce silt and clay into the sand at its upstream face; the filtered water leaving the bed-form may flush out fine sediment from its downstream face. Because the microflow system should be more intense in dunes than in ripples, these factors may offer a field method for distinguishing cross-bedding formed by ripples from that formed by dunes.

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TWO GENERATIONS OF SPHENE IN THE TUNK LAKE GRANITE, SOUTHEASTERN MAINE

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ABSTRACT

Preliminary study of the accessory mineral sphene, $CaTi(SiO_4)(O,OH,F)$, in the Tunk Lake granite southeastern Maine, indicates that it has formed in two different environments of crystallization. In the outer part of the concentrically zoned granite body which is exposed over a circular area of 70 square miles, sphene occurs as euhedral crystals which appear to have formed early in the crystallization history of the rock. In the center of the body sphene occurs as irregular masses which, along with biotite, secondarily replace primary hornblende, suggesting an origin by late-state recrystallization. The two occurrences indicate the complexity of accessory-mineral formation and the dependence upon other mineral reactions both during magmatic crystallization and late recrystallization.

INTRODUCTION

Accessory mineral formation has been considered by most petrologists to be early in the crystallization history of granitic igneous rocks since early workers noted that these minerals occur as well-formed crystals enclosed by apparently younger quartz, feldspar and ferromagnesian minerals. Moorhouse (1956) has suggested that many accessory minerals may be expected to crystallize late because they are formed from constituents present in small amounts in magmas; his most convincing evidence is their typical occurrence in late-formed pegmatites. The importance of textural evidence is emphasized by Schermerhorn (1958) in his discussion of Moorhouse's (1956) interpretations.

Although he emphasizes that accessory minerals form late, Moorhouse (1956) also documents and clearly supports the concept that accessory minerals may have complex crystallization histories in magmatic rocks. Silver and Deutsch (1963) have demonstrated that this is true for zircon while more recent studies have emphasized the variability of environments of crystallization of accessory minerals (for example Lee and Dodge, 1964 and Snetsinger, 1967).

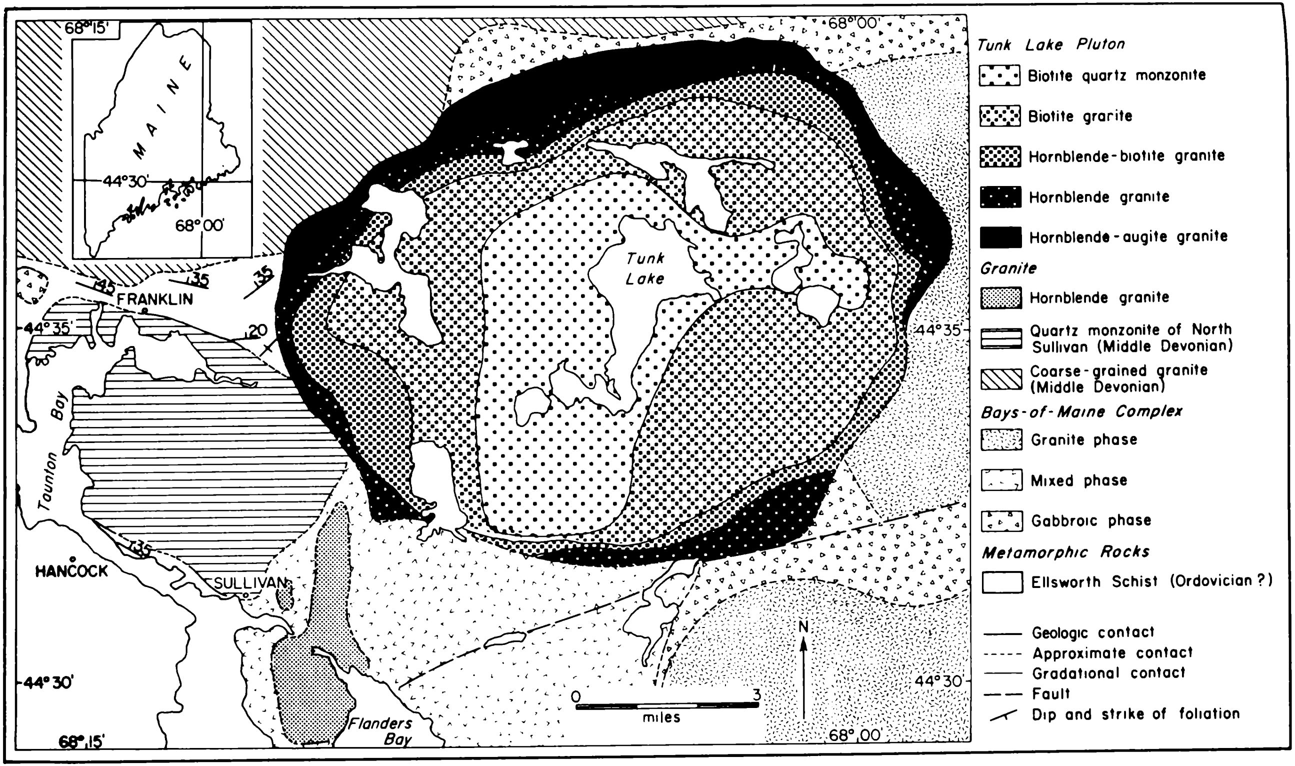

FIGURE 1—Geologic map of the Tunk Lake granite pluton (Karner, 1968).

In the Tunk Lake granite pluton most accessory minerals appear to have crystallized early (Karner, 1963, 1968). Preliminary textural evidence is given here that sphene, $CaTi(SiO_4)(O,OH,F)$, has formed both early and late in this granite in two quite different chemical and physical environments.

GEOLOGIC SETTING

The Tunk Lake granite body is intruded into Paleozoic igneous and metamorphic rocks in southeastern Maine (Karner, 1968). Its area of outcrop is 70 square miles and nearly circular (Figure 1). Six concentric zones are recognized. Numbered from the margin inward they are:

Zone I—Aegirine augite and magnetite granite chill zone.
Zone II—Hornblende-aegirine augite granite.
Zone III—Hornblende granite.
Zone IV—Hornblende-biotite granite.
Zone V—Biotite granite.
Zone VI—Biotite quartz monzonite.

The zones are gradational and form a continuous sequence from margin to core. Modal averages derived from thin sections from 140 locations in the granite body are given in Table I. Field and modal studies show that the Tunk Lake body is best interpreted as a single intrusion in which the zones have developed by differentiation of an originally homogeneous magma. Crystallization began along the margins where a sample of the original magma was frozen against the enclosing rocks forming a chill zone. The mineralogy of the marginal rocks indicates that they crystallized at relatively high temperature compared to the core which contains minerals formed at lower temperatures in the presence of abundant water. The water is believed to have streamed from the partially crystalline margin to the still molten core as a hot silica-rich fluid. This accounts for the enrichment of SiO_2 in the core, the most striking, chemical difference between it and the marginal rocks.

OCCURRENCE OF SPHENE

Sphene is not uniformly distributed throughout the Tunk Lake granite. Approximate modal data for sphene are compared with data for total mafics and accessories in Table II. Sphene is most abundant in zone V, 0.196 per cent, and decreases in abundance toward the core and toward the margin. Sphene also makes up the largest proportion of the total mafics and accessories in zone V, 4.46 per cent. This proportion decreases toward the core and toward the margin to zone II but increases in zone I. In zone I, the chill zone, some specimens contain abundant sphene, up to 0.5 per cent or 16 per cent of the total mafics and accessories, the largest proportion of any of the samples studied. However, many samples in zone I contain little or no sphene. Zone II, which contains the highest percentage of ferromagnesian minerals, contains the least sphene.

Titanium distribution in the granite body is less well known.

TABLE I

MODAL AVERAGES FOR ZONES OF THE TUNK LAKE PLUTON

Areal extent expressed as percentage of total outcrop area. Mean mineral abundances expressed as volume percentages with 95% confidence intervals. (From Karner, 1968)

Zone	Areal Extent	Quartz	Perthite	Albite in Perthite*	Oligoclase	Total Mafics and Accessories	Aegirine augite	Hornblende	Biotite-chlorite	Magnetite	Hematite	Zircon	Sphene	Allanite-epidote	Others**
I		34.1	57.5	42.6	5.5	2.9	0.37	0.39	0.48	0.91	0.27	0.13	0.01	0.11	0.17
		±3.0	±3.5	±1.6	±2.6	±0.6	±0.25	±0.25	±0.27	±0.32	±0.16	±0.06	±0.02	±0.08	
II	8.7	22.2	64.8	45.1	5.0	8.0	1.3	3.6	1.0	0.90	0.66	0.12	0.01	0.13	0.25
		±3.2	±3.2	±1.7	±2.8	±2.0	±0.4	±0.5	±0.6	±0.35	±0.55	±0.04	±0.02	±0.04	
III	10.2	28.1	62.4	44.4	4.1	5.5	0.22	3.0	1.0	0.67	0.21	0.11	0.09	0.08	0.06
		±1.6	±2.3	±1.0	±1.6	±0.5	±0.31	±0.6	±0.5	±0.17	±0.10	±0.03	±0.04	±0.04	
IV	13.2	29.3	51.8	33.3	13.8	5.1		1.7	2.4	0.56	0.13	0.09	0.17	0.03	0.04
		±1.8	±3.6	±1.5	±3.5	±0.6		±0.5	±0.6	±0.10	±0.06	±0.01	±0.07	±0.02	
V	40.4	32.2	44.3	30.7	19.1	4.4		0.49	3.1	0.46	0.02	0.07	0.20	0.04	0.06
		±1.8	±2.0	±1.2	±1.8	±0.4		±0.16	±0.3	±0.06	±0.02	±0.02	±0.07	±0.02	
VI	27.5	34.9	34.9	21.0	26.6	3.7	Tr.	0.12	2.7	0.58	0.03	0.04	0.13	0.06	0.04
		±1.1	±1.7	±2.3	±1.6	±0.7		±0.12	±0.6	±0.13	±0.02	±0.02	±0.06	±0.03	
AVG***		31.3	46.3	31.2	17.6	4.7	0.13	1.1	2.5	0.58	0.12	0.07	0.08	0.06	0.07

*Expressed as percentage of total perthite

**Includes olivine + riebeckite + apatite

***Weighted according to areal extent of the zones.

TABLE II

ABUNDANCE OF SPHENE IN THE ZONES OF THE TUNK LAKE PLUTON

Mineral Abundances Expressed as Volume Percentages

Zone	Sphene	Total Mafics and Accessories	Sphene Percentage of Total Mafics and Accessories
I	0.013	2.9	0.45
II	0.013	8.0	0.16
III	0.094	5.5	1.71
IV	0.166	5.1	3.26
V	0.196	4.4	4.46
VI	0.130	3.7	3.52

Calculations from mineral composition suggest approximate TiO_2 values of about 0.4 per cent for all of the zones (Karner, 1968). Wet chemical analyses of rocks from four individual locations in zones I, II, V, and VI give 0.27, 0.29, 0.45 and 0.30 per cent respectively (Karner, 1968). Sphene distribution is clearly not entirely dependent on titanium abundance but this may partially account for the high sphene content of zone V. Calcium appears to vary in the granite body increasing from about 0.5 per cent in zone I to values near the average of 0.9 per cent further inward (Karner, 1968). The analyzed specimens mentioned above gave CaO values for the specimens from zones I, II, V, and VI of 0.55, 0.73, 1.10 and 0.55 per cent respectively. Sphene distribution, especially the high content of zone V may be partly related to calcium distribution.

In zone I sphene occurs as wedge-shaped crystals, usually in clusters with other ferromagnesian or accessory mineral grains. These clusters are generally interstitial to larger feldspar and quartz grains. A typical occurence is shown in Figure 2A.

Further within the granite body in zones II, III and IV, sphene usually occurs as irregularly-shaped grains associated with hornblende which has been partly surrounded and replaced by biotite (Figure 2B). In zones V and VI sphene also occurs as irregular grains but wedge-shaped crystals are common (Figure 2C). In the granite body, particularly in zones III, IV and V, the shape of irregular sphene grains in some thin sections can be seen to be controlled by the cleavage of hornblende which the sphene replaces. In this respect the sphene is similar to biotite which has obviously replaced hornblende in these zones (Figure 2B).

CRYSTALLIZATION HISTORY

The two occurrences of sphene can be related to the crystallization history of the Tunk Lake granite by using the abundance and textural data cited above. In zone I, where crystallization generally took

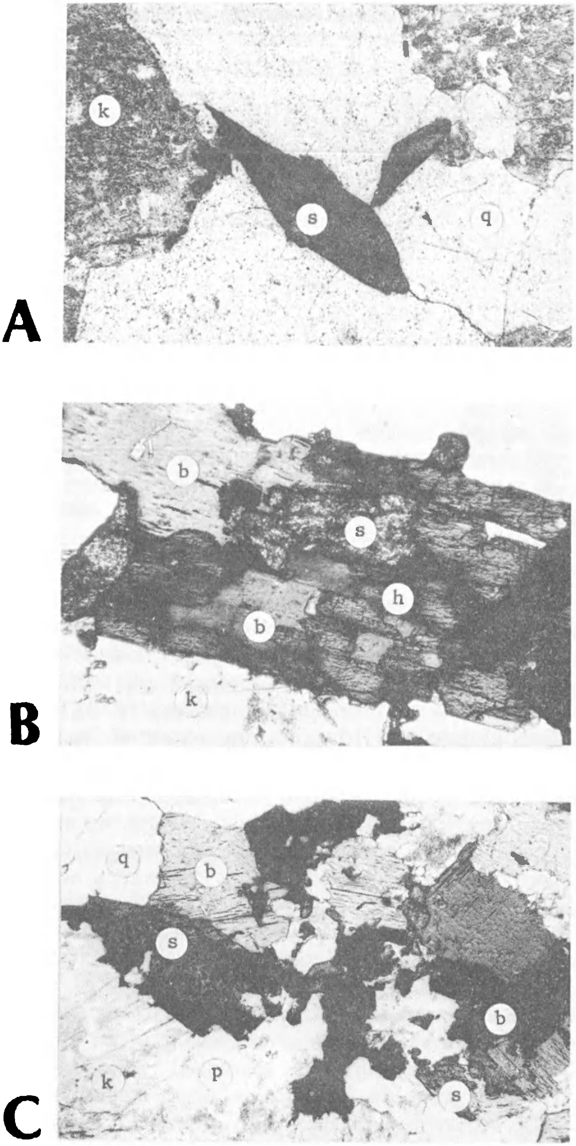

FIGURE 2—Typical occurrences of sphene in the Tunk Lake granite. A. Sphene crystals interstitial to perthite and quartz in zone 1, location 279, 14X. B. Hornblende replaced by biotite and sphene in zone V, location 238, 33X. C. Irregular and well-formed sphene crystals associated with biotite in zone V, location 238, 14X. Key: b = biotite, h = hornblende, k = perthite, p = plagioclase, q = quartz, s = sphene. (For precise location of specimens in zones see Karner, 1968.)

TABLE III

ASSEMBLAGES OF TITANIUM-BEARING MINERALS IN ZONES III AND VI OF THE TUNK LAKE PLUTON.

Zone III

Hornblende $(NaCa_2MgFe_3^{+2})\ (Al_{0.4}Fe_{0.4}^{+3}Ti_{0.2})\ Al_2Si_6O_{22}(OH,F)_2$
Magnetite-Ilmenite $Fe_3O_4\ Fe_2TiO_4$

Zone VI

Biotite $K_2(MgFe_3^{+2})\ (Fe^{+3}Al_{0.5}Ti_{0.5})\ Si_6Al_2O_{20})\ O_2(OH)_2$
Magnetite $(Fe_{2.8}Ti_{0.2})O_4$
Sphene $CaTi\ (SiO_4\ (O,OH,F)$

place at high temperature in a relatively dry magma, the form and interstitial position of sphene suggests early formation. Its sporadic occurrence in zone I may reflect titanium or calcium abundance or stability of other minerals which contain these elements such as magnetite-ilmenite (titanium) or plagioclase (calcium).

Further within the granite body sphene is rare or absent in zones II and II which consist of aegirine-augite and hornblende granites containing an opaque mineral which is probably magnetite-ilmenite relatively rich in titanium (Buddington, and Lindsley, 1964) since the granites are of relatively high-temperature origin. Calcium is believed to have been preferentially incorporated in early aegirine-augite and hornblende and titanium incorporated in these minerals as well as in magnetite-ilmenite.

In zones IV and V sphene again becomes abundant and typically occurs as irregular grains which along with biotite replace well-formed hornblende grains. The sphene and biotite are interpreted to be solid-state recrystallization products of primary hornblende and possibly magnetite-ilmenite. Recrystallization is believed to be the result of increased water-vapor pressure which facilitated adjustment to lower temperatures as cooling proceeded. Elements released from the primary minerals allowed precipitation of the secondary minerals. In the central parts of zone V and in zone VI sphene and biotite occur as primary minerals as the result of an overall lowering of crystallization temperature by increased water-vapor pressure. Table III gives the titanium-bearing mineral assemblages for zones III and VI. The occurrence of irregular sphene in zones IV and V is the result of the transition between these two assemblages particularly by recrystallization processes. Specifically, sphene is texturally related to the hornblende-biotite transition in which calcium and possibly titanium is released. This reaction has also been interpreted as the source of calcium which caused secondary plagioclase to crystallize in these zones (Karner, 1966). Other workers including Schermerhorn (1956) and Pratt (1967), have related sphene to recrystallization of other minerals, primarily biotite.

The two generations of sphene in the Tunk Lake granite show that the crystallization of this accessory mineral: (1) is complex (2) is dependent upon both high-temperature, liquid-crystal reactions and low-temperature, recrystallization processes (3) and is intimately related to reactions between other minerals.

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COCCOLITHS FROM THE NIOBRARA FORMATION (UPPER CRETACEOUS) OF NORTHEASTERN NORTH DAKOTA

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INTRODUCTION

Coccolithophorids are minute flagellate protists which range in size from 1 to 30 microns in diameter. In most respects, they are typical flagellates; they reproduce in sexual and asexual cycles; they may or may not possess chloroplasts (depending upon the species and the part of the reproductive cycle in question); and they may or may not encyst during part of their life history. They are important to the micropaleontologist, however, for yet another reason; coccolithophorids are covered by a number of small variously shaped, calcareous elements collectively called coccoliths. Although fossil coccoliths were first illustrated by Ehrenberg in 1836 (Bramlette and Sullivan, 1961, p. 130), their importance as a sediment source has only recently been realized (*e.g.*, Bramlette, 1958).

During a study of the literature dealing with fossil and recent nannoplankton the writers were impressed with the vast number of coccolithophorids present in the modern marine environment (*e.g.*, Ekman, 1953, p. 271; Bramlette, 1958). Because fossil coccoliths have been positively identified in rocks as old as Jurassic and were reported by Dawson (1874) from Cretaceous beds in Manitoba, it became apparent that they should occur in Mesozoic marine sedimentary rocks in North Dakota. A sample of calcareous shale from the Niobrara Formation of Upper Cretaceous age was hastily prepared and the suspicion confirmed.

The purpose of the present paper is, therefore, to discuss the occurrence of coccoliths in the Niobrara Formation of eastern North Dakota, present a list of genera thus far collected and identified, describe a simple method of preparing slides, and suggest future problems related to their study in the Cretaceous of the Midcontinent.

Subsequent to the initial preparation of this paper the writers became aware of the report of coccoliths from Cretaceous rocks in Minnesota by Woodward and Thomas (1895) and from shale in the Colorado Group by Goodman (1951). The publication of Trexler's (1967) excellent paper on Upper Cretaceous nannoplankton made us aware of McClung's (1898) figures of coccoliths from chalk in the Niobrara of Kansas and Black's (1965) summary of recent and fossil coccoliths.

COLLECTION AND PREPARATION

Upon discovery of coccoliths in the Niobrara, a method was developed to sample as much of the formation, in continuous section, as possible. Samples of the unit were obtained from Clarence G. Carlson, Geologist with the North Dakota Geological Survey, who was engaged in a study of the economic potential of the formation (Carlson, 1964). The test hole which presented the most complete Niobrara section was Carlson's Test Hole E-1, located in SE¼, NE¼ sec. 19, T. 158 N., R. 56 W., about 2 miles west of Edinburg, Walsh County, North Dakota. The well penetrated to a depth of 166 feet, all of which was in the Niobrara Formation except the first 34 feet which was glacial drift. A complete log of the well is given by Carlson (1964, p. 39-40). Samples for the present study were collected at odd two-foot intervals from a depth of 41 feet to 165 feet. Considerable care was taken to prevent samples from contaminating one another, a major problem considering the small (1-20 microns) size of the coccoliths.

Edwards (1963) described a rather elaborate technique for extraction of calcareous nannoplankton fossils by washing and centrifuging the sample several times. Although the results are good, the process is rather laborious, and a somewhat simpler technique was developed to accomplish essentially the same end-product. Samples were prepared by placing a small (2-3 g) sample in a vial of water. sealing the vial, and placing it in a Bendix sonic cleaner for disaggregation. When the sample was broken down, it was removed, stirred, and allowed to settle. Settling times were picked from the grain settling time chart developed by Krumbein (1935). Preliminary sampling was done at a depth of seven centimeters at time intervals of 1, 3, and 20 minutes and 1.5, 4.5 and 7.5 hours. The samples were taken using a paper drinking straw as a pipette so that it could be discarded after each sampling and prevent contamination. The drop of water thus collected was placed on a glass slide and allowed to dry. Using Caedax, the samples were covered by cover slips to complete the preparation. Having prepared several slides in this manner, it was determined that the best samples were drawn off at time intervals of 20 minutes and 1.5 hours so that these two times were used for all of the remaining preparations. The samples prepared in this manner contained very few particles except coccoliths, calcareous spicules, and occasional calcite crystals.

The prepared slides were viewed under a Leitz phase contrast microscope at a magnification of 1250 diameters. A small cross was inscribed in the center of each slide and the coccoliths photographed were located by means of stage micrometer readings with reference to the center of the cross. Specimens were photographed using both phase and bright field illumination.

