PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

Official State Academy
(Founded December, 1908)

VOLUME XXI
1967

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

PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

Official State Academy
(Founded December, 1908)

OFFICERS

President	William E. Dinusson
President-Elect	Paul Leiby
Secretary - Treasurer	Ben G. Gustafson
Historian	George A. Abbott

Additional Members of Executive Committee:
Harald N. Bliss
Myron Freeman
F D. Holland, Jr.
Richard M. Marwin
Franz H. Rathmann

VOLUME XXI
1967

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

Printed by
THE UNIVERSITY OF NORTH DAKOTA PRESS
GRAND FORKS, NORTH DAKOTA

PREFACE

The order of the papers and abstracts appearing in this issue of the Proceedings follows the general format of recent issues, in that the A. Roger Denison Student Research Competition papers are presented first, followed by the Invitational Paper, then the Professional Papers and Abstracts. They were presented before the Fifty-ninth Annual Meeting of the North Dakota Academy of Science, May 5 and 6, 1967, held at the University of North Dakota, Grand Forks. For the convenience of the reader there is an author index included in this issue.

All regard has been paid to the authors who contributed papers. Editorial changes have been kept minimal—essentially, to assure consistency within a given manuscript. It is assumed that the style of each paper follows that appropriate for the respective field. The content of the papers and conclusions drawn are the responsibility of the authors. It is suggested that authors strive to follow the directives suggested by the editorial committee (Proc. N. Dak. Acad. Sc., **20**: 7-10, 1966) in order to expedite the printing of the Proceedings.

F. A. Jacobs

Grand Forks, North Dakota

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

ABSTRACTS

TABLE OF CONTENTS

TABLE OF CONTENTS

HYDROLOGY OF THE CHARLOTTE LOBE DELTA

Kirth Erickson

Department of Geology

University of North Dakota, Grand Forks, North Dakota

First Place Winner

A. Rodger Denison Student Research Competition

The Charlotte Lobe Delta is located between Lake Charlotte and the terminus of the Charlotte Lobe of the Martin River Glacier in south-central Alaska. "Constant Creek" and "Roaring River" are meltwater streams which traverse the delta and discharge into Lake Charlotte. Fluctuations in these streams and the lake were measured during the summer of 1966 to determine their effects on the water table in the delta. Field study techniques included discharge measurements, leveling and mapping. The temperature was recorded on a thermograph. Water table and surface water fluctuations were observed by means of ground wells and stage gages, respectively.

The discharge of "Constant Creek" at the principal gaging station was rated on the basis of four measurements. A fifth measurement taken 300 feet downstream revealed an abnormally high loss of 7 cubic feet per second between the two stations. This amounts to an exceptionally high infiltration rate of 510 gallons per day per square foot.

Water level changes in "Constant Creek" correlate directly with temperature fluctuations, but show a lag of 8 to 10 hours. This lag is assumed to be a function of the distance between the stage gage and the drainage source.

During the observation period the levels of the delta water table rose rapidly as a result of a flash flood from "Roaring River." The rise in the water table was first observed in well #8, nearest the source, and finally in well #1, on the opposite side of the delta. The flood raged for nearly two days and then virtually ceased. Variations in one stream had no effect upon the other during the flood, indicating that no connection existed between them within the glacier. Furthermore, the delta water table varied in direct relationship to stream flow, indicating that the volume of flow in streams crossing the Charlotte Lobe Delta is the dominant control of the water table level.

INTRODUCTION

General—This paper is a summary of research undertaken during the summer of 1966 on the hydrology of the Charlotte Lobe Delta. The delta is located between Lake Charlotte and the terminus of the Charlotte Lobe of the Martin River Glacier, 60 miles east of Cordova in south-central Alaska (Figure 1). The work on the delta was directed toward a study of the meltwater streams from the glacier, the related ground water table, and the levels of Lake Charlotte.