SYSTEMATIC LIST OF NIOBRARA COCCOLITHS

Although discussion of the taxonomic problems related to the study of coccoliths is not germane to the present study, a few reservations on use of the present scheme should be mentioned. Perhaps the most complete taxonomic treatment of the group was presented by Deflandre (1952, p. 452-459) in which he subdivided the Class Coccolithophorida into two orders, based on crystal structure, and five families, based on gross morphology of the elements. Electron microscope studies of the group, however, have tended to challenge this classification. Until a more thorough study of coccoliths is undertaken, using both light and electron microscopy, therefore, it would seem wise to adopt the somewhat more generalized classification of Bramlette and Sullivan (1961, p. 138). They subdivided the group into two families, Coccolithophoridae and Braarudosphaeridae, and referred to a number of other genera *incertae sedis.*

The specimens collected from the Niobrara Formation were studied and photographed under phase contrast conditions which proved to be the most satisfactory available means of observing minute detail. Photographs of the specimens were then compared to the descriptions and illustrations of genera and species many of which were described by Deflandre (1952), Bramlette and Sullivan (1961), and Bramlette and Martini (1964). The generic assignments thus made are believed to be reliable. It was apparent, however, that much more thorough study of the Cretaceous forms in the Midcontinent would have to be made before specific assignments could be made with confidence. A list of genera identified from the Niobrara Formation is here given:

Family: Coccolithophoridae
  Arkhangelskiella species A
  Arkhangelskiella species B
  Coccolithus
  Cretarhabdus species A
? *Cretarhabdus* species B
  Cyclococcolithus
  Markalius
  Zygodiscus
  Zygolithus species A
? *Zygolithus* species B
  Zygrhablithus
Genera *incertae sedis*
  Marthasterites
  Micula
  Stephanolithion
  Tetralithus

RESULTS AND FUTURE WORK

The genera thus far identified from the Niobrara Formation in North Dakota compare rather closely with those studied by Bramlette and Martini (1964) from the Upper Cretaceous. There seems to be enough similarity of Niobrara forms to Cretaceous forms from other areas to encourage further, more detailed study of their stratigraphic

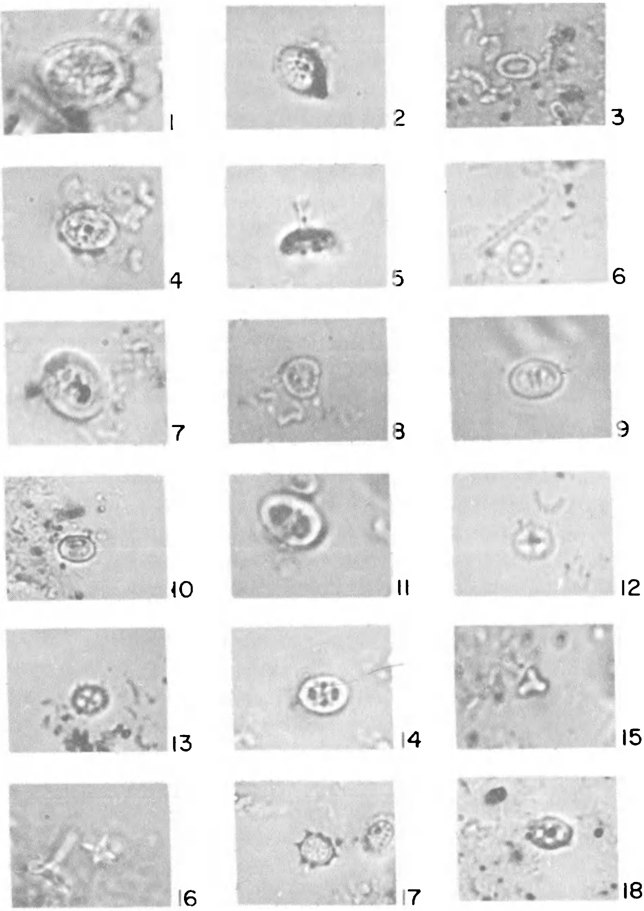

PLATE 1—All specimens were collected from the Niobrara Formation in Test Hole E-1 (Carlson, 1964). The depth recorded for each specimen indicates the sample position in the well as measured from the surface. All specimens were photographed under phase contrast illumination and are illustrated 2500X.

Figure 1. *Arkhangelskiella* species A, 41 feet; 2. *Arkhangelskiella* species B, 55 feet; 3. *Coccolithus* species, 41 feet; 4, 5. *Cretarhabdus* species A, 55 feet, apical and oblique lateral view; 6. ?*Cretarhabdus* species B, 55 feet; 7. *Cyclococcolithus* species, 41 feet; 8. *Markalius* species, 45 feet; 9. *Zygodiscus* species, 63 feet; 10, 11. *Zygolithus* species A, 41 and 49 feet, respectively; 12, 13. ?*Zygolithus* species B, 55 and 45 feet, respectively; 14. *Zygrhablithus* species, depth unknown; 15. *Marthasterites* species, 45 feet; 16. *Micula* species, 63 feet; 17. *Stephanolithion* species, 79 feet; 18. *Tetralithus* species, 51 feet.

value. The only genus reported herein which does not seem to have been reported previously from the Cretaceous is *Cyclococcolithus*. We agree, however, with Trexler (1967, p. 1360) when he implied the necessity of electron microscopy where he said (equally applicable in our case), "Further work is needed to establish the validity of the extended ranges indicated in this report."

Although the present study must be considered preliminary, it does serve to point out the abundance and a considerable degree of diversity of coccoliths in the Niobrara Formation. Undoubtedly, they can also be collected from other fine-grained rocks in North Dakota. An indication of this is the zones of "white specks" widely used as stratigraphic marker beds by petroleum geologists in the northern Midcontinent. We believe, as did Goodman (1951) and others, that the source of the "white specks" in the Niobrara is probably crushed coccolithophorids. Another "zone" of the "white specks" occurs lower in the column in the Greenhorn Formation, often known by drillers as the beds of the "second white specks."

Future work should be directed toward identifying other coccolith-bearing strata in the Midcontinent and establishing, where possible, first occurrences of genera. This would considerably enhance their stratigraphic value. This might become increasingly important in connection with renewed interest in drilling for shallow petroleum prospects (see Anderson, 1967; Kornfeld and Travis, 1967) in the "Muddy" or Newcastle Formation of Lower Cretaceous age. Stratigraphic zonation and paleogeographic interpretations might also aid in seeking silt lenses or other stratigraphic traps in the Mowry or Greenhorn Formation (between the Niobrara and Newcastle Formation) shoreward toward the east side of the Williston Basin.

Black (1965, p. 135-136) has summarized the distribution and abundance of coccolithophorids in modern oceans. He emphasizes the well-known biologic principle (Fischer, 1961) of greater variety of forms but lower number of individuals in tropical waters when compared with boreal or austral water which characteristically have a lesser variety of forms, but greater concentrations of individuals. Trexler (1967, p. 1361) has suggested that the great variety of forms (46) in the Benton-Niobrara interval of Colorado would indicate "warm surface currents from the South from the ancestral Gulf of Mexico." We found 15 different forms assignable to eight genera in our preliminary study. If the techniques employed did not cause us to miss some forms, we would suggest that the abundance of coccoliths but lack of as great a diversity as reported by Trexler, might indicate boreal waters in the late Cretaceous sea of northeastern North Dakota.

Finally, identification of coccoliths from a new area immediately points up the need for more thorough descriptions based, not only on study with the light microscope, but also on electron microscope observations (Hay and Sandberg, 1967). Only when the two techniques are combined in a single taxonomic study will significant classification leading to more reliable paleoecologic and paleogeographic interpretations be made possible.

ACKNOWLEDGMENTS

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A CHEMICAL APPROACH TO THE STUDY OF ARTHRITIS

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Doctors admit that they have no cure for arthritis. Their efforts are directed to the relief of pain and the prevention of stiffened joints. In severe cases they may resort to drastic surgery, replacing parts of joints with metal or plastic. The victims of arthritis may suffer cruelly for many years.

Many suggestions have been made as to possible causes of arthritis but no satisfactory explanation has come to the author's attention. He is a Professional Chemist; not a physician. This paper attempts to present a plausible approach to a chemical explanation of certain types of arthritis and possibly other obscure physical ills. The suggestion came from a careful consideration of the extremely vital and diversified role of the element phosphorus in the body metabolism, together with a study of dietary data. This has indicated that serious imbalances between the intake and excretion of body phosphorus are very common, becoming more serious as people grow older. Thus it seems obvious that serious metabolic disturbances must result from prolonger phosphorus deficiency.

The biochemical importance of phosphorus is truly astonishing. It is well summarized in NUTRITIONAL DATA (1). Phosphorus has more important functions than any other element in the body. This is not generally appreciated except by the biochemists who are actively investigating its behavior. They have reported at least 14 vital functions of phosphorus and others are added as research continues. Organic phosphates and pyro phosphates are high energy molecules making possible the explosive release of muscular energy under the catalytic action of the enzyme phosphatase. Phosphorus is involved in carbohydrate metabolism, fat metabolism, protein metabolism, brain and nerve metabolism. Inorganic phosphates constitute one of the most important buffers regulating the pH of the blood.

Phosphorus is involved in skeletal growth and in tooth development, as well as in the action of vitamins and hormones. Phosphorus is distributed in every cell of the body and is vitally concerned with the functions of these cells.

Whole blood contains from 35-45 mg per 100 ml of blood, of which 3-5 mg are inorganic phosphorus. The inorganic phosphorus is that portion which is readily available for chemical reaction and is most directly affected by the intake of phosphorus; but, it is in constant exchange with the organic phosphorus in the blood, and there is a constant replacement of the inorganic phosphorus if it is eliminated by disease or nutritional conditions.

The level of inorganic phosphorus is influenced by many factors affecting assimilation, absorption, excretion, and the mobilization of the phosphorus. Because of this, the levels in the blood do not necessarily guarantee a normal nutritional status of the phosphorus. On the other hand, an abnormally low inorganic phosphorus value in the blood is rather positive indication of disturbed phosphorus nutrition.

The amount of phosphorus excreted daily in the urine of an adult is from 0.8-1.3 g, with an average of about 1.1 g (2).

Increased alkalinity in the presence of food calcium, magnesium and iron, may cause the precipitation of insoluble phosphates in the feces (3).

In view of the extremely complicated body metabolism, it would be presumptous to consider phosphorus imbalance as the only possible causal factor in arthritis. Phosphorus assimilation and chemical behavior are known to be intimately related to the action of vitamins, enzymes, and hormones. Nevertheless, in the absence of other accompanying diseases and under normal hygienic and nutritional conditions, phosphorus deficiency might well be the determining factor.

So extremely vital is the behavior of phosphorus to the very maintenance of life itself, that any serious shortage must be made up by taking the necessary phosphorus from other less vital tissues. This withdrawal, "mining" or shifting of phosphorus from other parts of the body, is the basic idea underlying the suggested explanation of certain types of arthritis. Unlike vitamin deficiencies that are corrected by diet or vitamin suppliments, a phosphorus deficiency if not corrected in similar manner will force the body to extract the phosphorus needed to sustain life from other parts of the body itself. Such a process, conceivably may go on for a long time before the victim becomes aware of it. Even if weakening effects have been produced, they are naturally attributed to other causes. Not until positive pain is felt in muscles and joints does the person become anxiously concerned. Actual damage then already has occurred. When accompanied by painful swelling the conditions may be diagnosed as rheumatoid arthritis. If the phosphorus extraction has gone so far that it is tapping the richest phosphorus reserve—cartilage, bone and joint—it may then be diagnosed as degenerative osteo-arthritis.

Some have suggested that arthritis may result from diminished enzyme and hormone activity as people, particularly women, grow older. Even if this should prove to be true, it does not preclude the possibility that the enzymes and hormones that themselves contain active phosphorus may not suffer damage from the phosphorus withdrawal.

It is commonly assumed that standard balanced diets provide all necessary phosphorus. But such an assumption is no more justified than in the case of the vitamins, amino acids, and minerals, that now are regularly supplimented in diets. We do not wait until the patient has developed beri beri before giving him Vitamin B_1. Nor should we wait until he is suffering from painful and crippling arthritis before correcting phosphorus imbalance.

If, then, arthritis truly is a phosphorus deficiency disease, as it appears to be, this hypothesis should be subjected to proper clinical tests, but only by competent physicians working in close cooperation with expert biochemists, and expert dieticians. Only such a competent team would be able to deal with the complicated factors involved. They should be able to determine the best compounds for use as phosphorus suppliments, whether organic, inorganic, or both. Possible disturbances or side effects of such additions obviously should be investigated.

In advanced cases of degenerative osteo-arthritis, it is questionable if restoring phosphorus equilibrium could effect a complete cure. But even if the body repair mechanism could not restore damaged tissues, it would seem possible that the degenerative processes could be arrested and some measure of relief afforded.

In conclusion, the author hopes that this publicity of his hypothesis may stimulate prompt investigation to determine if it has any sound basis.

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TRANS-AXIAL COORDINATION OF HETEROCYCLIC AROMATIC AMINE N-OXIDES WITH OXOVANADIUM(IV) IONS

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ABSTRACT

The coordination of 4-substituted pyridine and quinoline 1-oxides trans to the vanadyl oxygen atom in several oxovanadium (IV) chelates has been investigated. Using an infrared procedure, variations in absorption attributable to vanadium-vanadyl oxygen stretching modes ($\bar{\nu}_{V-O}$) were recorded for adduct formation with bis (2,4-pentanedionato)oxovanadium(IV), bis(1-phenyl-1,3-butanedionato-oxovanadium(IV), and bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV). For the first two oxovanadium(IV) chelates, and in a variety of solvents, a linear correlation was established between the displacement of $\bar{\nu}_{V-O}$ upon adduct formation and a set of substituent parameters, σ_{PyNO}, defined in terms of ligand pK_{BH^+} values. (Bis(1,1,1-trifluoro-2,4-pentonedionato)oxovanadium(IV) apparently forms adducts with the N-oxide ligands, but the complex was insufficiently soluble to adequately investigate the variations of $\bar{\nu}_{V-O}$.

INTRODUCTION

The distorted square pyramidal structure of bis(2,4-pentanedionato)oxovanadium(IV) (1,2) bis(1-phenyl-1,3-butanedionato) (3) and presumably of bis(1,3-diphenyl-1,3-propanedionato) (4) and bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV) (5) are readily susceptible to the coordination of diverse donor molecules at the site opposite the vanadyl oxygen atom (trans-axial coordination) (5,8). This property has evoked numerous attempts to utilize bis(2,4-pentanedionato)oxovanadium(IV) as a reference acid in establishing a quantitative measure of base donor strengths (5, 6, 8). Carlin and Walker (5), for example, found a linear relationship between the stability constants for adducts of the oxovanadium (IV) chelate with several substituted pyridines and the respective ligand conjugate acid dissociation constants, K_{BH^+}.

As a continuation of work directed to evaluation of donor properties of substituted heterocyclic aromatic amine N-oxides toward oxovanadium(IV) ions (9), interactions of representative ligands with several bis(beta-diketonato)oxovanadium(IV) chelates were investigated. The results of an infrared investigation of adducts between bis(2,4-pentanedionato)-, bis(1,1,1-trifluoro-2,4-pentanedionato)-, and bis(1-phenyl-1,3 butanedionato)oxovanadium(IV) and a series of substituted pyridine and quinoline N-oxides are reported here.

EXPERIMENTAL

Preparation of starting materials.—Bis(2,4-pentanedionato)-, bis(1,1,1-trifluoro-2,4-pentanedionato)-, and bis(1-phenyl-1,3-butanedionato)oxovanadium(IV) were prepared by the neutralization of acidic solutions of oxovanadium(IV) sulfate in the presence of the respective *beta* diketones according to established procedures (10, 11). The resulting blue precipitates were collected, washed free of ionic impurities, dried, and recrystallized from benzene or toluene.

Bis(2,4-pentanedionato)oxovanadium(IV). Calculated for $VO(C_5H_7O_2)_2$: C = 45.30%, H = 5.32%. Found (12) C = 45.34%, H = 5.28%.

Bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV). Calculated for $VO(C_5H_4F_3O_2)_2$; C = 32.19%, H = 2.16%, F = 30.55%. Found (12): C = 32.26%, H = 2.30%, F = 30.27%.

Bis(1-phenyl-1,3-butanedioato)oxovanadium(IV). Calculated for $VO(C_{10}H_9O_2)_2$: C = 61.71%, H = 4.66%. Found (12): C = 61.73%, H = 4.61%.

4-Cyano- (13), 4-methoxy-(14), 4-methyl (14), 4-nitro- (14), and the parent pyridine 1-oxide (14), were obtained commercially. The 4-methoxy compound was recrystallized from an acetone-ether solvent prior to use. Pyridine 1-oxide was resublimed under reduced pressure and subsequently handled in a glove bag to reduce contamination by water from the atmosphere. The remaining N-oxides were employed as received. 4-Bromo- and 4-chloropyridine 1-oxides were prepared by treating the 4-nitro substituted compound with the approproiate acetyl halide and working up the resulting mixtures as outlined by Ochiai (15). 4-Carbomethoxypyridine 1-oxide was prepared via peroxide oxidation of the corresponding pyridine (16).

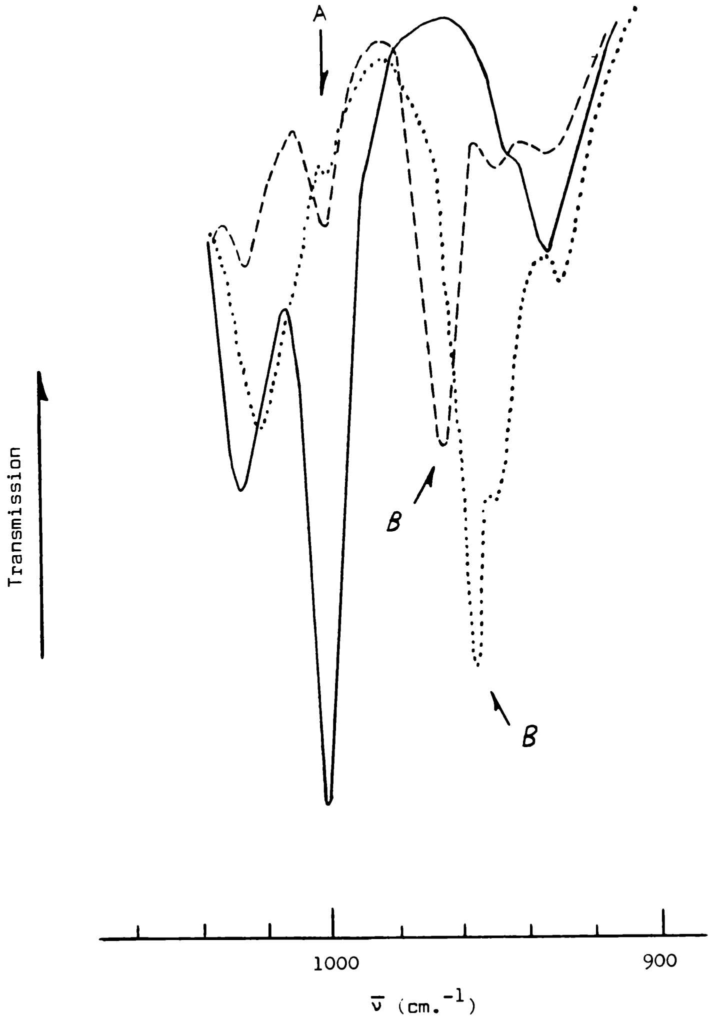
Quinoline, iso-quinoline and 4 methylquinoline 1-oxides were prepared by the oxidation of the corresponding nitrogen bases with hydrogen peroxide in glacial acetic acid as outlined by Ochiai (15). 4-nitro-, 4-chloro-, and 4-methoxyquinoline 1-oxides were likewise prepared by procedures outlined by Ochiai (15, 17).

Procedure for spectrophotometric studies.—Stock solutions of the appropriate oxovanadium(IV) compounds were prepared in diverse solvents. For each system investigated, successive portions of a base donor were added to one aliquot of the respective oxovanadium(IV) stock solution. The infrared and/or the visible spectra were then recorded for each solution after each addition. The formation of an adduct could thus be followed by the development of bands not previously observed in either the spectrum for the free chelate or that for the free ligand.

No decomposition of the oxovanadium(IV) adducts was noted over periods well beyond those necessary for recording of their spectra. Subsequently however, decomposition was indicated by the formation of dark residues and by changes in both the infrared and visible spectra of the solutions (18).

Infrared spectra were recorded on a Beckman IR-4 recording spectro-photometer equipped with sodium chloride optics or on a Perkin-Elmer model 421 grating spectrophotometer. Visible spectra were obtained on a Cary model 14 recording spectrophotometer.

Figure 1 — Infrared spectra of bis(2,4-pentanedionato)oxovanadium(IV) in CH_2Cl_2 (————) showing the effect of addition of approximately 1:1 mole ratio of 4-methyl- (........) and 4-chloro- (- - - - -) pyridine 1-oxides. $\bar{\nu}_{V-O}$ for unperturbed (A) and perturbed (B) bands are indicated.



RESULTS AND DISCUSSION

Displacements of the vanadium-vanadyl oxygen stretching band, $\bar{v}_{v-o}$, upon coordination of substituted pyridine 1-oxides with bis-(2,4-pentanedionato)oxovanadium(IV) in dichloromethane are illustrated in Figure 1. Addition of successive portions of a base brings about reduction in the intensity of band A, the unpreturbed value of $\bar{v}_{v-o}$, and concomitant increase in the intensity of the adduct bands indicated by B. Analogous spectral changes are observed for this system in the solvents acetonitrile, bromoform, and chloroform. Similar spectral changes are also recored for interaction of the oxovanadium(IV) chelate with substituted quinoline N-oxides in dichloromethane solution.

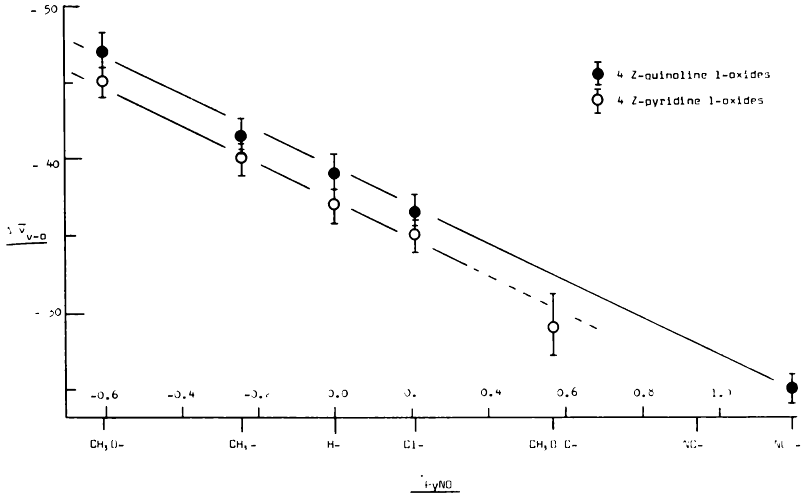
The transfer by a central metal atom of electronic perturbations induced by changes in a relatively distant substituent on a coordinated ligand molecule is not new. The vanadium-vanadyl oxygen moiety, for example, has been investigated as an "indicator group" with which to probe certain structural and bonding features for a series of oxovanadium(IV) complexes (20, 21, 22). However, the wide variety of complexes and respective molecular symmetries provided data compatible with only a series of rather qualitative correlations.

In contradistinction, the adducts of bis(2,4-pentanedionato)oxovanadium(IV) with 4-substituted pyridine 1-oxides and quinoline 1-oxides are found to manifest the "indicator function" in a more systematic manner. A linear correlation of $\Delta\bar{v}_{v-o}$ (defined as $\bar{v}_{v-o}$ for the N-oxide adduct minus $\bar{v}_{v-o}$ for the free Lewis acid) with a set of substituent parameters, σ_{PyNO} (23), has been established (24). This relationship is depicted in Figure 2. Larger negative values of $\Delta\bar{v}_{v-o}$ indicate a relative decrease in the vanadium-vanadyl oxygen "bond order" as the 4-substituent on the hetercyclic aromatic amine N-oxide ligand becomes more activating.

No points for the highly deactivator substituted 4-nitropyridine 1-oxide or 4-cyanopyridine 1-oxide are included in the correlation of Figure 2. Under the experimental conditions employed, no absorption bands assignable to $\bar{v}_{v-o}$ of the adducts were identified. Moreover, adduct formation with saturated solutions of 4-carbomethoxypyridine 1-oxide is just discernable by the infrared technique. Therefore a relatively larger uncertainty is indicated for the corresponding data point. The more negative pK_{BH^+} of 4-nitropyridine 1-oxide (-1.7) (25) and 4-cyanopyridine 1-oxide (-1.17) (26) compared to that of the 4-carbomethoxypyridine 1-oxide (0.41) (27) is in accord with the relative donor strengths suggested by the trend in observed values of $\Delta\bar{v}_{v-o}$. Limited solubility of the ligands in the solvents employed must surely also be an important factor contributing to lack of observable adduct formation.

Quinoline 1-oxides seem to react more completely with bis(2,4-pentanedionato)oxovanadium(IV). Bands attributable to the adduct

Figure 2. - Correlation of $\Delta \bar{\nu}_{v-o}$ values with σ_{PyNO} for bis(2,4-pentanedionato)oxovanadium(IV) adducts with substituted heterocyclic aromatic amine 1-oxides in dichloromethane solvent.



in the region expected for $\bar{\nu}_{v-o}$ became more intense in the presence of lesser relative amounts of quinoline N-oxide upon addition of successive portions of the solid ligands. A greater solubility of the ligands in the solvents employed is probably a major contributing factor. In the presence of a near saturated solution of 4-nitroquinoline 1-oxide, a clearly defined absorption band appears in the region expected for $\bar{\nu}_{v-o}$ which is not present in the spectrum of either the ligand or the chelate. This is contrast to observations for solutions of 4-nitropyridine 1-oxide in the presence of the oxovanadium(IV) chelate.

The relative stability of adducts between bis(2,4-pentanedionato)-oxovanadium(IV) and the heterocyclic aromatic amine oxides is not totally unexpected. A comparison of the corresponding ligand pK_{BH^+} values (Table I) indicated the 4 substituted quinoline 1-oxides are indeed stronger Lewis bases than the corresponding substituted pyridine 1-oxides. Hence, the greater displacement of $\bar{\nu}_{v-o}$ from values near 1000 cm.$^{-1}$ for the free oxovanadium(IV) chelate are explicable. The greater the interaction between the Lewis acceptor site and the ligand oxygen trans to the vanadyl oxygen, the greater the perturbation in the V-O bonding. The greater the perturbation in energy of the vanadium-vanadyl oxygen vibrational mode, the larger is the values of $\Delta \bar{\nu}_{v-o}$.

Support for the above qualitative statement derives from a consideration of the thermodynamic parameters associated with adduct formation with bis(2,4-pentanedionato)oxovanadium(IV). Splinter and Tobias (28) report values of 14±2 l/mole (IR) 11±4 l/mole.

(Optical) for the stability constant corresponding to the reaction in chloroform.

$$VO(PD)_2 + PyNO \longrightarrow PyNO\ .\ VO(PD)_2$$

$$K_{eq} = \frac{[PyNO\ .\ VO(PD)_2]}{[VO(PD)_2]\ [PyNO]}$$

These are to be contrasted with values reported (5) for the stability of adducts with pyridine, (58 1/mole), 4 methylpyridine (140 1/mole) 3-methylpyridine, (65 1/mole) and quinoline (2 1/mole) in nitrobenzene. Corresponding values of $\Delta \bar{\nu}_{V-O}$ observed for adducts of the pyridine bases are -39, -48, and -47 cm^{-1} respectively, as compared to -37 cm^{-1} for the pyridine 1-oxide adduct, all recorded in dichloro methane. Calorimetric measurements provide enthalpies of adducts formation (5) of -7.4, -8.8, -8.1 and -4.3 kcal/mole for the nitrogen bases, respectively, in nitrobenzene solution. Similar measurements for the series of substituted pyridine 1-oxide adducts give values of ΔH ranging from -3 to -9 kcal./mole (29). It is premature at this time to discuss further the relative stabilities of the N-oxide adducts of bis(2,4-pentanedionato)oxovanadium(IV). This must await the results of equilibrium measurements now in progress.

TABLE I

REPRESENTATIVE VALUES OF LIGAND pK_{BH^+} FOR SUBSTITUTED PYRIDINE AND QUINOLINE 1-OXIDES

	Ligand $pk_{BH}+$	
Ligand Substituent (Z)	4-Z-Pyridine 1-oxide[a]	4-Z-Quinoline 1-oxide[b]
CH_3-	1.29	1.44
H-	0.79	0.86
Cl-	0.36	0.47
NO_2-	-1.7	-1.39

[a] Reference 25.

[b] R. Whyman, D. B. Copley and W. E. Hatfield, J. Am. Chem. Soc., *89*, 3135 (1967).

Plots of $\Delta \bar{\nu}_{V-O}$ values are shown in Figure 3 for the N-oxide adducts of bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV) and bis(1-phenyl-1,3-butanedionato) oxovanadium(IV).

4-substituted pyridine 1-oxides appear to be ineffective in displacing solvent molecules from the trans-axial site of bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV) in acetonitrile solution. In less basic solvents, however, adduct formation with the N-oxides does occur, as is suggested by the data of Figure 4. The displacement of optical absorption maxima indicated are similar to those observed for solutions of bis(2,4-pentanedionato)oxovanadium(IV) upon formation of N-oxide adducts. Enthalpies for adduct formation between bis(1,1,1-trifluoro-3,4-pentanedionato)oxovanadium(IV) and

Figure 3.—Variation of $\Delta\,\bar{\nu}_{V-O}$ with σ_{PyNO} for adducts of bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV) {$VO(TFPD)_2$} and bis(1-phenyl-1,3-butanedionato)oxovanadium(IV) {$VO(\varphi, BD)_2$} with 4 substituted pyridine 1-oxides.

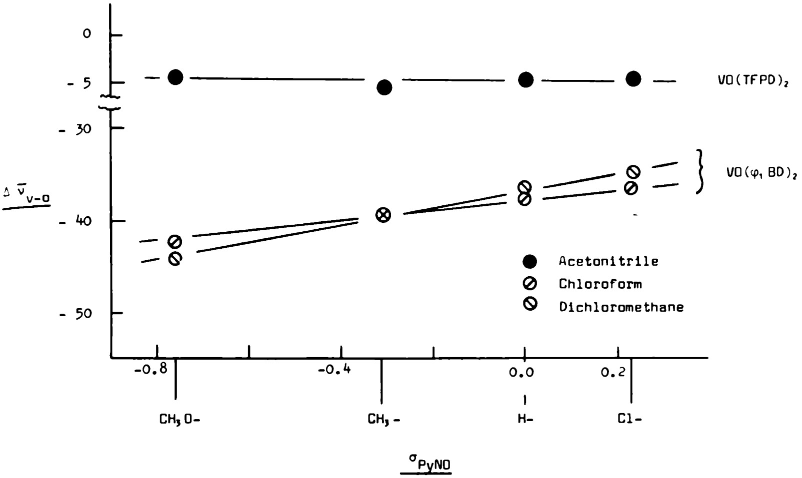
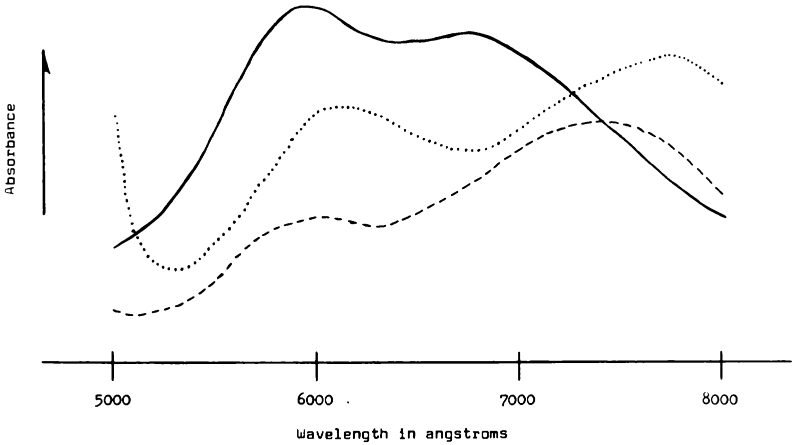


Figure 4. Optical absorption spectra of bis(1,1,1-trifluoro-2,4-pentanedionato)-oxovanadium(IV) in dichloromethane (————) with added acetonitrile (- - - - -) and 4 methylpyridine 1-oxide (........).



pyridine, 3-methylpyridine and 4-methylpyridine have been measured (15). The value -8.8, -8.5, and -10.2 kcal/mole, respectively, suggest a stronger degree of interaction, although infrared data on

$\bar{\nu}_{v-o}$ suggests just the opposite. Unfortunately, the trifluoromethyl substituted compound has only limited solubility in the halocarbon solvents useful for infrared investigation of $\bar{\nu}_{v-o}$. Thus the effects of adulct formation as portrayed in values of $\Delta\ \bar{\nu}_{v-o}$ could not be unequivocally investigated. The results of further optical measurements, however, will be the subject of a future correspondence.

Bis(1-phenyl-1,3-butanedionato)oxovanadium(IV) is more readily soluble in the solvents used than is bis(1,1,1-trifluoro-2,4-pentanedionato)oxovanadium(IV), but not as soluble as bis(2,4-pentanedionato)oxovanadium(IV). Thus the assignment of $\bar{\nu}_{v-o}$ for the N-oxide adducts of the phenyl substituted chelate are less certain than those for the parent oxovanadium(IV) chelate. Of interest is the observation that pyridine 1-oxide ligands readily form adducts with the phenyl substituted ligand even in acetonitrile solution. The apparently anomalous behavior of the trifluoromethyl substituted compound toward formation of adducts with the pyridine 1-oxides is probably related to the electronic distortions introduced by the pressence of the highly electronegative fluorine atoms. A decreased electron density on vanadium results which in turn allows an increase in strength of the vanadium-vanadyl oxygen bond relative to that expected upon adduct formation. Rather pronounced changes in the carbonyl and carbon-carbon double bond stretching frequencies are noted for copper (II) and nickel (II) complexes with beta-diketones when a methyl group is replaced with a trifluoromethyl group (30). On the other hand, substitution of a phenyl group has only a slight effect on these same bonds. The change in $\bar{\nu}_{v-o}$ upon replacing a methyl group with a trifluoromethyl group in bis(beta-diketonato)oxovanadium(IV) acids is very pronounced ($\bar{\nu}_{v-o}$ shifts from about 999 cm^{-1} to 930 cm^{-1} for spectra recorded from potassium bromide disks (9) and Nujol mulls or to 972 cm^{-1} in acetonitrile). Substitution of a phenyl group has, as before, little effect ($\bar{\nu}_{v-o}$ occurs at 995 cm^{-1} for spectra recorded from potassium bromide disks or Nujol mulls and at 999 cm^{-1} in dichloromethane).

In the presence of substituted quinoline 1-oxides, absorption bands in the region expected for $\bar{\nu}_{v-o}$ of adducts with bis(1-phenyl-1,3-butanedionato)oxovanadium(IV) are broad and highly structured. For this reason, data on these complexes are not included in Figure 4. It may be that steric interaction between the larger ligand molecules and the phenyl substituents of the chelate ring is taking place to such an extent that trans-axial association with the oxovanadium(IV) ion is hindered. Optical spectrophotometric work now in progress should clarify this point.

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SORPTION OF H_2O VAPOR AND HYDROGEN ⇆ DEUTERIUM EXCHANGE ON 4-CHLORO-3,5-DIMETHYLPHENOL-FORMALDEHYDE HEPTAMER[1]

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ABSTRACT

Adsorption and desorption isotherms of water vapor on 4-chloro-3,5-dimethylphenol-formaldehyde heptamer are measured at 25° and 35°, which demonstrated some slopes in the region of monomolecular layer sorption. The total monolayer sorption was approximated to be 1.25 moles of H_2O per mole of heptamer, for both adsorption and desorption. The differential heats at the beginning of adsorption and at the end of desoprtion, were found to be 17 and 30 kcal/mole, respectively. These values decrease rapidly to the value of water condensation as the amount sorbed approaches one mole. The results indicate the presence of only one active phenolic hydroxyl group per linear heptamer and may lead to a clarification of the structural and chemical properties of phenolic polymers. The H⇆D exchange as determined by successive adsorption of D_2O or H_2O vapor followed by complete desorptions on the solid sample under vacuum at 10^{-7} mm demonstrated complete reversibility of ~ 7 labile hydrogens of the hydroxyl groups of the heptamer illustrating a precise method to check structure of phenol-formaldehyde polymers.

INTRODUCTION

Surface studies of high polymeric compounds attracted many researches in a variety of branches of science especially where the clarification of physico-chemical properties might be related to molecular structure.

Gravitational sorption method by means of high vacuum technique was used in this laboratory in similar studies on a number of natural and synthetic high molecular weight compounds including proteins (1-3), humic acid from lignite (4-6), starch[2] and poly-L-glutamic acid (7,8).

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[1] This work was supported by a North Dakota State University Research Grant.

[2] In preparation for publication.

The results of those studies showed that the high polymeric compounds with large surface areas, which was achieved through lyophilization, used as the adsorbents interacting with a particular vapor or gas adsorbate, have demonstrated isotherms containing a number of slopes in the total monomolecular region. The appearance of slopes was concluded to be related to the kinds of functional group-sites present in the adsorbent polymeric compound. Therefore, it seems possible to determine functionalities by proper choice of adsorbate after sorption isotherms are established with the highest precision. This information on complex compounds is not readily obtained by titrimetric or spectroscopic methods. It was established that to get the proper resolution the isotherms should be determined at different temperatures. Furthermore, as the result of successive adsorption-saturation of D_2O, followed by complete desorption, all the labile hydrogen could be exchanged for deuterium (1,2,7) or vice versa. These data may reveal information on the molecular structure of compounds as well as to provide possible information on the mechanism of reactions.

For the purpose of specific studies it was suggested to extend them to model compounds. Poly-L-gutamic acid (7,8) and some of its derivatives (still under investigation) were the first model compounds studied in which the characteristic functional groups ($-NH_2$,-COOH and -CO-NH-) were confirmed by the appearance of slopes in the isotherms. Furthermore, it was established that the adsorptivity of H_2O vapor and exchangeability of labile hydrogen by deuterium are of the same magnitude on this compound.

In this paper is reported results of similar investigations on a model linear phenol-formaldehyde polymer, namely, the linear 4-chloro-3,5-dimethylphenol-formaldehyde heptamer.

EXPERIMENTAL

Apparatus and Procedure. The quartz helical balance having a sensitivity of 1.498 mg/mm, thermostated at 40° by circulating water in the surrounding jacket, was extended by a glass thread and attached to a sample pan. The sample was also thermostated by another circulating water jacket to maintain the desired temperature. The sample compartment is attached to the vacuum system consisting of a cold-trap and mercury diffusion and mechanical vacuum pumps, providing for pressures down to 10^{-7} mm for the desorption of samples.

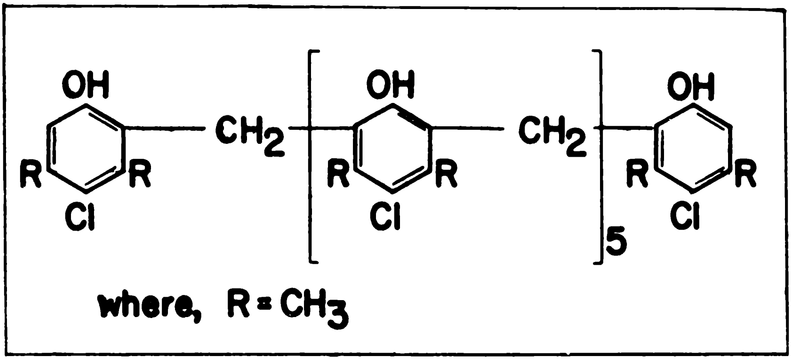
A mercury manometer sealed to the vacuum system permits reading the pressure, using a cathetometer, to a precision of ± 0.005 mm. A McLeod gauge was used for the measurement of very low pressures in the case of desorption. Weight changes in a sample, during desorption and adsorption, were obtained by the use of a traveling microscope with a precision of ± 0.002 mm. The reference point was the position of the sample at complete desorption (dry weight).

The general procedure was to determine the dry weight of the sample after it had been desorbed during a prolonged period of time (36-48 hours) at 10^{-7} mm of pressure at 25° or 35° until it reached a constant weight. The system was then closed and the sample was exposed to increments of vapors (H_2O or D_2O) for adsorption at a particular temperature. Each increment of exposure to vapor was maintained until equilibrium was reached at which pressure and increase in weight (or decrease in weight in the case of desorption) were recorded as functions of time. A plot of the weight sorbed vs. equilibrium pressure up to saturation, or experimentally permissible pressure, represents the isotherm .

The procedure for hydrogen-deuterium exchange was as follows: a completely dry (desorbed) sample was exposed to D_2O (or H_2O) vapor at approximately 20° until it adsorbed a maximum. The temperature was then raised to 35° and the sample was completely desorbed. The increase in weight due to the exchange of deuterium for the labile hydrogen present in the sample was determined. Repeated adsorptions of D_2O, followed by complete desorptions cause increase in the weight which were plotted versus the sum of the amounts of adsorbed D_2O (or H_2O for the reverse exchange).

Materials.

1. *4-chloro-3,5-dimethylphenol-formaldehyde heptamer* is a linear polymer of the following formula:



The compound was synthesized by Peterson, Guilbert and Burke using the corresponding trimer and 2,6-bis (hydroxymethyl)-4-chloro-3,5dimethylphenol which was then purified from pyridine-water (9). The molecular structure of the heptamer was confirmed by elemental analysis, nuclear magnetic resonance spectroscopy and infraded spectroscopy. A sample of this model heptamer was used without any pretreatment of any kind. The heptamed was desorbed to a constant weight.

2. *Water.* Triple distilled water was additionally redistilled many times in the vacuum system before use for sorption-saturation.

3. *Deuterium Oxide.* K and K Laboratories, Inc., Plainview, N.Y. (99-99.8% purity). The D_2O was redistilled two or three times in the vacuum system before use for sorption-saturation.

RESULTS AND DISCUSSION

a. *Determination of dry weight of sample.* Several original samples of 4-chloro-3,5-dimethylphenol-formaldehyde heptamer were used to determine weight loss and stability. Each sample was put in the sample pan attached to the helical spring balance and completely desorbed under vacuum for a long period of time at pressure of 10^{-7} mm and at different temperatures. The original desorption caused the average loss of weight in the samples at 50° was 3.2%.

Readsorption of H_2O vapor up to 4-4.5% followed by complete desorption at 35° caused a further total loss of weight; $\sim$ 0.050%. Repetition of similar operations (adsorption-desorption of H_2O) caused only the negligible loss 0.002-0.0008%/hour in the range of 25-35°respectively, which was taken into account.

Therefore, it was concluded that the original total loss of weight, $\sim$ 3.25%, was probably due to residual solvent, the presence of volatile monomers, air and moisture. The further loss may be due to sublimation.

The completely desorbed samples of 4-chloro-3,5-dimethylphenol-formaldehyde heptamer weighed about 210 mg, which were used for the determination of sorption isotherms of H_2O vapor at 25° and 35°.

b. *Adsorption and desorption of H_2O vapor at 35°.* Using this procedure, the adsorption and desorption isotherms were determined. Typical data of the amounts sorbed, in moles per mole of heptamer versus the equilibrium pressure, are represented by curves 2 and 2a in Figure 1. From this figure it is evident that the shape of the isotherms are of sigmoid type, which began as the curvatures adjoining the slopes of increased equilibrium pressures; two in adsorption and three in desorption isotherms. There is remarkable hysteresis in the desorption case in the range of equilibrium pressures investigated. An extension of the final slopes to the saturation pressure of H_2O at 35° for the determination of the total monolayer amount of sorption (1,4) gives $\sim$ 1.15 mole of H_2O per mole of heptamer for desorption but for adsorption it is much lower (not shown in the figure). This may indicate that the upper slope in the adsorption isotherm is not the final one.

c. *Adsorption and desorption of H_2O vapor at 25°.* The desorbed sample was then saturated with H_2O vapor at the lower temperature and completely desorbed at 25°. It should be mentioned that the reproducible isotherms are obtained only if the sample is resaturated and compltely desorbed at the isothermic temperature. This was also observed with the natural polymers (1,4).

Employing the same procedure, adsorption and desorption isotherms of H_2O vapor at 25° were determined and are shown by curves 1 and 1a respectively. The type of isotherms are the same,

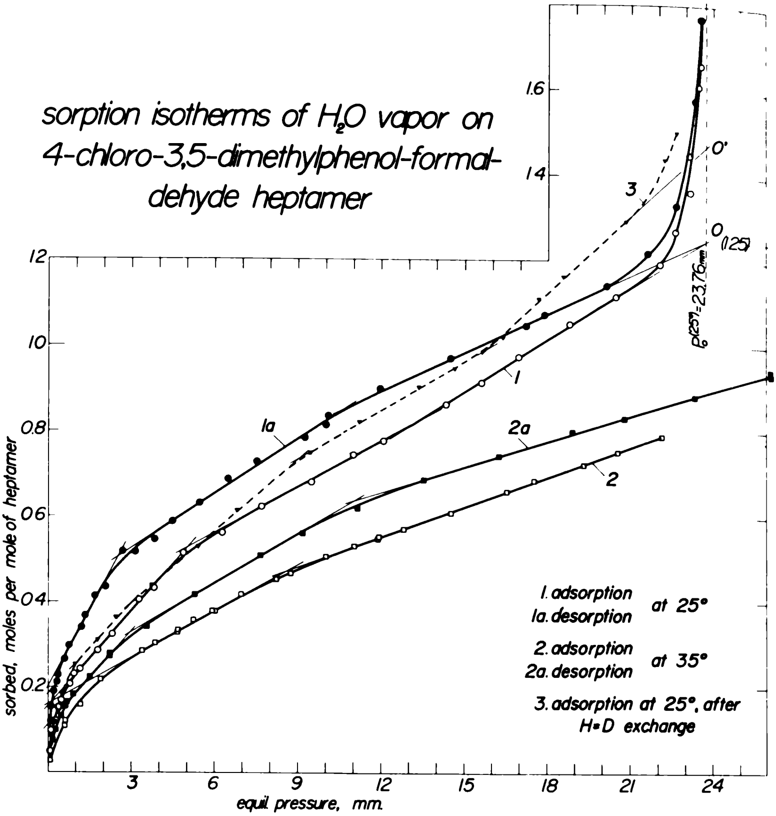


FIGURE 1—See text.

however they run at higher amounts of sorption. The hysteresis in desorption is also observed. Now, since at this temperature it was possible to approach more closely the region of the saturation pressure at H_2O at 23.76 mm, it is evident that the third upper slopes are the final slopes in total monolayer sorptions. It is also interesting that their extension to the saturation pressure coincide at the same point O, which represents the amount of total monolayer sorption, equal to 1.25 moles of H_2O per mole of heptamer. Experimentally, both isotherms, before reaching the saturation pressure, turn into the multilayer-condensation region of the amount sorbed. This was observed also in earlier investigations (1-6).

Appearance in the isotherms of 2-3 slopes, obtained by approximation, could be exhibited also by rough smooth curves containing only definite final slopes. This may indicate the presence of 2-3 kinds of functional group-sites or sorption at the same kind of sites but in three stages. The last case is suggested as the most probable. 4-Chloro-3,5-dimethylphenol-formaldehyde heptamer contains only phenolic hydroxyl groups (-OH) which may be coupling with molecules of H_2O. In this case it may be expected, in the total monolayer, that at least seven H_2O will sorb. In reality, sorption reached only slightly more than one H_2O (1.25) per molecule of heptamer. This may indicate that only one OH-group is responsible for sorption, possibly only one end group hydroxyl.

Now, why should the isotherms appear to exhibit three slopes or stages of sorption? This might be due to the presence of other functionalities within the molecule. It is known that the presence of aromatic rings in a molecule increases the so-called lyophobic nature of the whole molecule. There may be a kind of shielding of the OH-groups from the H_2O by the aromatic rings.

The one end-group OH (the one at the right in the formula) may be imagined as being highly ionized and not being involved in intramolecular hydrogen bonding. If this is the case, it may, at least to some degree, clarify a mechanism of condensation. This hopefully could be proved by further investigations, which are underway, using nuclear magnetic resonance spectroscopy to study the hydrogen-deuterium exchange.

d. *Differential heats of adsorption and desorption of* H_2O *vapor in range of 25 to 35°.* Isothermic data of adsorption and desorption at 25 and 35° were used for calculation of differential heats of sorption by the Clausius-Clapeyron equation assuming the vapor to behave as an ideal gas. Naturally, data are relative, especially at very low pressures, never-the-less serving good for comparison between adsorption and desorption as shown in Figure 2.

The relatively high initial heat for adsorption, around 17 kcal/mole, rapidly dropped to 12.5 kcal/mole at 0.2 mole of H_2O adsorbed per mole of heptamer. It rises slightly up to 13 kcal/mole at 0.5 mole H_2O adsorbed. With further adsorption, the heat approaches the value for the condensation of H_2O.

As desorption of H_2O proceeds, the heats of desorption become remarkably high. The highest measured heat of desorption occurs when 0.15 mole of H_2O is still present per mole of heptamer. The value at this point is about 30 kcal/mole, indicating strong bonding between the H_2O and the heptamer. At higher contents of H_2O, the heats of desorption become less and finally approach the heat of condensation of water, $\sim$10.5 kcal/mole.

The hysteresis effect noted in desorption, accounting for the difference in the heats of adsorption and desorption, is suggested as being due to a high energy of activation in the desoprtion process.

e. *Hydrogen⇆deuterium exchange on heptamer.* After completion of the isotherms the sample was completely desorbed and the

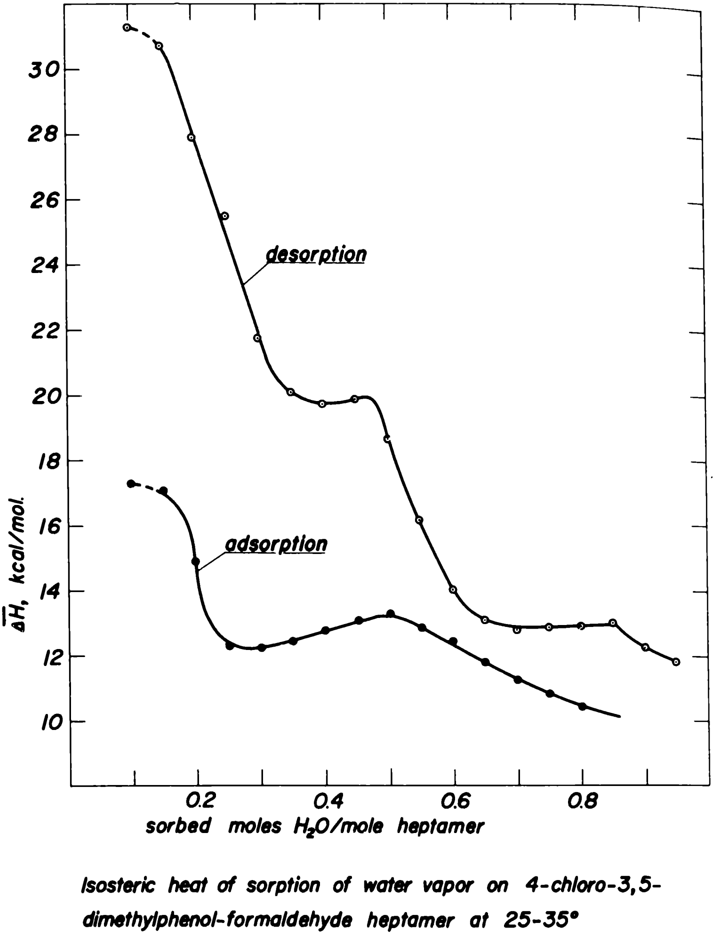


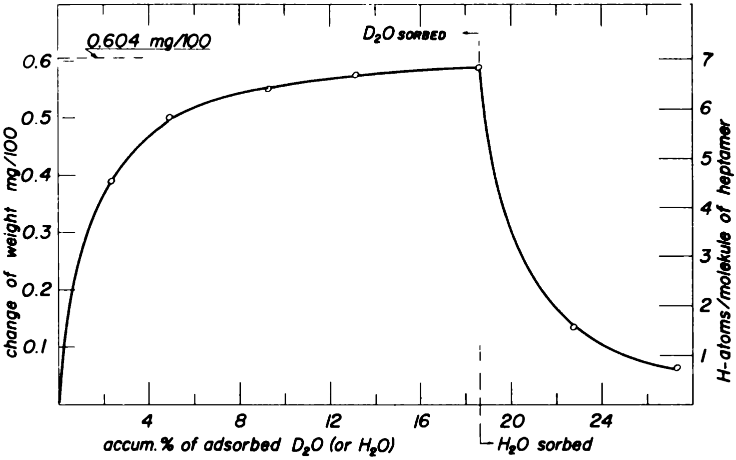
FIGURE 2—See text.

dry-weight was rechecked. It was then exposed to the deuterium oxide vapor for adsorption-saturation. During adsorption of D_2O it is expected that some exchange of labile hydrogen in OH-groups with deuterium from D_2O will occur according to the following equation:

$$\underbrace{R\text{-}(OH)_7}_{\text{original heptamer}}+nD_2O \longrightarrow \underbrace{R\text{-}(OD)_7}_{\text{deuterized heptamer}}+7DOH+n\text{-}7\ D_2O$$

As a result of this exchange reaction, the weight of deuterized heptamer increases by a difference in attomic weights, D-H=1.0066 (1). This increase in weight was recorded. Further successive readsorption-saturation with D_2O vapor, followed by complete desorption at

10^{-7}mm of pressure, were conducted. The data of $H \leftrightarrows D$ exchange are presented in Figure 3.



Changes in the weight of 4-chloro-3,5-dimethylphenol-formaldehyde heptamer due to Hydrogen⇄Deuterium exchanges occuring during succesive adsorptions, followed by complete desorptions at 35°

FIGURE 3—See text.

The first operation of saturation-desorption resulted in an exchange of ∼ 60% of theoretical. The amount of D_2O adsorbed was only 3%. This amount of D_2O could provide for a maximum of 30% exchange. The remaining 30% of exchange must have occurred through collision with D_2O vapor molecules which surround the sample and which is present in an amount 8-10 times greater than the amount actually adsorbed on the sample. A similar observation has been established previously by W. Hnojewyj in exchange studies on poly-γ-benzyl-L-glutamate. From these it can be concluded that the exchange process is of a statistical nature.

The five successive operations of adsorption followed by complete desorption was enough to achieve practically 100% exchange of the seven labile hydrogens present in the heptamer by deuterium (see Figure 3). The exchange is also reversible as it was tested by further adsorption-saturation of H_2O vapor, followed by complete desorption, and shown as continuation of the same curve presenting change of weight of heptamer versus accumulative % of sorption in Figure 3.

f. *General remarks.* It is not surprising that on this model compound the sorptive ability is poor but, on the other hand, that it exhibited excellent exchangeability. In previous works on proteins it was found, for most of the proteins investigated, the high exchangeability meant high adsorptivity. It is, however, not the case for this model compound. Therefore, it is again suggested that presence of so many aromatic rings resulted in poor adsorptivity but did not disturb the exchangeability. This was also shown to be the case in work with some monomers (10).

After reverse hydrogen-deuterium exchange on the heptamer it was noted that there was some increase in the adsorption of H_2O. This adsorption isotherm is shown by curve 3 (dashed line) in Figure 1. Extrapolation of the upper slope to the saturation vapor pressure (point O') represents the adsorption of $\sim$1.48 moles of H_2O per mole of heptamer. This result may indicate that during many operations of adsorption - desorption and hydrogen - deuterium exchange some additional active sites may become available for sorption of H_2O.

CONCLUSION

This investigation has shown that 4-chloro-3,5-dimethylphenol-formaldehyde heptamer is adsorbing only about one H_2O molecule in total monolayer region in range of 25-35°. The labile hydrogens of this heptamer are easily exchangeable by deuterium from adsorbed as well as surrounding vapor of D_2O. This technique concerning the exchangeability may be entended for structural studies to unknown complex compounds.

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NORTH DAKOTA FLEAS. I. *HYSTRICHOPSYLLA* FROM GRAND FORKS COUNTY

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INTRODUCTION

In his comprehensive volume on Siphonaptera of western North America, Hubbard (1947) called attention to the scarcity of published flea records for North Dakota. Furthermore, he suggested that the state would be a fertile area for the study of fleas. That observation appears to be as correct today as it did some 20 years ago. To the best of our knowledge only 11 species of fleas have been reported from North Dakota. The parasites are given in Table I along with host and locality records, and documenting references.

The Roman numeral "I" in the title of this paper simply reflects our optimism concerning future collections and reports of North Dakota fleas.

METHODS

During the fall of 1967, one of us (S.A.P.) received several fleas preserved in 70 per cent ethanol. These ectoparasites were taken from a *Clethrionomys gapperi* trapped on October 21, 1967, near Arvilla, in Grand Forks County. The fleas were bleached in 10 per cent potassium hydroxide, followed by standard methods of dehydration, clearing, and mounting.

RESULTS AND DISCUSSION

One of the female fleas collected from the red-backed vole was unusual in two respects, *i.e.*, its large size (5.5 mm in length) and and possession of a double spermatheca (Figures 1, 3). An appreciation of its size can be gained by comparing this specimen with the common cat flea, *Ctenocephalide felis* (Figure 2). According to Holland's (1957) revision of the genus *Hystrichopsylla,* this female is *H. dippiei dippiei* Rothschild. The flea has been deposited as No. 331 in the University of North Dakota Parasitology Collection.

In the same depository there is a slide bearing a male *Hystrichopsylla* approximately 4 mm in length (UND Parasit. Coll. No. 202). Based on the morphology of sternum IX with its slightly oblique apex (Figure 4), this specimen also fits the subspecific characteristics of *H. dippiei dippiei.* According to the slide label, the flea was taken from a chloroformed bumblebee nest near Inkster, Grand Forks County, on July 30, 1963, by Dr. G. C. Wheeler. One can speculate on the presence of a flea in such a location but it probably reflects the parasite's abandonment of a frightened and retreating rodent. It is well known that fleas leave dead hosts, but Stark and Kinney (1962) have shown that abondonment also occurs when rodents are

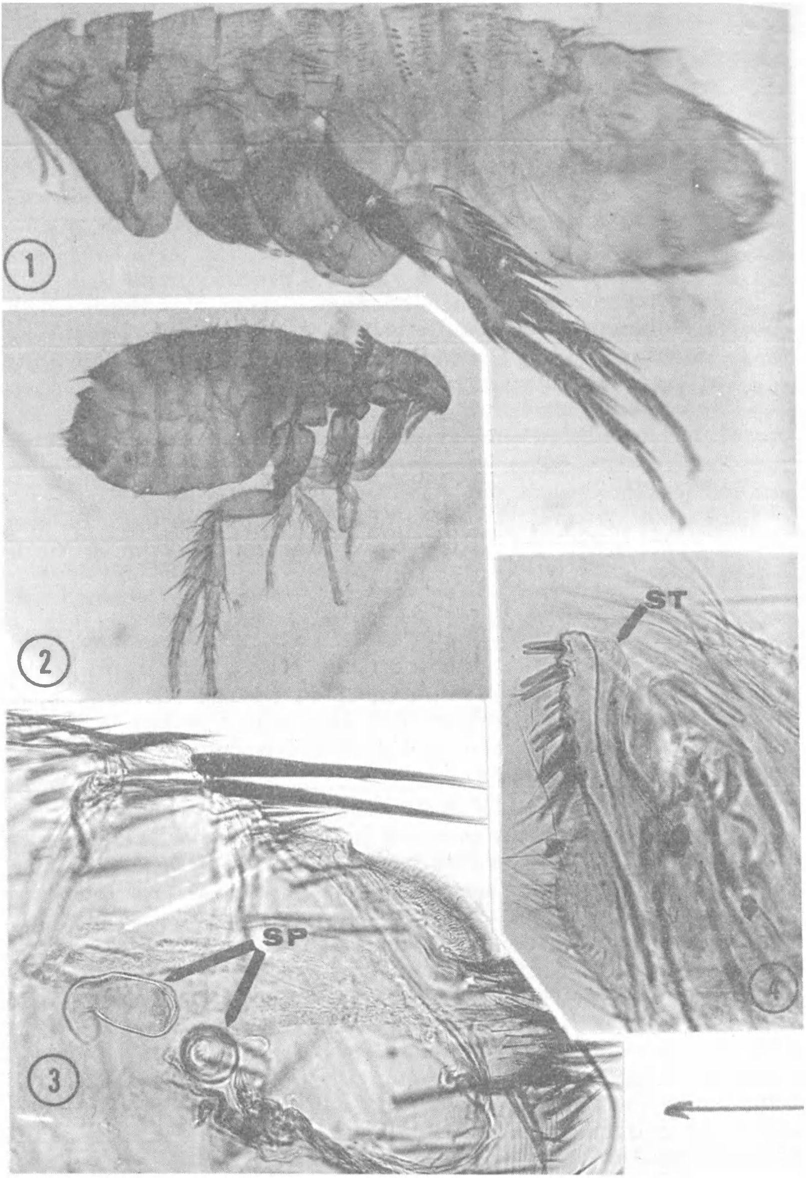

FIGURE 1—*Hystrichopsylla dippiei dippiei*, female. X18.
FIGURE 2—*Ctenocephalides felis*, female. X18.
FIGURE 3—*H. d. dippiei*, female, showing double spermatheca (SP). X91.
FIGURE 4—*H. d. dippiei*, male, showing apex of sternum IX (ST). X91.

TABLE I

PUBLISHED RECORDS OF NORTH DAKOTA FLEAS

Parasite	Host	Locality	Reference
DOLICHOPSYLLIDAE			
Foxella ignota albertensis (Jordan & Rothschild)	*Mustela l. longicauda*	McHenry Co.	Jordan (1937)
	No host recorded	Ward Co.	Hubbard (1947)
Opisocrostis t. tuberculatus (Baker)	*Citellus franklini*	McHenry Co.	Jordan (1937)
	C. r. richardsoni	Divide Co.	Prince (1943)
Opisocrostis labis (Jordan & Rothschild)	*C. r. richardsoni*	Divide Co.	Prince (1943)
Oropsylla rupestris (Jordan)	*C. r. richardsoni*	Divide Co.	Prince (1943)
Thrassis bacchi (Rothschild)	*C. r. richardsoni*	Divide Co.	Prince (1943)
	C. r. richardsoni	Rolette Co.	Prince (1943, 1944)
	C. tridecemlineatus	Rolette Co.	Prince (1943)
HYSTRICHOPSYLLIDAE			
Epitedia wenmanni (Rothschild)	*Mustela l. longicauda*	McHenry Co.	Jordan (1937)
Neopysylla inopina Rothschild	*Citellus franklini*	McHenry Co.	Jordan (1937)
Hystrichopsylla d. dippiei Rothschild	No host recorded	Rolette Co.	Holland (1957)
	Clethrionomys gapperi	Grand Forks Co.	(this paper)
PULICIDAE			
Cediopsylla inaequalis inaequalis (Baker)	*Lepus townsendi campanius*	SW North Dakota	Voth & James (1966)
Hoplopsyllus affinis (Baker)	*L. t. campanius*	SW North Dakota	Voth & James (1966)
Pulex irritans Linnaeus	*L. t. campanius*	SW North Dakota	Voth & James (1966)

frightened, excited, or otherwise disturbed. There was no indication that the bumblebee nest was inhabited by rodents at the time of the collection, nor any evidence that the flea was alive at the time of anesthesia (Wheeler, personal communication).

This appears to be the second report of this flea in North Dakota (Table I). The previous record (Holland, 1957) failed to note the host. In general, members of this genus are found infrequently on their hosts since they are nest-dwellers, but even there they occur in low numbers (Holland, 1957).

Host records indicate that *H. dippiei dippiei* parasitizes small rodents, especially *Citellus, Microtus,* and *Peromyscus.* Although this flea has been reported recently as far east as New Brunswick (Brown, 1968), it appears to be more typical of the prairie and Rocky Mountain areas of Alberta, Saskatchewan, Montana, and Wyoming. To the best of our knowledge, our report constitutes a new eastward extention of the known range of this parasite in the United States, and one of the more easterly records within the continent.

ACKNOWLEDGEMENTS

We wish to thank Mr. William Harper, undergraduate major in wildlife management, for donating to the Biology Department those fleas collected from *Clethrionomys gapperi.* We are also indebted to Dr. William L. Jellison, Hamilton, Montaña, for examining our specimens of *Hystrichopsylla dippiei dippiei.*

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ASPECTS OF THE DETERMINATION OF THE pH OF MARSH WATERS

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INTRODUCTION

This study was undertaken to determine the reliability of pH indicating systems in the measurement of the pH of prairie marsh waters.

It is generally difficult to carry sensitive portable battery-operated pH meters in the field with the other equipment needed in wildlife research. Battery operated instruments are readily jarred out of alignment and are adversely affected by dust and by accidental immersion in water or mud. The breakage of electrodes or other electronic components at remote sites where laboratory standardizing equipment and chemicals are not available also causes problems. Therefore, field biologists are interested in the use of convenient indicator papers and indicator dye-color comparators for the determination of the hydrogen ion concentration of prairie marsh waters.

The accuracy and theoretical significane of nonelectrometric pH determination has been discussed by Bates (1964) and Ellis *et al.* (1948). Frank (1953) reports that indicator papers show no major deviation from true pH values when dealing with well-buffered solutions. Poorly buffered systems are misread by several pH units.

METHODS

The glass electrode pH meter was used as the standard in this study for the evaluation of indicator papers and indicator dye-color comparators. Fifty-one water samples were collected from various types of prairie wetlands within a 50-mile radius of Jamestown, North Dakota. These wetlands ranged from permanent spring fed lakes to temporary pools that were the result of snow melt and runoff from spring rains. Dominant vegetation ranged from predominantly emergent species of cattails (*Typha*) and bulrushes (*Scirpus*) to predominantly submerged species of pondweeds (*Potamogeton*).

For 36 samples the pH was immediately determined at the collection sites with pH paper (pHydrion) and with a battery powered electrical pH meter (Beckman N-2). The pH of the other 15 samples was determined in the field with the battery powered electrical pH meter alone. All samples were brought to the laboratory and preserved within 4 to 6 hours after sampling, with the addition of chloroform at the rate of 5 ml per liter. The pH was again determined in the laboratory with a line powered pH meter (Beckman E-76) and with a mechanical pH Color Comparator-Indicator Dye Kit (Hach 17-N) utilizing a wide range indicator dye. Also, the paper values were spot-checked in the laboratory.

RESULTS

Table I presents a comparison of preliminary pH data obtained by glass electrode meter and indicator dye-color comparator from water samples collected at 15 marsh sites. Table II presents the comparison of pH data obtained from 36 sites using the above two methods plus indicator paper.

TABLE I

COMPARISON OF ELECTRICAL AND CHEMICAL pH DETERMINATIONS

Site	Meter	Comparator	Difference
1	9.3	9.5	(+)0.2
2	8.5	8.5	0.0
3	8.1	8.0	(−)0.1
4	8.4	8.5	(+)0.1
5	8.0	8.0	0.0
6	8.1	8.0	(−)0.1
7	8.7	9.0	(+)0.3
8	8.1	8.0	(−)0.1
9	9.4	9.5	(+)0.1
10	8.0	8.0	0.0
11	8.7	9.0	(+)0.3
12	8.6	8.5	(−)0.1
13	8.5	8.5	0.0
14	8.6	8.5	(−)0.1
15	8.5	8.5	0.0
Avg.	8.5	8.5	0.0
Range	8.0-9.4	8.0-9.5	(−)0.1 to (+)0.3

DISCUSSION

The data from both the battery powered and line powered electrical meters agreed within 0.05 pH units, showing an average pH of 8.45 with a minimum pH of 7.5 and a maximum pH of 9.7. Hence, these values are listed in a single column in the tables. Data from the mechanical comparator also shows an average pH of 8.45, with a minimum pH of 7.5 and a maximum pH of 10.0. Determinations by means of paper indicate an average pH of 8.1, with a minimum pH of 6.5 and a maximum pH of 10.5. The comparator showed a maximum range difference of one pH unit from the electrically determined pH while the paper determinations resulted in a maximum range difference of 3.4 units.

Thus, for water quality surveys on the northern prairie, a me-

TABLE II

COMPARISON OF ELECTRICAL, MECHANICAL AND PAPER pH DETERMINATIONS

				Differences from Meter	
Site	Meter	Comparator	Paper	Comparator	Paper
16	7.8	7.7	9.0	(−)0.1	(+)1.2
17	9.0	8.5	8.3	(−)0.5	(−)0.7
18	9.0	9.0	8.0	0.0	(−)1.0
19	8.3	8.0	8.6	(−)0.3	(+)0.3
20	8.7	9.0	6.5	(+)0.3	(−)2.2
21	8.1	8.0	8.5	(−)0.1	(+)0.4
22	8.0	8.0	8.5	0.0	(+)0.5
23	8.3	8.5	6.8	(+)0.2	(−)1.5
24	9.0	8.7	8.5	(−)0.3	(−)0.5
25	8.5	8.5	8.0	0.0	(−)0.5
26	8.0	8.0	8.3	0.0	(+)0.3
27	8.6	9.0	8.5	(+)0.4	(−)0.1
28	8.0	8.3	7.7	(+)0.3	(−)0.3
29	8.1	8.0	8.0	(−)0.1	(−)0.1
30	7.6	7.5	8.5	(−)0.1	(+)0.9
31	7.5	7.6	6.8	(+)0.1	(−)0.7
32	8.9	9.3	8.5	(+)0.4	(−)0.4
33	8.5	9.0	8.5	(+)0.5	0.0
34	9.7	10.0	10.5	(+)0.3	(+)0.8
35	7.9	8.0	7.0	(+)0.1	(−)0.9
36	8.8	8.7	9.2	(−)0.1	(+)0.4
37	9.2	9.5	9.0	(+)0.3	(−)0.2
38	8.6	9.0	8.5	(+)0.4	(−)0.1
39	7.6	7.5	7.5	(−)0.1	(−)0.1
40	8.1	8.0	7.0	(−)0.1	(−)1.1
41	8.4	8.5	6.5	(+)0.1	(−)1.9
42	8.2	8.0	7.5	(−)0.2	(−)0.7
43	7.9	7.8	6.8	(−)0.1	(−)1.1
44	8.0	8.0	8.0	0.0	0.0
45	8.3	8.0	7.6	(−)0.3	(−)0.7
46	7.9	8.0	8.2	(+)0.1	(+)0.3
47	8.2	8.0	7.2	(−)0.2	(−)1.0
48	8.6	8.5	8.7	(−)0.1	(+)0.1
49	8.6	8.5	8.5	(−)0.1	(−)0.1
50	8.5	8.5	8.5	0.0	0.0
51	8.5	8.5	9.2	0.0	(+)0.7
Avg.	8.4	8.4	8.1	0.0	(−)0.3
Range	7.5-9.7	7.5-10.5	6.5-10.5	(−)0.5 to (+)0.5	(−)2.2 to (+)1.2

chanical pH color comparator-indicator dye kit is of sufficient accuracy and has immense practical value for field use or for operations in isolated sites from a base camp where repairs to more complicated equipment would present a problem. Its dimensions are such (e.g., 6 1/2 x 5 1/2 x 1 1/2) that it can be easily carried in a pack or even in one's pocket.

SUMMARY

A mechanically operated color comparator-indicator dye kit is of sufficient accuracy and low cost to warrant its adoption instead of indicator papers or electical meters for the determination of the pH of alkaline marsh waters in the field. It also has the advantage of being easy to carry and capable of withstanding rough handling or adverse field conditions.

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PRIMARY PRODUCTION AND CHEMICAL AND PHYSICAL CHARACTERISTICS OF LAKE ASHTABULA RESERVOIR, NORTH DAKOTA

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INTRODUCTION

This study describes the chemical and physical characteristics of the water of Lake Ashtabula, a reservoir on the Sheyenne River, North Dakota, and attempts to relate these to the standing crops and production rates of phytoplankton.

Upon completion of the Garrison Diversion project, irrigation waters from Lake Sakakawea (Garrison Reservoir) will flow through about 8 existing North Dakota reservoirs, including Lake Ashtabula. Data reported here will provide a basis for assessing changes these irrigation waters may have upon the reservoir.

There are 18 reservoirs having a usable capacity of 5,000 acre-feet or more in North Dakota (Martin and Hanson, 1966). For the most part, studies of the quality of their waters have not been made. A

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[1] M.S. Candidate, 1967.

study of Lake Sakakawea was included by Neel, Nicholson and Hirsch (1963) in their work on the effects of 6 Central Missouri River mainstream reservoirs on water quality. The North Dakota Game and Fish Department has done some work on Lake Ashtabula, especially in relation to stocking with fish and determining seasonal fishing conditions. Tubb, Copes and Johnston (1965) listed species of fish in the Sheyenne River and Lake Ashtabula. The U.S. Geological Survey has made sporadic chemical and physical measurements in Ashtabula and the Sheyenne River (U.S.G.S. 1954-63; 1964-66).

Lake Ashtabula (Figure 1) spreads northward behind Baldhill Dam, located 8 miles northwest of Valley City in Barnes County, North Dakota, in the NW1/4 Sec. 18, Twp. 141 N., R. 58W, and is described by Wells (1959). The dam was built and is operated by the U.S. Army Corp of Engineers. Storage began 30 July 1949. The dam is an earth-fill structure 500 m long and 17.3 m high. The normal pool depth at the head of the dam is 12.1 m and the deepest part of the reservoir, slightly over 15.1 m, is in the old river channel. Water is normally released from the reservoir from 8.5 m below the surface at normal pool. The reservoir decreases in depth about 0.19 m/km in the upstream direction.

The length of the reservoir at normal full pool is 43.5 km and its maximum width is 0.96 km. Storage capacity is 0.0873 km^3 (70,700 acre-ft) and maximum capacity is 0.1437 km^3 (116,500 acre-ft). The surface area of the reservoir at the normal pool level is 2197.5 ha (5,430 acres). The drainage area of the Sheyenne River above the dam is 10,717.5 km^2 (4,138 miles^2).

A 10-20 m-wide band of submergent vegetation, consisting of largely *Potomogeton* species, was found along the shores of the lake from the dam to Station 4. *Potomogeton pectinatus* and *P. natans* were common species at all stations. *Potomogeton perfoliatus* was common at Station 1 but sparse at the other stations. During the summer phytoplankton were predominantly blue-green with *Aphanizomenon* always being a major component. The diatom *Stephanodiscus* was prominent in early spring and late fall.

METHODS

Chemical and physical measurements were taken from April 1966 to April 1967. Phytoplankton production was measured from June to December 1966 and from May to June 1967. Most measurements were taken at Station 2 located 4.3 km from the dam (Figure 1). Occasional samples were taken at Stations 1, 3 and 4 located 0.8, 16.4 and 24.1 km from the dam to detect possible variations in conditions.

Water temperatures were measured with a portable thermistor unit. Daily maximum-minimum air temperatures were obtained from records of the dam-tender, U.S. Corps of Engineers. Water transparency was measured with a standard 20.0 cm Secchi disc.

A Van Dorn 2-liter sampler was used to collect water samples. Chemical analyses of samples included measurements of pH with an

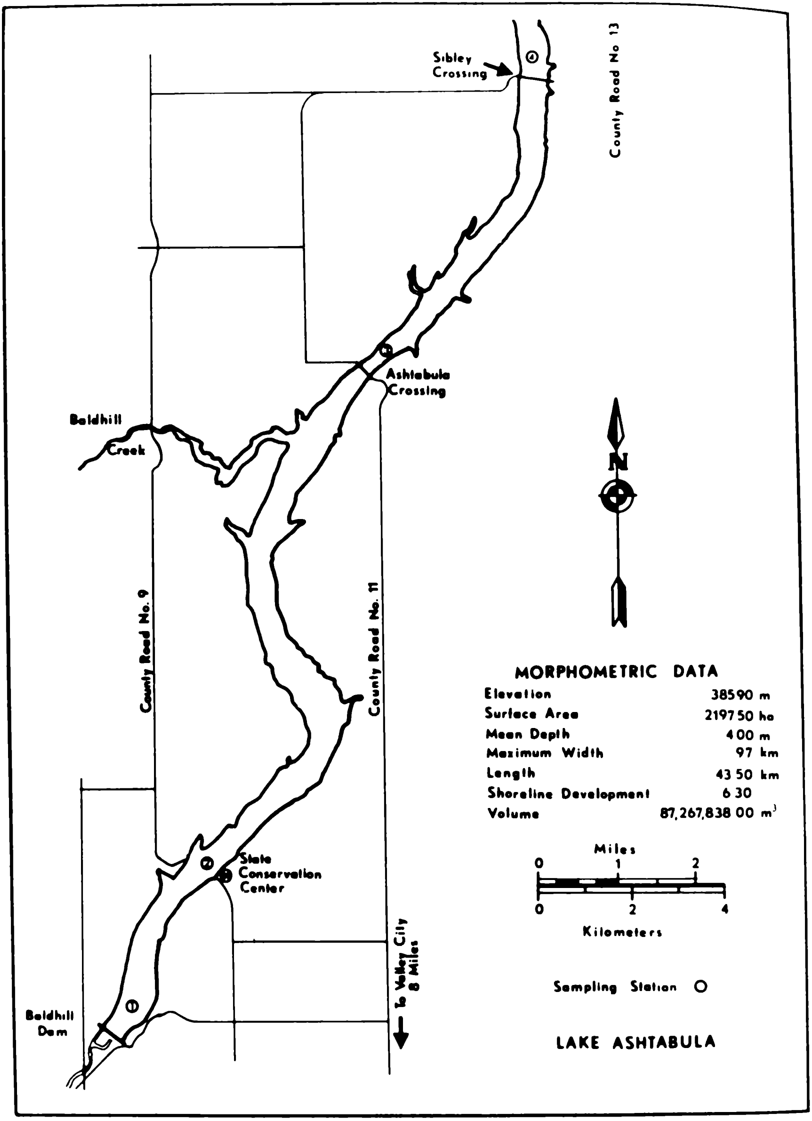

FIGURE 1—Map of Lake Ashtabula showing locations of the 4 principal sampling stations.

Instrumentation Laboratories pH meter; dissolved oxygen, using the azide modification of the Winkler method (American Public Health Association, 1965); and alkalinity, using 0.02 N sulfuric acid and phenolphthalein and methyl orange as indicators (A.P.H.A., 1965). Total phosphate, sulphate, ammonium nitrogen, nitrate nitrogen and nitrite nitrogen were determined with a Bausch and Lomb Spectronic 20 from procedures and chemicals of the Hach Chemical Company of Ames, Iowa.

Total dissolved solids were determined by filtering 50 ml of lake water through a No. 42 Whatman filter paper and evaporating the filtrate from a crucible at 103°C (A.P.H.A., 1965).

Primary productivity of phytoplankton was measured by the light-and-dark-bottle oxygen change method. For each 1-m depth, three glass-stoppered 250 ml B.O.D. bottles were filled with water collected with the Van Dorn sampler. One bottle was used to measure the original concentration of dissolved oxygen. The other two, one clear and one dark, were clipped to a chain attached to a buoy anchored at the sampling station and suspended at the depth from which the water was taken. The dark bottles were made by wrapping black adhesive tape over aluminum foil-covered bottles which were then pointed with white lacquer to limit heat absorption. The necks and tops were covered with aluminum-foil caps.

The bottles were usually suspended from 0900 to 1500 hr on clear and partly-cloudy days. During periods of intense phytoplankton production, they were suspended for only 3-4 hr to prevent formation of oxygen bubbles that appeared in the clear bottles suspended at the surface or at the 1-m depth.

Gross production of photosynthesis, expressed as g O_2/m^2 per day, refers to total oxygen produced, including that due to phytoplankton respiration, for a 1 m^2 column of water extending from the surface to the bottom of euphotic zone, for a 24-hr period. Net production is total oxygen produced, less that due to respiration of organisms within the dark bottles. Phytoplankton standing crops, expressed as mg chlorophyll "a"/liter, were measured in 1967 samples by the method of Odum (1958). During 1966, 1-liter water samples were filtered through a No. 40 silk plankton net and re-filtered through the Millipore membrane, pore size 0.45 μ. The initial filtration through the plankton net caused a loss of smaller plankton, so a correction factor of 1.3 was determined to adjust the 1966 chlorophyll determinations to actual chlorophyll content.

Statistics reported as significant were at the 0.05 level.

RESULTS

Except for periods of a few days when stratification occurred, water temperatures were uniform from top to bottom during ice-free periods (Figure 2), indicating that winds, which averaged 10 mph, caused the relatively shallow waters of the reservoir (mean depth, 4m) to mix freely.

Water temperatures could be accurately predicted during ice-free periods from mean air temperatures (Figure 3). The equation expressing this relationship was: $Y = -5.60 + 1.04X$, where Y is the surface water temperature in °C and X is the 10-day mean air temperature. Air temperature explained 92% of the variation in water temperature.

During periods of ice-cover with low releases of water from the lake, warmer water temperatures occurred near the bottom than near the surface (Figure 2). A colder layer of water was recorded near the bottom at Station 2 on 27 February 1967. This layer apparently resulted because large amounts (320 acre-ft/day) of water were drawn out of the reservoir beginning 10 February, causing colder water from the upper reservoir to move along the bottom at Station 2. A warm water layer of 5° C occurring from 10 to 20 March 1967 at depths ranging from 2-5 m, with cooler water above and below may have been the result of unusually warm weather during 1 to 4 and 9, 10 March.

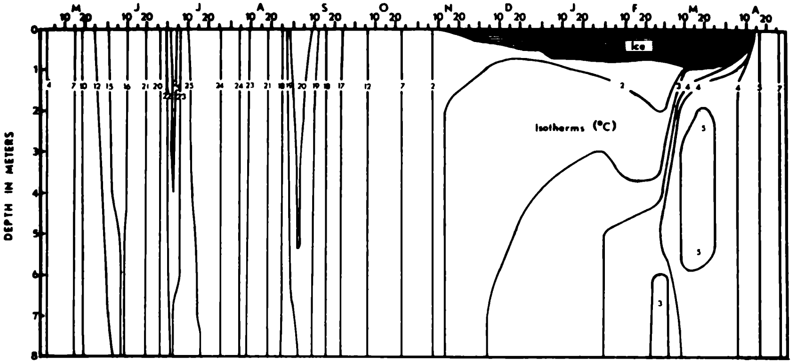

FIGURE 2—Water temperatures at Station 2 from May 1966 to April 1967.

Transparency depths at Station 2 ranged from 0.5-3.8m and were affected by both silt and density of phytoplankton. Silt was important during high water flows, particularly during the spring and following storms with strong winds. The significant correlation coefficient between Secchi disc measurements and chlorophyll content was -0.397 for all data combined, demonstrating that phytoplankton standing crop had some influence upon transparency. Transparency values at Station 4 were generally less than 1 m, increasing to average values of 2 m near the dam, as silt, carried into the reservoir by the river, gradually settled out (Figure 3).

Alkalinity concentrations, expressed as total $CaCO_3$, ranged from lows of 140-160 ppm at all water depths in April-May 1966 and April 1967, to highs of 330 and 440 ppm at the surface and bottom, respectively, in March 1967 (Figure 4). We attributed this increase in alkalinity from spring to just before the ice melted to four probable

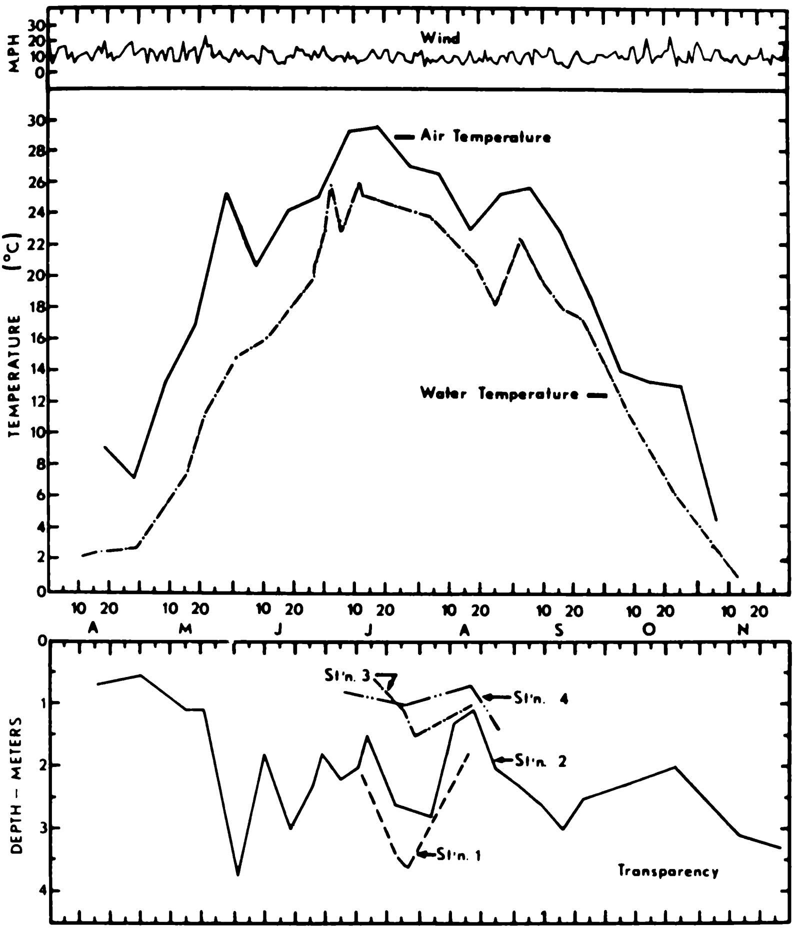

FIGURE 3—Mean daily wind velocity, air-water temperature at Station 2 and Secchi disc transparency from 1 April to 30 November 1966.

causes: 1) Incoming water from the major tributaries of Baldhill Creek and the Sheyenne River was high in alkalinity during summer and fall. For example, alkalinity averaged 270 ppm at Station 3 and 190 ppm at Stations 2 and 1 during July and August 1966. 2) In summer, evaporation rates were high and releases of water from the reservoir were low. 3) Year-round decomposition activities, particularly during ice-cover, caused resolution of precipitated carbonates. 4) In winter, ice formation resulted in a concentration of solids. A decrease in alkalinity resulted in the spring when large volumes of melt-water, low in mineral content, entered the reservoir. The exchange rate (volume of water entering the reservoir divided by the volume of water of the reservoir at normal pool) during April 1967 was 1.06 and the alkalinity decreased from 340 to 150 ppm during this month.

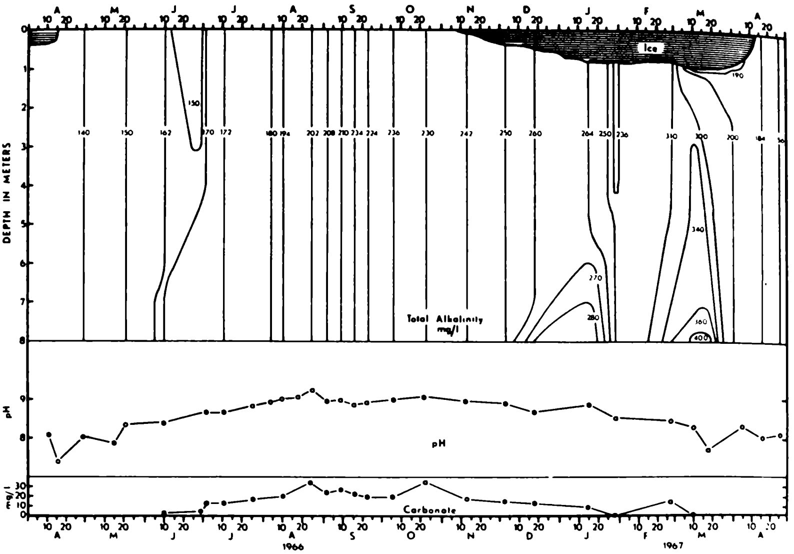

FIGURE 4—Total alkalinity, pH, and surface carbonate at Station 2 from April 1966 to April 1967.

In general, fluctuations of sulfate, ammonia, nitrite, nitrate, total phosphate and dissolved solids followed those of alkalinity with low concentrations following spring run-off and highest concentrations being reach just before ice break-up (Figures 5, 6). Concentrations of ammonia, nitrite, nitrate and phosphate tended to fluctuate considerably during ice-free periods. No significant correlations were found between the fluctuations of any nutrient and rates of gross photosynthesis.

The pH values were lowest during April and May 1966 when they ranged from 7.5-8.3, then gradually reached highs of 8.8-9.2 from 25 June 1966 to 15 January 1967 when they began gradually decreasing to approximately 8.0 in March and April 1967 (Figure 4). High pH levels during June to mid-January probably resulted from photosynthetic activity removing CO_2 from the water, causing precipitation of $CaCO_3$ (marl) and formation of hydroxides.

Dissolved oxygen concentrations, ranging from 6.4-13.9 ppm at

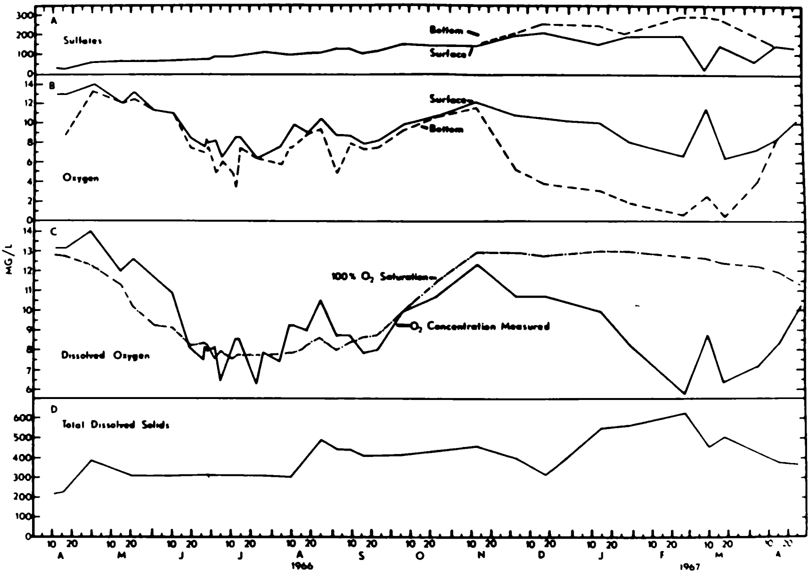

FIGURE 5—Sulfates, dissolved oxygen and total dissolved solids from April 1966 to April 1967. The broken-line in part C indicates oxygen concentrations at which the surface water would be saturated; the solid line indicates oxygen measurements at the surface.

Station 2 during ice-free periods, were nearly uniform from top to bottom, reflecting the constant mixing of the water (Figure 5). Supersaturated oxygen levels often occurred during April through September 1966, indicating intense photosynthetic activity of phytoplankton (Figure 5). During ice-cover, oxygen concentrations dropped rapidly, reaching lows of 8.2 ppm at the surface on 28 January 1967 and 0.4 ppm at the bottom on 27 February 1967.

Phytoplankton standing crop and production.—Standing crops in the euphotic zone at Station 2 from 14 July 1966 to 30 June 1967 ranged from 0.0-43.0 μg chlorophyll/liter, with peaks of 43.0, 38.0, and 41.0 μg chlorophyll/liter occurring on 11 to 18 August, 6 October 1966 and 27 June 1967, respectively (Table I). No chlorophyll was detected from late December 1966 to early May 1967. The average standing crop of 23.6 μg chlorophyll/liter in the euphotic zone was nearly the same as the average standing crops of 22.9 μg chlorophyll/liter from surface to bottom during 14 July to 3 December 1966, reflecting the fact that the water of the lake was freely circulating and that phytoplankters were distributed throughout the water column. Although average standing crops were generally uniform at all depths, they were occasionally greater or smaller in the euphotic zone than in the

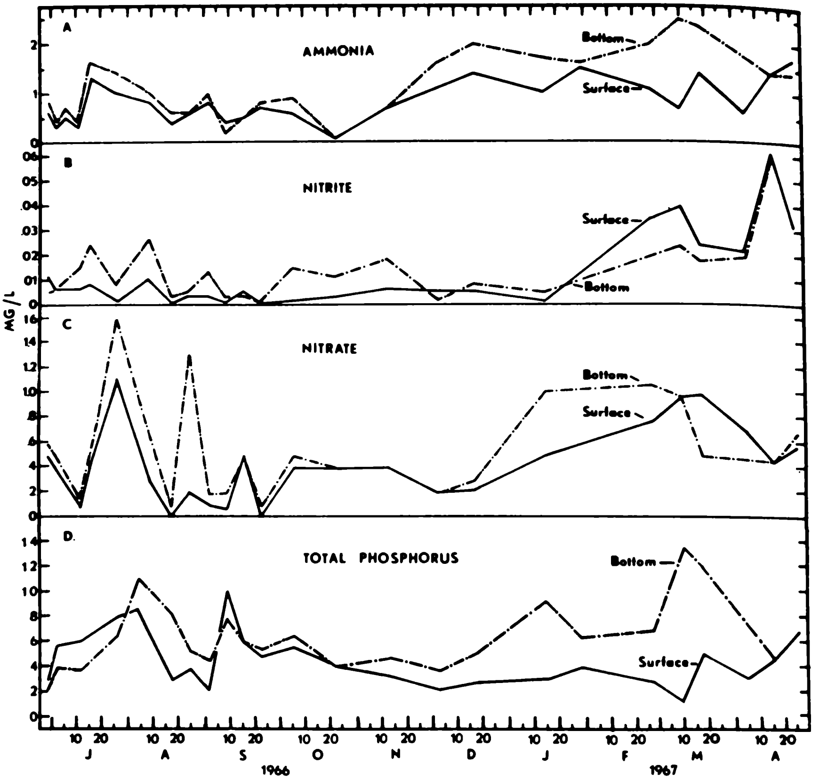

FIGURE 6—Ammonia-, nitrite-, and nitrate-nitrogen, and total phosphate at Station 2 from June 1966 to April 1967.

dystrophic zone. These variations of standing crops with depth resulted when changes in wind velocities caused fluctuations in water stratification and when active growth or death phases of populations of phytoplankton influenced the buoyancy of phytoplankton cells.

Gross photosynthesis of the euphotic zone at Station 2 from June to December 1966 and from May to June 1967 ranged from 0.1-3.6 g O_2/m^3 per day; respiration ranged from 0.1-1.5 g O_2/m^3 per day (Table I). Average gross photosynthesis of 0.13 mg O_2/liter per hr (range 0.04-0.26 mg O_2/liter per hr) in the euphotic zone from May to December (the 8-month period from May to June 1967 and June to December 1966) was 40.7% of the average optimal photosynthesis of 0.32 mg O_2/liter per hr (range 0.05-0.85 mg O_2/liter per hr). Optimal photosynthesis was the maximum rate of photosynthesis and occurred at either 0 or 1 m depths. An average assimilation ratio of 4.3g C/g chlorophyll per hr was obtained by dividing average optimal

TABLE I.

PHYTOPLANKTON STANDING CROP, PHOTOSYNTHESIS, AND RESPIRATION RATES IN THE EUPHOTIC ZONE OF LAKE ASHTABULA.

	1966															1967										
	June		July		August				September			October		Nov.	Dec.	May		June								Average
	27	28	12	14	4	11	18	25	2	16	23	6	23	13	3	13	21	1	5	9	17	23	27	29	30	
Phytoplankton:																										
ug chlorophyll/L surface to bottom				16.2	13.1	41.6	42.9	32.6	26.5	16.1	11.8	35.6	21.7	9.1	7.8											22.9
ug chlorophyll/L euphotic zone				21.0	16.0	43.0	43.0	26.0	29.0	13.0	12.0	38.0	27.0	7.8	7.8	1/	10.5	10.7	10.7	9.0	14.0	32.0	41.0	37.0	35.0	23.0
Gross Photosynthesis:																										
mg O_2/L/hr	0.11	0.12	0.22	0.22	0.12	0.25	0.26	0.20	0.17	0.11	0.14	0.13	0.10	0.05	0.02	0.05	0.09	0.10	0.10	0.04	0.05	0.21	0.13	0.13	0.11	0.13
gm O_2/m^2/day	7.77	7.97	13.88	13.49	7.70	17.86	18.19	13.52	11.17	6.63	8.51	5.95	4.31	1.38	0.61	2.32	4.99	5.47	5.12	2.98	4.20	18.60	11.99	10.59	9.06	8.57
ug O_2/ug chlorophyll/hr				10.5	7.2	5.8	6.0	7.5	5.8	8.2	11.7	3.4	3.8	6.4	2.2	1/	8.6	9.4	8.7	4.5	3.6	6.5	3.2	3.5	3.2	6.2
Black-Bottle Respiration:																										
mg O_2/L/hr	0.06	0.02	0.08	0.04	0.03	0.08	0.08	0.05	0.06	0.03	0.04	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.08	0.02	0.03	0.03
gm O_2/m^2/day	6.48	1.92	8.16	3.60	2.40	2.88	9.12	9.12	6.48	3.12	4.80	0.60	0.84	0.00	0.00	0.48	0.79	0.91	1.08	0.79	0.79	3.67	11.35	2.62	3.24	3.41
Optimum Photosynthesis:																										
mg O_2/L/hr	0.26	0.27	0.40	0.46	0.35	0.74	0.85	0.50	0.24	0.35	0.51	0.26	0.45	0.09	0.06	0.15	0.20	0.19	0.16	0.05	0.10	0.54	0.20	0.24	0.28	0.32
ug O_2/ug chlorophyll/hr				21.9	21.9	17.2	19.8	19.2	8.3	26.9	42.5	6.8	16.7	11.5	7.7	1/	19.0	17.7	14.9	5.6	7.1	16.9	4.9	6.5	8.0	15.28

1/ no measurement made

photosynthesis by average chlorophyll standing crop and by using a photosynthetic quotient of 1.2 (Strickland, 1960) to convert oxygen values to carbon values. This ratio compares favorably with values reported by Odum (1959).

It is believed that winds shifted phytoplankton about so that average production was about equal in all areas of the lake. We therefore estimated average net production for the entire lake from May to December was 20.7 kg O_2/ha per day, assuming average respiration and photosynthesis rates at Station 2 were representative of the entire lake. When we converted the oxygen to carbon using a photosynthetic quotient of 1.2, net production for the entire lake was 6.5 kg C/ha per day. Using Ryther's (1956) estimate that the amount of dry organic matter was twice that of carbon, we determined that net production for the entire lake was 13.0 kg organic matter/ha per day (11.6 lbs organic matter/acre per day). The total net production for the lake from May to December was 3560 kg organic matter/ha (3176 lbs organic matter/acre). Using computations similar to these for net production, we estimated gross photosynthesis from May to December for the lake was 10,555 kg organic matter/ha (9,417 lbs organic matter/acre).

There was negative production from 13 November 1966 to 18 March 1967 since respiration rates then exceeded production rates. The rate of oxygen loss from the entire lake during that period, used to measure loss of organic material produced before formation of ice, was 2.5 kg organic matter/ha per day. The amount of organic material lost from 1 January to 31 March 1967 was estimated as 152 kg organic matter/ha or approximately 4.3% of the net production from May to December.

Gross photosynthesis and respiration rates generally increased as chlorophyll standing crops became larger (Table II, A). No explanation is presently available for the apparently low respiration rate of 2.5 g O_2/m^2 per day in the range of 30.1-40.0 mg chlorophyll/m^3. However, with larger standing crops ranging from 20.1-55.0, net production tended to remain rather constant at 6.1-6.4 g O_2/m^2 per day when the high net production rate in the range of 30.1-40.0 mg chlorophyll/m^3 was eliminated because of the apparently low respiration rate mentioned above. Although maximum production of 6.1-6.4 g O_2/m^2 per day was associated with larger standing crops, the efficiency of net to gross production was only 51-58%, as compared with the efficiency of 65-82% associated with lower standing crops of 5.1-20.0 mg chlorophyll/m^3. The average gross photosynthesis per gram chlorophyll decreased with increasing chlorophyll standing crops (Table II, B).

Although combining measurements from various seasons and a variety of environmental conditions caused considerable scatter in the data upon which the averages in Table II were based, there appeared to be a consistent trend of low standing crops having greater

TABLE II

A. COMPARISON OF CHLOROPHYLL STANDING CROP, GROSS PHOTOSYNTHESIS, RESPIRATION AND NET PRODUCTION IN THE EUPHOTIC ZONE OF LAKE ASHTABULA. VALUES COMPILED FROM 1966-1967 DATA[1]

Chlorophyll (mg Chlorophyll/m^3)			Gross Photosynthesis (g O_2/m^2/day)[2]	Respiration (g O_2/m^2/day)[2]	Net Production (g O_2/m^2/day)[2]
Range	Average	Number of Observations			
5.1-10.0	8.2	3	1.7	0.3	1.4
10.1-20.0	14.4	15	7.3	2.6	4.7
20.1-30.0	25.8	4	10.6	4.5	6.1
30.1-40.0	35.5	4	11.1	2.5	8.6
40.1-55.0	45.5	6	12.5	6.1	6.4

B. COMPARISON OF CHLOROPHYLL STANDING CROP WITH RATE OF OXYGEN PRODUCTION PER UNIT WEIGHT OF CHLOROPHYLL

Chlorophyll (mg Chlorophyll/m^3)			Average Gross Photosynthesis (mg O_2/mg Chlorophyll/hr)
Range	Average	Number of Observations	
5.1-25.0	14.5	18	8.6
25.1-35.0	28.6	5	6.4
35.1-55.0	46.2	9	5.2

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[1] includes data from July to November, 1967.

[2] to convert to g O_2/m^3/day divide values by 4.4 m which was the average depth of the euphotic zone.

efficiency of net to gross production and greater rates of production per unit chlorophyll than larger standing crops.

Factors affecting photosynthesis. — Since pH levels of the lake water were generally greater than 8, and free CO_2 is absent or extremely low at these levels, it was thought the lack of free CO_2 might limit photosynthesis. Wright (1960) found evidence of CO_2 limitation of photosynthesis when the density of the standing crop of phytoplankton was high in Canyon Ferry Reservoir, Montana. In Lake Ashtabula, there was a direct relationship of chlorophyll standing crop and photosynthesis to pH (Table III and Figure 7). The correlation between pH and chlorophyll was 0.325 and between pH and gross photosynthesis was 0.256. Both correlations were significant. The increase in pH with the increase in chlorophyll standing crop resulted from removal of CO_2 by phytosynthesis. The increase in photosynthesis with increase in pH suggests that the lack of CO_2 at high pH levels did not limit photosynthesis for if it had, there should have been a leveling-off of the pH-photosynthesis curve with increasing pH.

An attempt was made to determine the effects of various environmental factors upon photosynthesis. A multiple regression equation was calculated from 1966-67 data using gross photosynthesis as the dependent variable and average nitrate nitrogen, sulfate, total dissolved solids, total phosphate, total and phenolphthalein alkalinity, chlorophyll standing crop, water temperature and pH in the euphotic zone, and Secchi disc measurements as the independent variables.

Chlorophyll standing crop and water temperature were the only variables significantly influencing photsynthesis. The equation best expressing this relationship was $P = -2.92 + 0.23C + 0.38W$, where P is gross photosynthesis (g O_2/m^2/day) and C is μg chlorophyll/liter and W is water temperature in °C. Chlorophyll and water temperature together explained 65.1% of the variation in photosynthesis, with chlorophyll explaining 38.2% and water temperature 26.9% of the total.

Predicting photosynthesis. — Accurate daily photosynthesis predictions based on environmental factors cannot be made because these factors fluctuate unpredictably from day to day. However, monthly averages of these factors should reduce fluctuations, permitting their use in calculating photosynthesis. Therefore three methods were tested to predict monthly photosynthesis in the euphotic zone using various combinations of temperature, Secchi disc, chlorophyll standing crop and optimum photosynthesis measurements.

In method one, $P = F \times O \times D \times T$, where P = average gross photosynthesis for the month (gm O_2/m^2 per day); F = ratio of average to optimal gross photosynthesis for the month; O = average optimal photosynthesis for the month; D = depth of the euphotic zone in meters, estimated by establishing monthly ratios between

TABLE III

RELATIONSHIP OF pH WITH CHLOROPHYLL STANDING CROP AND GROSS PHOTOSYNTHESIS IN THE EUPHOTIC ZONE OF LAKE ASHTABULA. DATA FROM 1966-1967[1]

pH			Chlorophyll (mg/m^3)	Gross Photosynthesis ($g\ O_2/m^2/hr$)
Range	Average	Number of Observations		
8.2-8.5	8.5	5	10.0	.28
8.6-8.9	8.8	13	21.5	.61
9.0-9.3	9.1	10	36.2	.91

[1] includes data from July to November, 1967.

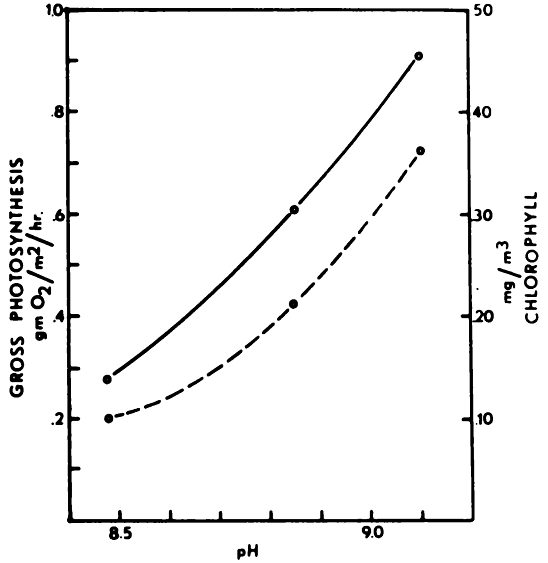

FIGURE 7—Relationship of pH with chlorophyll standing crop (broken line) and gross photosynthesis (solid line) in the euphotic zone of Lake Ashtabula.

Secchi disc readings and the actual depth of the euphotic zone measured from light-and-dark bottle experiments and then multiplying these ratios by the observed Secchi disc measurement; and T = average number of hours of sunlight for the month.

In method two, P = F_c x O_c x C x D x T, where F_c = ratio of average to optimal gross photosynthesis per weight of chlorophyll standing crop for the month; O_c = average optimal gross photosynthesis for the month (mg O_2/μg chlorophyll per hr); and C = average standing crop of chlorophyll for the month (μg chlorophyll/liter).

In method three, P = -2.92+0.23C+0.38W, where C = average standing crop of chlorophyll for the month (μg chlorophyll/liter); W = average water temperature in the euphotic zone in °C; and the constants were from the multiple regression equation.

All three methods resulted in greater predicted values than the actual measured average monthly photosynthesis (Table IV). Method one was best, with average percentage deviations of calculated from actual measured values of 9%, compared with 16 and 23% in methods two and three, respectively.

DISCUSSION

Phytoplankton standing crop and production rate data indicate that Lake Ashtabula is very productive. The average phytoplankton standing crop from May to December was 101 mg chlorophyll "a"/m^2

TABLE IV

ACTUAL MEASURED AND CALCULATED GROSS PHOTOSYNTHESIS (gm $O_2/m^2/day$). VALUES ARE AVERAGES FOR THE MONTH AND WERE FROM 1966-1967 DATA[1]

Month	Number of Samples	Average Measured Photosynthesis (g $O_2/m^2/day$)	Average Calculated Photosynthesis (g $O_2/m^2/day$)		
			Method[2] 1	Method[2] 2	Method[2] 3
June	10	7.2	7.6	9.0	9.6
July	7	9.4	10.6	10.2	9.5
August	8	11.9	11.7	12.6	11.7
September	5	8.9	11.2	12.6	10.6
October	2	5.1	5.1	5.1	8.1
Average		8.5	9.3	9.9	9.9

[1] Includes data from July to November 1967.

[2] See text for explanation of the methods.

or, by converting chlorophyll standing crop to dry organic matter (ash-free dry weight), 121 kg dry organic matter/ha (108 lb/acre), since 1.0 μg chlorophyll "a" equals 0.12 μg ash-free dry weight as determined by Wright (1959). The average standing crop of phytoplankton in Lake Ashtabula was about 3 times that of 32 mg total chlorophyll/m^2 reported for five Wisconsin lakes (McConnel and Sigler, 1959); about 0.2 the average standing crop of 450 mg chlorophyll/m^2 during July and August reported for a South Dakota sewage lagoon by Bartsch and Allum (1957); and about 1.5 times the average standing crop of 68 mg chlorophyll/m^2 during April to October in Canyon Ferry Reservoir, Montana, as computed from the data of Wright (1958, 1959).

The average gross photosynthesis from May to December was 2.2 g C/m^2 per day or 4.4 g dry organic matter/m^2 per day. For the entire year, assuming no photosynthesis from January to April, average gross photosynthesis was 1.4 g C/m^2 per day or 2.8 g dry organic matter/m^2 per day. Average annual photosynthesis in Lake Ashtabula is the same as that reported for a small (15 ha) lake in Denmark that received large quantities of purified sewage (Steeman Nielsen, 1955). In the Danish lake, production may have been limited by intense surface blooms of green algae restricting light penetration and by the development of pH levels greater than 10 having an "injurous effect upon phytoplankton."

Since the multiple regression analysis did not indicate any significant relationships of productivity with various macronutrients, particularly nitrogen and phosphorus, it is suggested that nutrients did not limit photosynthesis. Further evidence that nutrients were not limiting is that from June to October, phytoplankton populations, as measured by chlorophyll standing crops, were always present in appreciable amounts and only occasionally were smaller than 10 μg chlorophyll/liter in the euphotic zone. This almost continual alga bloom probably was a result of: 1) warm water temperatures favoring both alga growth and bacterial decomposition of dead cells, both in the water and at the mud-water interface, releasing nutrients back into the water, and 2) continual water circulation which assured nutrients would not be depleted for long, as the result of localized concentrations of phytoplankton populations. It is possible that some nutrient not measured, or an undetected temporary lack of a measured macro-nutrient, was important in limited photosynthesis.

The inverse relationship of large standing crops of phytoplankton with rate of oxygen production per unit of chlorophyll has been previously reported by Findegg (1966) and Wright (1959). Two possible explanations are offered for this in Lake Ashtabula. 1) Large standing crops of 25-55 mg chlorophyll/m^3 were associated with heavy blooms which probably contained many cells in an inactive or senescent state whose chlorophyll was extracted along with that of active cells thus causing the efficiency of oxygen production per unit chlorophyll to be lower than the efficiency for standing crops in which most of the cells were in an active growth state. 2) Some self-inhibiting substance may have been produced by the phytoplankton cells themselves which would inhibit photosynthesis with increasing population size. Fogg (1965) reviews recent information on the self-inhibiting substances produced by phytoplankton.

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IMMUNOLOGICAL BEHAVIOR OF BOVINE CARBONIC ANHYDRASE ISOZYMES[1]

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SUMMARY

Three electrophoretically pure components (BCA-I, BCA-II, and BCA-III) of bovine red cell carbonic anhydrase (Carbonate hydrolyase, EC 4.2.1.1) were obtained by preparative disc electrophoresis. BCA-II and BCA-III were used as antigens to elicit antibody production in rabbits. The antisera were tested by the Ouchterlony double-diffusion method. The results indicate that, in contrast to the immunological behavior of human carbonic anhydrase, electrophoretically separable forms of bovine carbonic anhydrase are immunologically not distinguishable.

INTRODUCTION

Multiple forms of bovine red cell carbonic anhydrase have been isolated earlied by column chromatography or gel electrophoresis

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(Nyman and Lindskog, 1964; Tappan et al., 1964; Ashworth, 1967), but all the forms reported have similar activities. This seems to indicate a certain heterogeneity of the protein associated with a single specific activity. In contrast, human red cell carbonic anhydrase exhibits a multiplicity both in electrophoretic mobility and activity (Nyman, 1961; Rickli et al., 1964) and the fractions may also be distinguished immunologically (Wistrand and Rao, 1968).

In the present study the electrophoretic heterogeneity of bovine red cell carbonic anhydrase was compared with its immunological behavior. Three highly purified isozymes of bovine carbonic anhydrase (BCA-I, BCA-II, and BCA-III) were obtained on the preparative scale by polyacrylamide gel electrophoresis. It was found that rabbits are able to produce antisera against these isozymes, but the antisera were not distinguishable by the double diffusion method of Ouchterlony. The immunogenicity of the molecule, like the catalytic activity, seems to be unaffected by the conformational changes producing the electrophoretic behavior. Urea-denatured enzyme forms a single band of precipitate with the antisera. This strongly suggests that the determinant with which the antibody combines may be a linear sequence of amino acids rather than a specific folding of the polypeptide.

MATERIALS AND METHODS

Preparation of purified isozymes. Purified powdered carbonic anhydrase (Sigma; prepared according to Keilin and Mann; 3500 Roughton-Booth Units per mg at 4°C) was subjected to preparative polyacrylamide gel electrophoresis in a Buchler Poly-Prep apparatus (Jovin et al., 1964) applying the Tris-glycine-polyacrylamide system of Ornstein and Davis (1964) with slight modifications to suit the preparative scale and the molecular properties of carbonic anhydrase. One hundred twenty milliliters resolving gel (7.5% monomer, pH 8.9) and 80 ml concentrating gel (3% monomer, pH 7.2) were polymerized at 2°C. In each run, 200 mg carbonic anhydrase was applied in 50 ml sample buffer (12.8 ml H_3PO_4, 2.85 g Tris, 3 g sucrose, and 1 ml 0.01% bromphenol blue solution in 100 ml) and electrophorized at 50 ma constant current and at 2°C for approximately 36 hr. The protein components were continuously eluted by a symmetrical flow of the elution buffer (0.1 M Tris, pH 8) at a rate of 0.75 ml/min and passed through an ultraviolet analyzer (Isco Model UA-2) to a fraction collector kept at 2-4°C. The enzymatically active portions of the eluate were dialyzed at 4°C against deionized water and lyophilized. Some preparations were desalinated and concentrated by ultrafiltration in a Zeineh microconcentrator (Biomed).

Measurement of enzymatic activity. Specific activities were measured according to Davis (1963) by a pH-recording electrometer (Heath Model EUW-301 with multi-speed chart drive) and a combination electrode (Sargent). Approximately 10 mg enzyme was dissolved in 95 ml 0.004 M phosphate buffer (0.002 M in NaCl), pH 7.0

at 3°C and introduced into a reaction vessel placed in ice water. Under constant stirring, 5 ml water saturated with CO_2 at 3°C was quickly injected by a syringe and the pH change resulting from the generation of H^+ recorded. A typical curve of activity measurement is represented in Figure 1. Enzyme units were obtained in ΔpH per min. Protein concentrations were determined by the E_{280}/E_{260} method.

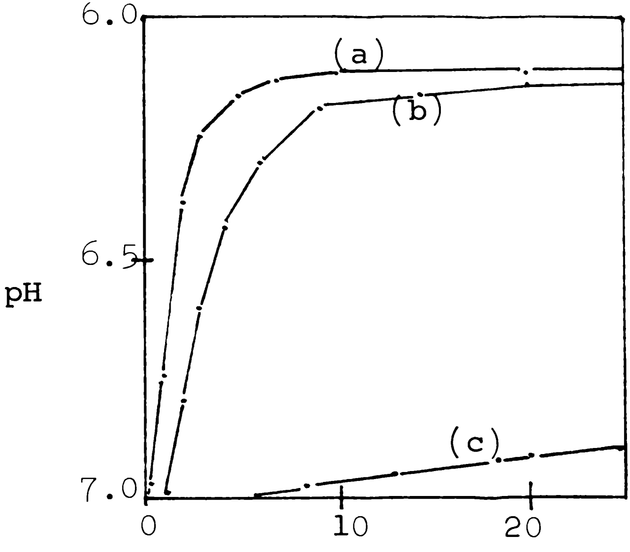

FIGURE 1—A typical run of carbonic anhydrase activity measurement at pH 7.00 and 3°C; 0.004M phosphate buffer; added ionic strength 0.002 M NaCl; enzyme concentration 3 x 10^{-8} M; substrate concentration 3.78 mM; (a): purified isozyme; (b): emulsified isozymes; (c): buffer blank.

Immunization procedure. Antisera were prepared against BCA-II and BCA-III by Freund's adjuvant method (1951). New Zealand rabbits weighing 3-5 kg were used in the experiments. The animals were injected intradermally with 1 ml Freund's Complete Adjuvant containing 10 mg lyophilized enzyme. Two weeks later, another 10 mg enzyme was administered with Freund's Incomplete Adjuvant. Control rabbits were injected with saline-adjuvant emulsions. Five weeks after the initial injection, the rabbits were bled from the marginal vein of the ear collecting 10 ml blood from each animal. The blood was allowed to clot at 4°C, centrifuged, and the serum preserved with merthiolate (0.01%). A booster injection of 2 mg enzyme was given to each test rabbit every 7 weeks.

Immunological Assay. The antisera were analyzed for anti-enzyme activity by th edouble diffusion method of Ouchterlony (1949) using Ago-Immuno-diffusion Plates (1% agarose; Mann, Lot No. S 4052, Pattern B). The center well was charged with 100 μl antiserum. The peripheral wells were filled with 25 μl portions of electrophoretically pure isozymes obtained directly from electrohoresis. The plates were incubated at 34°C for 12 hr in a humid atmosphere.

RESULTS

Electrophoretic resolution of bovine carbonic anhydrase. Bovine red cell carbonic anhydrase was resolved into three clearly distinguishable fractions by preparative gel electrophoresis, designated by us as BCA-I, BCA-II, and BCA-III. Protein recovery varied between 95 and 100% in the runs. BCA-III constituted 65%, BCA-II 30%, and BCA-I 5% of the total protein applied (200 mg in each run). With respect to specific activity, no significant differences could be detected between the fractions, and only minor losses of activity were observed during electrophoresis and emulsification with Freund's adjuvant (Figure 1). The stability of the fractions were practically identical.

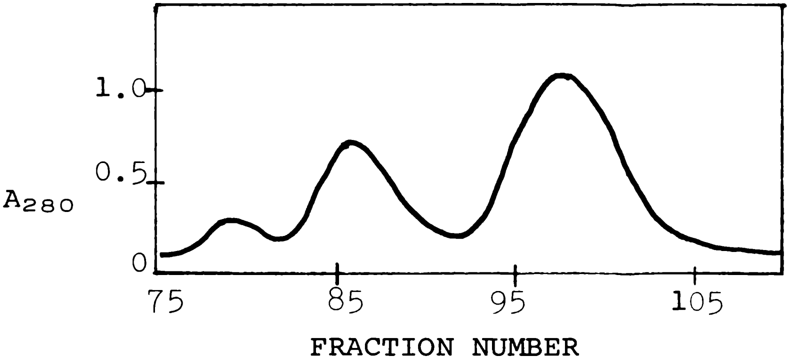

FIGURE 2—Electropherogram of bovine red cell carbonic anhydrase.

Specificity of the antisera. All the rabbits injected with the immunizing preparations developed antibodies. In the immunodiffusion tests identical single lines were obtained with each antiserum regardless which isozyme was applied. The results indicate that the antisera did not exhibit any distinguishable specificity for the isozymes. Figure 3 is a photograph showing a typical immunodiffusion experiment of the first series in which anti-BCA-II was charged in the center well and BCA-I, BCA-II, and BCA-III was charged in the top, right, and left peripheral wells, respectively. After the incubation period identical precipitation lines developed equidistant from their respective wells. Figure 4 shows a typical immunodiffusion experiment of the second series in which the center will is charged with anti-BCA-III serum. The peripheral wells are charged as in Figure

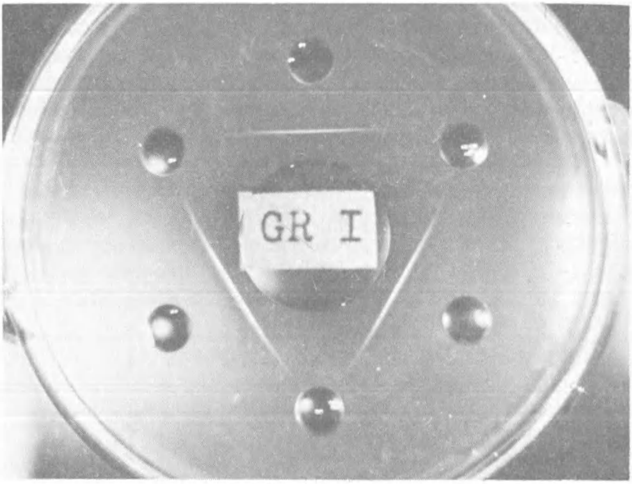

FIGURE 3—Immunodiffusion Test. Center well: 100 μl anti-BCA-II; upper well: 25 μl BCA-I; right hand well: 25 μl BCA-II; left hand well: 25 μl BCA-III. Incubated at 34°C for 12 hr.

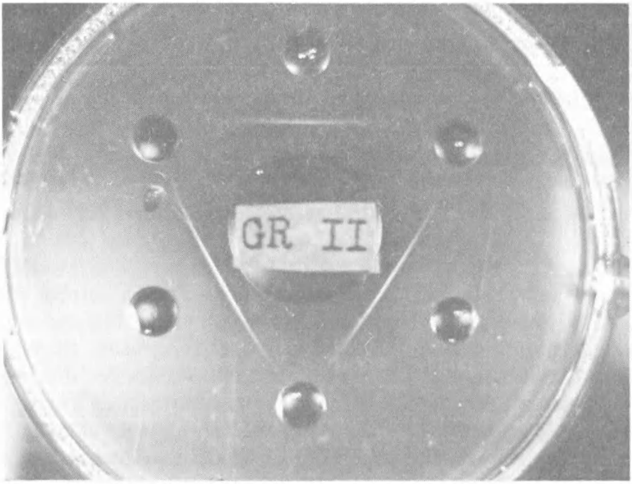

FIGURE 4—Immunodiffusion Test. Center well: 100 μl anti-BCA-III; upper well: 25 μl BCA-I; right hand well: 25 μl BCA-II; left hand well: 25 μl BCA-III. Incubated at 34°C for 12 hr.

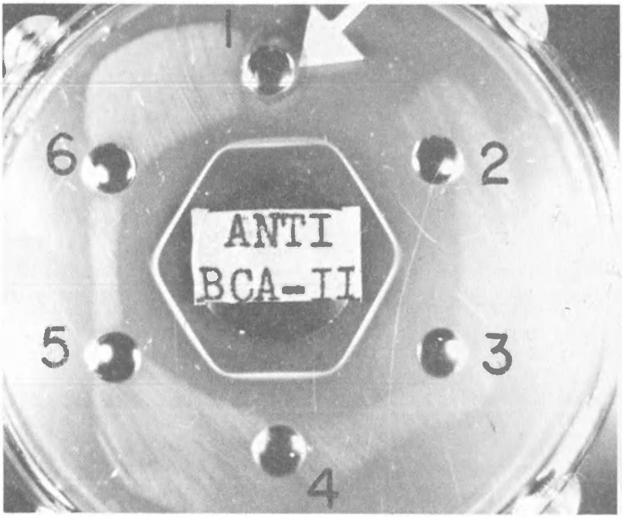

FIGURE 5—Immunodiffusion Test. Center well 100 μl anti-BCA-II. Peripheral wells: 1 and 4, 25 μl BCA-I; 2 and 5, 25 μl BCA-II; 3 and 6, 25 μl BCA-III. Incubated at 34°C for 24 hr.

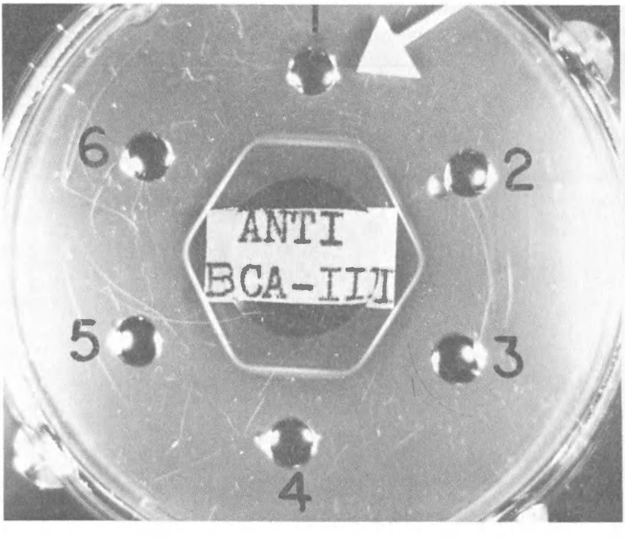

FIGURE 6—Immunodiffusion Test. Center well: 100 μl anti-BCA-III. Peripheral wells: 1 and 4, 25 μl BCA-I; 2 and 5, 25 μl BCA-II; 3 and 6, 25 μl BCA-III. Incubated at 34°C for 24 hr.

3, and the precipitation lines exhibit the same pattern as above. In both immunodiffusion plates, the alternate wells are charged with elution buffer. In Figures 5 and 6 the isozymes are charged adjacent to each other and the immunodiffusion test is carried out against anti-BCA-II and anti-BCA-III, respectively.

Figure 7 shows a control experiment in which the center well of the immunodiffusion plate is charged with the serum of a control rabbit, and the peripheral wells are charged as in Figures 3 and 4. Figure 8 is an immunodiffusion test with urea-denatured enzyme charged in five peripheral wells against anti-BCA-II, in the center well. The remaining peripheral well is charged with deionized water. The commercial enzyme was treated at room temperature with 8 M urea for 2 hr. The immunodiffusion behavior is identical with the patterns observed with non-denatured antigens.

DISCUSSION

The identification of bovine red cell carbonic anhydrase isozymes so far has rested either on column chromatography or on analytical gel electrophoresis. Tappan et al. (1964) applied a semi-quantitative technique electrophorizing the enzyme on a pair of analytical polyacrylamide columns (0.5 x 4.0 cm), with one column stained for protein and its mate cut into thin discs for elution and enzymatic assay. These results suggested the presence of three or four isozymes, while reports on chromatographic separations varied between two and four different forms of this enzyme. The application of preparative polyacrylamide gel electorphoresis with simultaneous continuous

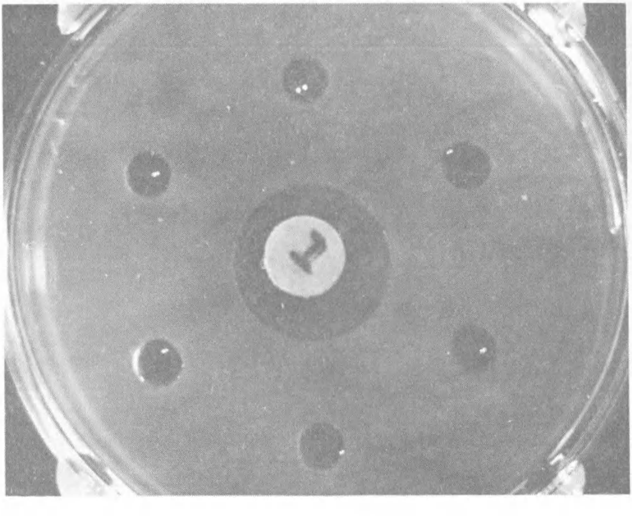

FIGURE 7—Immunodiffusion Test. Center well: 100 μl control serum; upper well: 25 μl BCA-I; right hand well: 25 μl BCA-II; left hand well: 25 μl BCA-III. Incubated at 34°C for 12 hr.

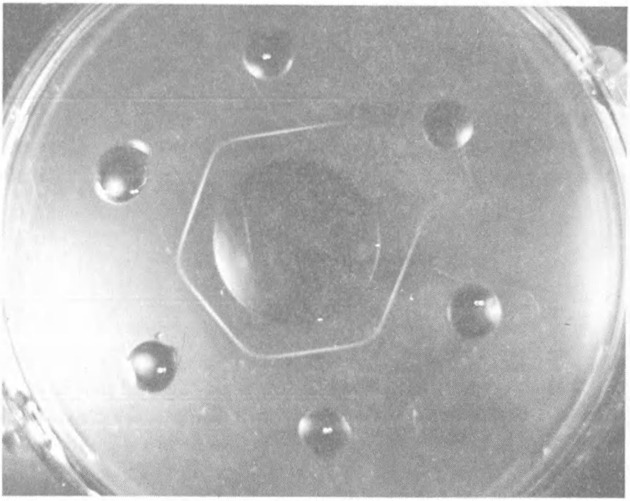

FIGURE 8—Immunodiffusion Test. Center well: 100 μl anti-BCA-III; peripheral wells: 25 μl urea-denatured BCA; control: deionized water. Incubated at 34°C for 12 hr.

elution, the large size of the sample, and the 95-10% recovery of the total protein seem to safeguard against ambiguities inherent in earlier techniques.

Multiple forms of enzymes with immunogenic properties offer a quite useful first approach to the analysis of antibody-antigen binding sites. Interaction between antigen and antibody depends on a relatively small portion of the antigen. Antibody combines with a volume of the antigen not larger than 34 X 12 X 7 A (Kabat, 1958; Anderer, 1963). On the other hand, differences in the electrophoretic behavior of isozymes are a good reflection of conformational variants. These variations may or may not induce changes in the immunogenic behavior. In the absence of such influence, indicated by the lack of immunospecific response against the conformers, we can safely conclude that the conformational changes at play do not change the configuration of the immunologic determinant. This insensitivity might be due to a specific linear sequence of amino acids, or to a protected portion of the folded polypeptide. In the case of carbonic anhydrase, there is an indication of such immutability of the determinant.

ACKNOWLEDGEMENTS

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SINGLE-STAGE BILATERAL ANDRENALECTOMY IN SHEEP

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Various techniques have been described for adrenalectomy of the ruminant animal. These techniques have involved both single and double incisions as well as being accomplished as single-stage operations or over a period of several days. Mansson and Obel (1958) achieved fair success in adrenalectomy techniques when a 7-21 day period was interposed between removal of the right and left adrenal. Goding and Denton (1957) were able to remove both adrenals in one operation period but noted mortality was high unless pre-operative preparation included treatment with cortisone and deoxycorticosterone acetate. Buck and Bond (1956) have refined the techniques of the preceding workers such that adrenalectomy could be accomplished with a single incision. Both adrenals are removed through an incision on the right side of the animal. Success with this type of operation was reported at 85% with a very limited number of animals.

The adrenalectomy procedure to be described in this article is a modification of the technique of Buck and Bond (1966). The purpose of modifying their technique was to obtain many successfully adrenalectomized ewes with the lowest possible mortality rate.

PROCEDURE

Sixty-nine ewes, of various breeds and ages, were adrenalectomized in a single-stage operation for use in a study on embryonic mortality.

Prior to surgery the ewes were fasted for approximately 24 hours. The paralumbar fossa on each side was closely clipped with a small animal clipper, using a number 40 blade. The neck was also clipped over the juglar furrow to facilitate subsequent intravenous injections.

An intravenous injection of 10 mg of dexamethasone[1] was given followed immediately by a "sedative dose" of chloral hydrate 12.5% magnesium sulfate 10.0%[2]. The dosage of the anesthetic solution averaged 50 ml per ewe.

The ewe was then placed in lateral recumbency on a hydraulically operated small animal operating table and secured. No preference was given as to the side on which the animal was placed.

The uppermost paralumbar fossa was then prepared for surgery by scrubbing and by the application of merthiolate or tincture of iodine. Lidocaine hydrochloride 2% with epinephrine 1:100,000[3] was then infiltrated along the proposed incision line followed by a second application of the antiseptic.

A horizontal incision commencing approximately 2 cm posterior to the last rib and continuing for about 12 cm caudad was made through the skin, fascia and lateral abdominal musculature. This incision was about 2 cm ventral to the transverse processes of the lumbar vertebrae. The peritoneal cavity was at no time invaded.

By digital manipulation, the adrenal gland was located and freed from its fascial attachments. Care must be taken that the gland capsule is not ruptured. The blunt dissection was continued until the only attachment remaining was to the large veins (posterior vena cava with respect to the right adrenal and the renal vein to the left).

No attempt was made to achieve visual exposure of the gland. An eight-inch curved Rochester-Pean forceps were then inserted and clamped across this attachment. A scalpel was carefully inserted and the attachments severed. One must make certain all adrenal tissue is removed.

The forceps were left in place and closure of the incision began. The abdominal musculature and fascia was closed using a continuous lock stitch. Just prior to placement of the last suture the forceps were removed. The skin was closed using simple continuous or horizontal matress sutures. A synthetic suture material[4] was used throughout. A penicillin-streptomycin combination[5] was placed in the incision line.

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[1] Azium 2 mg/ml—Schering Corporation, Bloomfield, New Jersey.

[2] Chloral-Mag—Norden Laboratories, Lincoln, Nebraska.

[3] Neurathesia—Fort Dodge Laboratories, Fort Dodge, Iowa.

[4] Vetafil-medium (0.3mm.)—Dr. S. Jackson, Washington, D.C.

[5] Antibiotic combination — Corvel, Division of Eli Lilly & Co., Indianapolis, Indiana.

The ewe was then turned over to the opposite side and the procedure repeated.

The majority of the ewes were able to ambulate and consume feed immediately following surgery. They were given a daily intramuscular injection of 5 ml penicillin-streptomycin for four days.

DISCUSSION AND RESULTS

Removal of both adrenals from a single right paralumbar incision, as described by Buck and Bond (1966) was unsatisfactory in our hands. Transection of a portion of the last rib as reported by the same authors proved unnecessary for our purposes.

The left adrenal was less difficult to remove due to its less intricate attachment to the renal vein as opposed to right adrenal's attachment to the posterior vena cava. The left adrenal also lies further caudad than does the right making it easier to reach and manipulate.

The method of anesthesia proved to be quite satisfactory in the absence of equipment necessary for inhalation products. Initially the chloral hydrate solution was used in sufficient quantities to achieve a surgical plane of anesthesia. When four of the first five ewes succumbed within 18 hours from what was presumed to be anesthetic overdose and/or surgical shock, the procedure was changed to the use of a small, sedative dose of the intravenous preparation plus infiltration of the incision site with a local anesthetic. The use of dexamethasone just prior to surgery was also felt to greatly reduce the incidence of surgical shock.

Of the sixty-nine ewes adrenalectomized, a total of six died as a direct result of surgery, two from excessive hemorrhage and four from surgical shock or the anesthetic procedure. This produced a survival rate of 91%. Only one of the last 64 ewes died a "surgical" death. If a ewe survived and appeared alert and active after 36 hours, she was presumed to have passed the surgical crisis.

Following adrenal removal, the ewes were maintained by daily intramuscular injections of 20 mg cortisol and 5 mg deoxycorticosterone. Reduction of this dosage by one-half resulted in death in six days in one ewe. Cortisol alone was not sufficient for maintenance. The addition of electrolytes to the drinking water was also found to be beneficial in maintaining the ewes.

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TRAFFIC LIGHT CONTROL BY COMPUTER SIMULATION[1]

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ABSTRACT

The handling of a large volume of traffic through a single-lane traffic light has for a long time been a serious problem to traffic planning officials. The need for a simple versatile system to determine the time cycle has long been recognized.

Data for this paper were collected by the author and another student by actual observation of the intersection studied. In the intersection under study, 90% of the traffic flow was in the North-South bound direction. With this unproportionate amount of traffic in these two directions, it was determined to develop a system to: a) reduce the queue length of North-South traffic; b) while yet, obtaining the minimum waiting line for the East-West direction; c) and also to accomplish all of this with a realistic model.

The result of this study was the design of a computer simulator based on a poisosn arrival time, with the capability to determine possible bottle-necks, and the correct time cycle for the North-South bound lanes. The fina lsystem allows for the complete simulation period of three hours. The program provides a minute by minute summary of cars passing through the intersection, queues, holdovers, and other necessary information. In verifying the results of this model with empirical data only small differences were noted.

INTRODUCTION

Since the advent of the digital computer in the early 1950's, a large number of new and powerful analytical tools have been created. Each has had a significant and lasting impact on a number of scientific areas. This report discusses a special class of one of these new analytical tools known as computer simulation. The purpose of this report is to show the development and use of this analytical tool. It will be shown that the computer model can be used to simulate an actual situation with ease and efficiency.

The three basic areas that will be discussed in this repart are: 1) the collection of empirical data; 2) the design of the simulation model; and 3) analysis and interpretation of the results.

No attempt has been made in this report to completely develop plans for a multi-light system and this model has no method for any outside occurances, (i.e. accidents, stalled cars, emergency traffic). Nevertheless, the system is designed to handle any two-lane traffic

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[1] This paper had been delivered as a student paper.

assuming that the cross-bound traffic is at a minimum and can be neglected without destroying the usefulness of the model. The basic structure of this model is completely up to the operator at run-time.

Since exact traffic records were not available to the author, a number of studies were made by him and another student to determine traffic flow rates and traffic flow pattern. The data used in this report is indicative of the existing situation and is reliable for the purpose of this report.

The next section deals with the development of the computer model. Each step of the model simulation is accompanied by a brief but comprehensive explanation. The concluding section presents information that is pertinent to the situation as a whole and recommendations for future use and expansion of the present model.

The need for this study is acutely evident at the very moment one comes to a traffic light and has to wait what seems to be an eternal amount of time. The methods purposed in this report should help to remedy the situation.

GENERAL DISCUSSION

The light that was used was located in the northern part of Fargo, North Dakota, on a main throughway in the North-South direction. Through the course of the study, there was a minimal traffic flow in the East-West direction. Since this condition occurs in many areas of the city, it was determined that a single channel simulator could be developed to handle the single lanes of traffic moving in the two opposite directions. It would be ridiculous to just make the model function of the two separate lanes. This would be a false assumption. A way of determining the interaction of the two lanes of traffic would be needed.

The first step was to obtain information concerning the operation of the system. It was decided to conduct a survey of this intersection and to study the actual flow patterns. The author and another student stationed themselves at the intersection for a number of hours each day for a week. Information concerning the limiting factors of the intersection (the maximum number of cars that would and could be passing through the intersection at one time), the turn patterns of the cars passing through the intersection (what percentage turned in each direction), and what effect it would have on the total flow through the intersection was obtained at this time.

Arriving units may form one line and be serviced through only one station, as in a doctor's office or they may be serviced through several stations, as in a barber shop or the check-out counters of a supermarket. Many times it would be too costly or even impossible to run a trial run on systems like these.

In many cases car counters are used in the study of traffic flow. However good they may be in the determination of an actual count, they cannot give any information on future operating conditions. Therefore, a system must be devised and used that can test the situation realistically, yet not cause any public inconvenience due to poor service during the test and experimental period. Also, the information required in such a test must be easily obtained inexpensively.

The various properties of a waiting line, as the number of cars in a line at any instant or the waiting time experienced by any arrival are somewhat random variables. They are random variables because of the chance way that the events occur. The time you would leave the office would be fixed, but the circumstances leading to your arrival home need not be fixed. These circumstances might include the condition of the street, the pedestrian that may walk in front of the car, and emergency traffic.

In the construction of this model it was decided to use two basic methods for producing the time flow rate of the intersection. Since arrivals are very sporadic, the poisson distribution was chosen to predict the traffic flow. This means for calculating the poisson distribution was a hybrid compute subroutine developed by Thomas H. Naylor in his recent book, *Computer Simulation Techniques.* This method will be discussed in greater length under the section "Description of the Computer Model."

The second variable was used to determine the portion of traffic that would make a left turn. In the survey it was concluded that right turns would not disrupt traffic to a large extent. It was also found, at the time time, that 15% turned left in the southbound lane while 20% tourned left in the northbound lane. These were included in the model in the form of a Monte Carlo technique, using a normal random distribution.

After designing the model into mathematical terms it was programmed. The language used was Kingston Fortran II, which is similar to Fortran IV but designed for the I.B.M. 1620 version H. The next step was programming and testing. The model was tested in comparison to the normal distribution and also to the actual system. Of all the comparisons made, the model best compared to the real system with the use of the poisson distribution. The results can be seen in the appendix.

DESCRIPTION OF THE COMPUTER MODEL

To completely understand the operation of the computer program it is advisable to become acquainted with the symbols commonly used in programming. This information is contained in Table I. Table II should also be useful in understanding the number of Kingston Fortran subroutines used in the model. The major limiting factor in the development of this model was the machine limitation of the I.B.M. 1620 version H. For this reason, a simulation program such as G.A.S.P. (General Activities Simulation Program) or G.P.S.S.II (General Purpose Digital Systems Simulator II) was used.

Two major consideration in the design of this model are the program size and the run time. The present program takes 39,981 core

TABLE I

SYMBOL TABLE DOCUMENTATION

Symbol Description

HDMX1—Maximum holdovers in the north lane
HDMX2—Maximum holdovers in the south lane
HLDS1—Sum of all holdovers in the north lane
HLDS2—Sum of all holdovers in the south lane.
QRED—Array to count arrivals during red light
QGREEN—Array to count arrivals during green light
QTOT—Array to count number of cars passing through system
LMTV—Maximum number of cars that can pass through system during green light
SPD—Speed limit for the intersection
DIST—Distance across the intersection
ITG—Number of seconds that light is green
ITR—Number of seconds that light is red
T1—Table number that heads the top of output
PI—Array that contains the 12 15-minute probabilities
PLTN1—Probability of left turn in Northbound traffic
PLTS—Probability of left turn in Southbound traffic
N—Denotes the northbound lane (subscript)
S—Denotes the southbound lane (subscript)
PNL—Position in lane of a left-turning car

TABLE II

LIBRARY OF SUBROUTINES USED IN THE PROGRAM

Name Description

AMAX—Determine the maximum number of a group
DISCI—Used for storing information on disc storage
DISCO—Used for retriving information on disc storage
EXP—Used to find ${}_{e}$arg
EXIT—Used for branching out at end of program
CHAR—Used with plotter to put titles on graphs
PLOT—Used to plot points on the incremental plotter
RAND—Generates pseudo random number from 0.0 to 1.0
SAVE—When placed in program all information will be stored on disc to be accessible at later time

positions of a possible 40 K. The run time is 27 minutes for the actual program plus an additional three minutes for each of the graphs. A system to consider the other two lanes of traffic would be impossible with the present computer and is unnecessary for this study.

The program consists of six basic parts. The first of these is the format requirements and the initialization section. In these sections the formats are defined for input, output, the plotting titles and the headings. In the initialization all of the storage arrays are initialized to zero and other variables are set to the proper index. Then the following is read in on punched cards: speed limit, length of the intersection, cycle time for the red and green lights, and the percentage of traffic that turns left for the north and south lanes.

The next portion of the program deals with the calculation for the arrivals in the traffic lane. This is done with a poisson generator, where 'delta time' is one second and each of the probabilities that are entered must be evaluated. For example, if the average flow of traffic for a given period was 360 cars per hour the probability that would be entered would be as (360 cars/hour)(1 hour/3600 seconds) = 0.1 cars/second. In this example 0.1 would be the probability entered.

In the survey that was conducted by the author it was found that there were changes in the flow that even an hourly prediction would not show. Since the intersection was on a busy throughway it was affected by sporadic peaks of traffic that lasted for very short periods of time, most of which were less than an hour long. For this reason, the model was designed to accept proabilities for traffic flow on the basis of a 15-minute period. The effects can be seen in the charts on the following pages. Chart 1 and 2 show traffic passing through the intersection while 3 and 4 show waiting queues that develop at the red light. It should be noted that these graphs are actual computer output from the I.B.M. 1627 Incremental Plotter. One should also note the build up to the limiting values of Chart 1 and 2 while at the same time the waiting line increases on Chart 3 and 4.

The next section checks for the interaction of the two opposite lanes of traffic. This is done in three steps. The first step determines if there will be a left turn in the north bound lane. If there will be the second step checks to find the number of cars in the south bound lane that would block the left turn. Depending on the amount of oncoming traffic, the turn is executed and the queue line is reduced or the position is checked and any cars ahead of the turning cars are removed from the line. This phase is then repeated for the south bound lane of traffic. In the last phase, the limiting factor of the intersection is noted. In this model there are a limited number of positions for each car, such as: 1) waiting; 2) passing through; 3) making a turn; 4) out of the system. Because of the size of the intersection only one car can be in it at a time. Based on this assumption the following equation for the limiting factor was developed.

LMTV=(seconds light is green)(speed in mph)(5280/3600)
(distance across intersection in feet)

This equation will determine the number of cars that can possibly pass through the intersection at one time.

The last part to be discussed here concerns the final recommendations that the program produces. This is the portion of the program

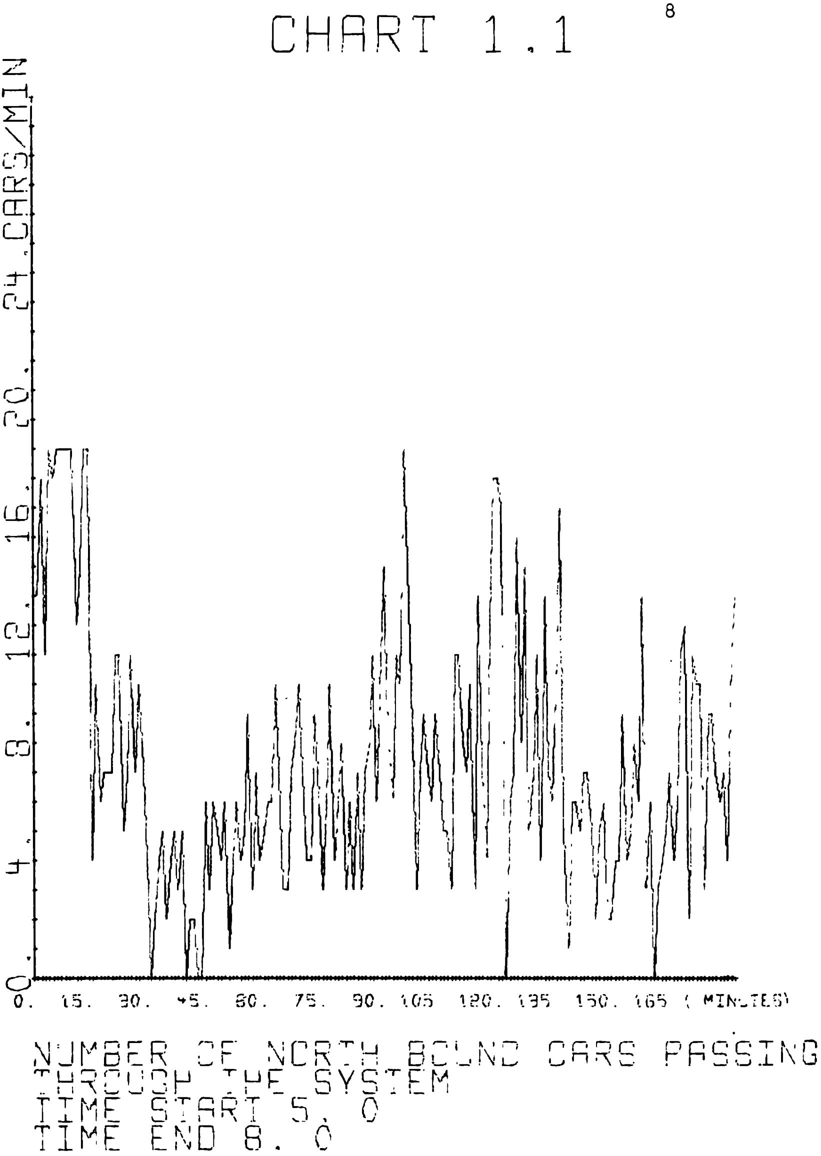

CHART 1.1

NUMBER OF NORTH BOUND CARS PASSING
THROUGH THE SYSTEM
TIME START 5. 0
TIME END 8. 0

FIGURE 1—See text.

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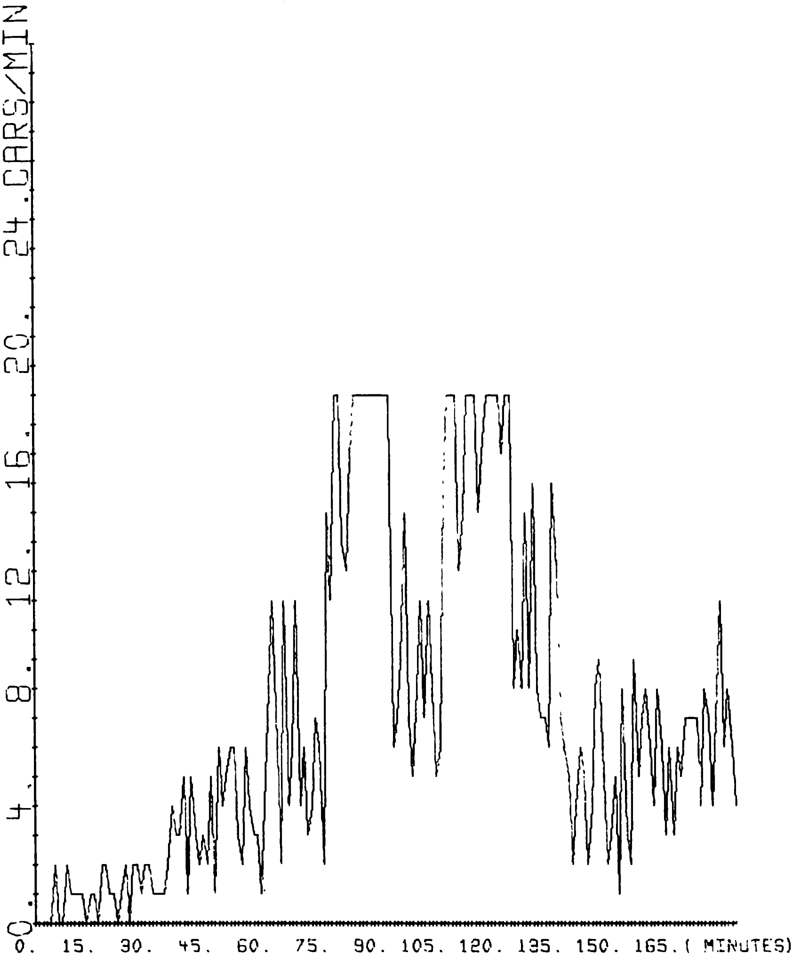
CHART 1.2



NUMBER OF SOUTH BOUND CARS PASSING
THROUGH THE SYSTEM
TIME START 5. 0
TIME END 8. 0

FIGURE 2—See text.

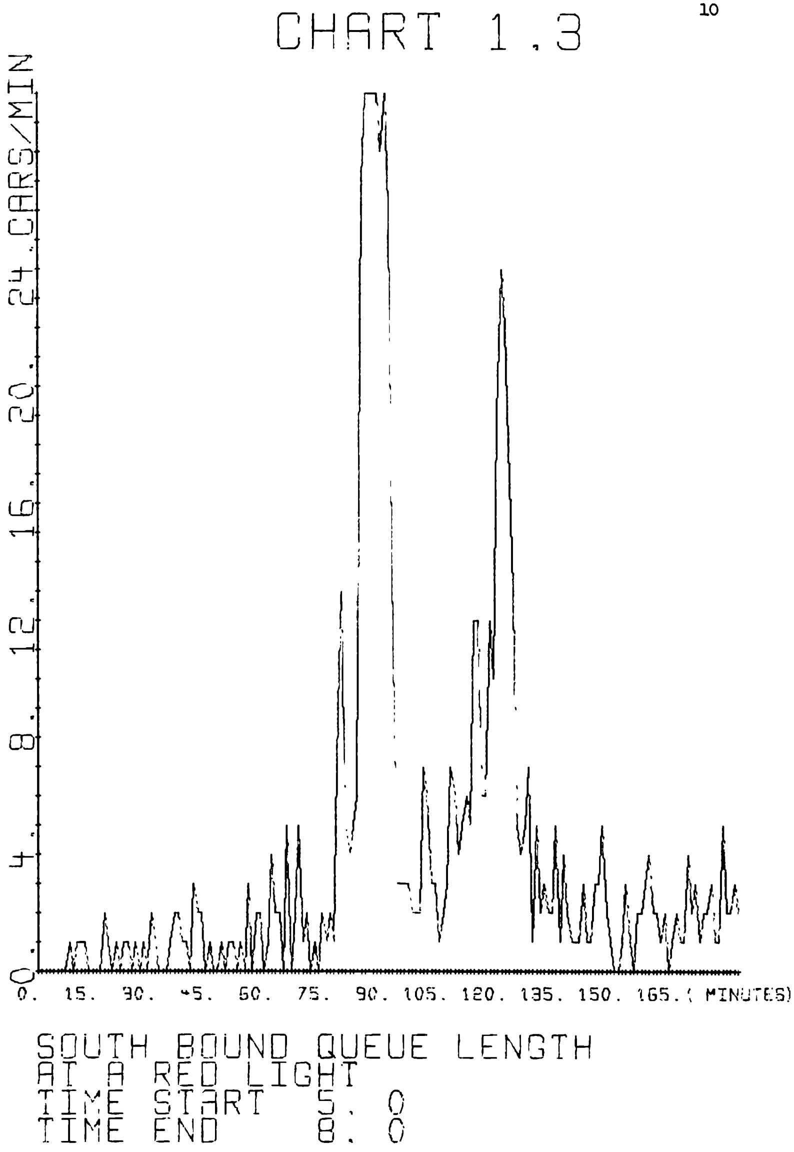


FIGURE 3—See text.

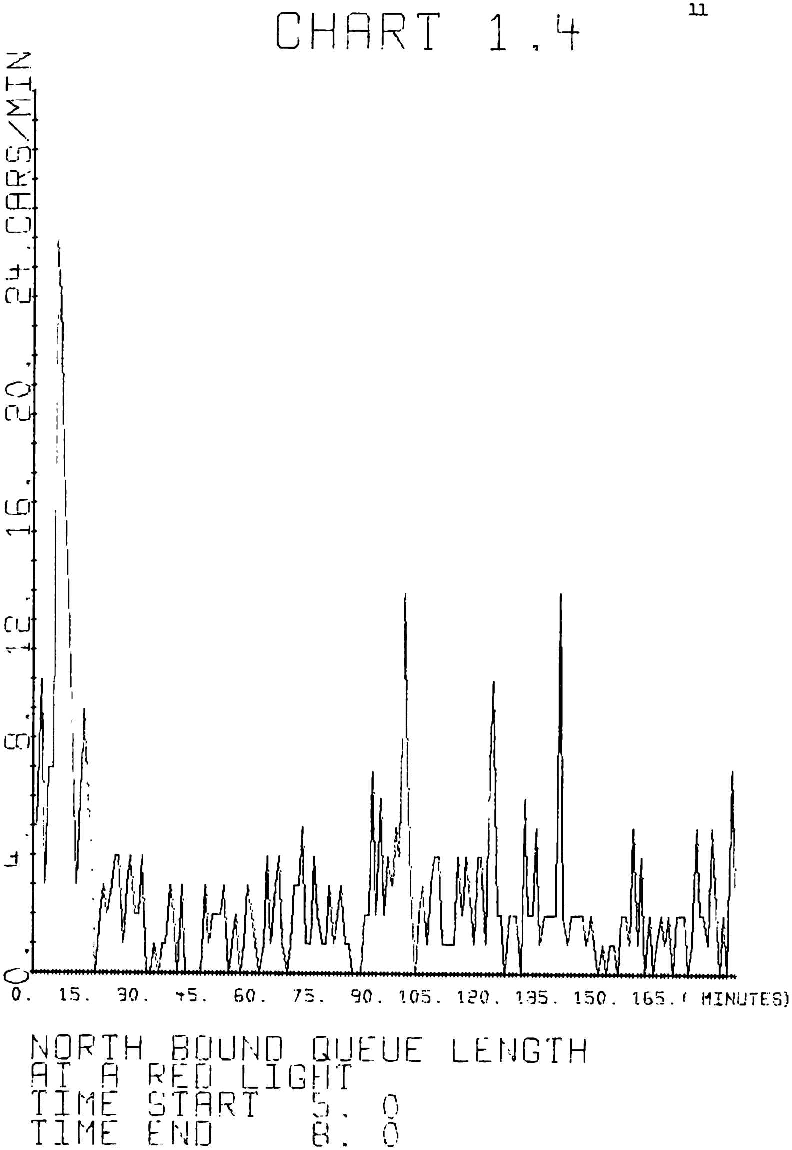


FIGURE 4—See text.

where the model tests a number of sums and maximum values for both lanes. These values include the peak holdover amount and the average holdover number. The program tests these limits to see if they exceed 50% and 15% of the limiting value, respectively. If any of them do exceed these limts the above equation is transposed to calculate the time for the green-light and the red-light cycle is reduced by 90%.

The only part of the program that has not been discussed is the output section. The major output is done by the means of a punched card and additional plotted graphs are made by the use of the I.B.M. 1627 Plotter.

CONCLUSION

Digital simulation has already been proven effective for urban traffic studies.[2] However, considerable effort is required to program the computer for simulation of any complexity. In the development of this particular system, production of an efficient model without going into the complex programming rituals that are necessary with some simulators such as G.A.S.P. and G.P.S.S.II has been tried. However, highly developed as they might be, they are expensive and require extremely sophisticated hardware. This is one advantage of

TABLE III

COMPARISON OF SYSTEM TO MODEL AT P=12%
NUMBER OF CARS THROUGH THE SYSTEM

Time	Actual Data	Poisson Distribution	Monte Carlo
5.16	14	8.0	6.0
5.17	9	6.0	6.0
5.18	2	8.0	7.0
5.19	7	7.0	7.0
5.20	4	6.0	7.0
5.21	6	14.0	6.0
5.22	6	6.0	8.0
5.23	8	7.0	7.0
5.24	8	6.0	7.0
5.25	8	7.0	7.0
5.26	7	5.0	5.0
5.27	16	8.0	6.0
5.28	5	6.0	8.0
5.29	8	5.0	7.0
5.30	6	15.0	14.0
TOTALS	114	115.0	121.0

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[2] Goode, H. H., "The use of a Digital Computer to Model a Signalized Intersection," *Proc. Highway Res. Board 35*. pp. 548-557. 1956.

this particular model. Under this system even the smaller cities can afford the benefits of computer simulation.

Simplicity of operation is the basis of this design. Nevertheless, how simple the design may be it produces most of the pertinent information that is needed in traffic planning such as the limiting values, queue lengths, and cycle times for the lights. The validity of this simulation model can be seen in the comparison tables that are presented (Tables III and IV).

TABLE IV
LENGTH OF WAITING LINE
COMPARISON OF THE SYSTEM TO THE MODEL

Time	Actual Data	Poisson Distribution	Monte Carlo
5.16	10	3.0	2.0
5.17	4	2.0	2.0
5.18	1	3.0	2.0
5.19	3	2.0	2.0
5.20	2	2.0	3.0
5.21	1	3.0	2.0
5.22	2	2.0	2.0
5.23	4	3.0	3.0
5.24	2	2.0	3.0
5.25	2	2.0	2.0
5.26	3	3.0	2.0
5.27	7	3.0	2.0
5.28	4	2.0	3.0
5.29	2	2.0	3.0
5.30	3	5.0	5.0
TOTALS	48	39	39

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EDITOR'S NOTE: This paper came in too late to be included with the student paper section of the Proceedings.

AUTHOR INDEX[1]

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[1] The numbers in standard type and in parentheses refer to abstracts; those in **boldface** to complete articles.

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