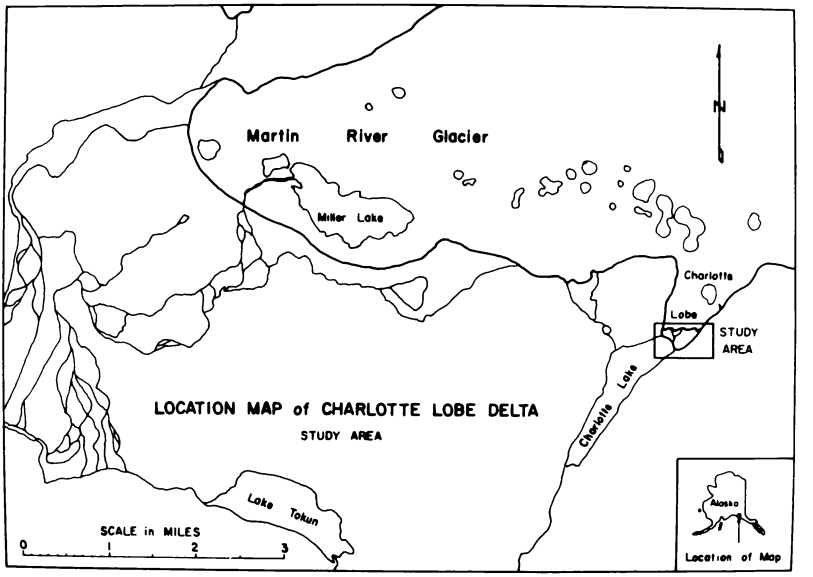


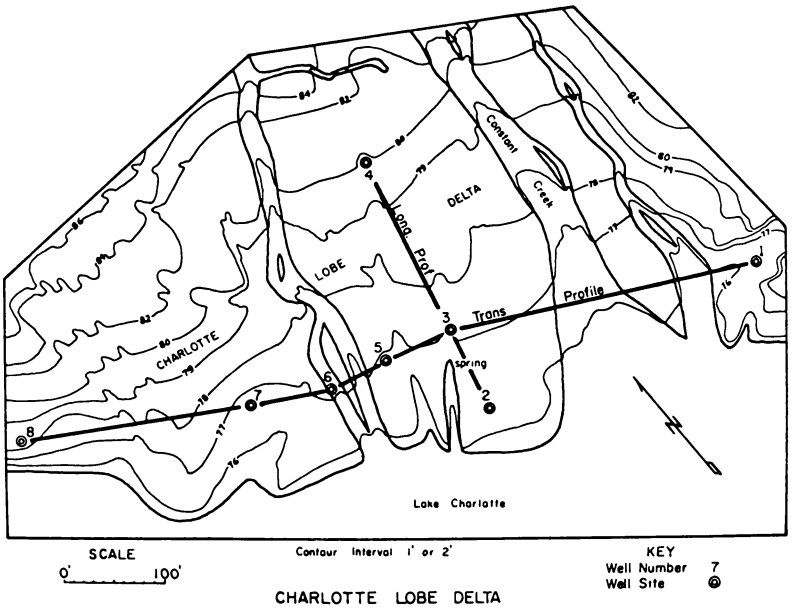
FIGURE 1.—Map of the Martin River Glacier showing the location of the study area.

Runoff from spring rains and from melting ice fill tunnel networks in this part of the glacier and constitute the stream flow which traverses the delta. The glacier is covered with up to a foot of rock debris and vegetation which continually slide down the ice terminus onto the delta, thereby blocking the tunnel outlets.

During the 1966 season a pressure head gradually built up within the glacier. When this head reached a critical pressure the plugs in the tunnel openings were breached and a flash flood was released onto the delta. Fluctuations of the ground water in the delta and in Lake Charlotte were observed during the draining of the tunnel network.

Purpose—The purpose of this investigation was to determine what hydrologic and physical factors control the water table level in the Charlotte Lobe Delta.

Field methods—Ground wells, which consisted of 4-foot lengths of one-inch aluminum pipe, were located on the active, vegetation-free area of the delta. Two staff gages were constructed by laying a 4-foot length of ¾-inch aluminum pipe on a leveling rod and marking every one-hundredth of a foot. A small cut was made with a hacksaw at each one-tenth foot mark and two cuts were made at each one-foot mark. These graduations were then labeled with an indelible marker. One gage was placed in Lake Charlotte and the other in "Constant Creek" (Figure 2).



CHARLOTTE LOBE DELTA

FIGURE 2.—Topographic map of the Charlotte Lobe Delta, after the flood of July 8th, 9th, and 10th, 1966, showing the location of the well profiles.

Levels were run from a reference point to the staff gages and to the ground wells so that the elevations of the water table and lake level could be compared. A topographic map was subsequently drawn of the portion of the delta in which the wells were located in order to observe the relationships of the water table to the surface of the delta.

"Constant Creek", which flowed at a fairly uniform rate throughout the period of investigation, was rated by taking four individual discharge measurements at various gage heights at a selected site on the channel, and plotting the discharges on a graph of gage height vs. discharge (Figure 3). This rating curve was utilized in determining the infiltration rate of the delta sediments. Temperatures were recorded on a thermograph at the Miller Lake camp (Figure 1).

INVESTIGATIONS

Well locations.—Two rows of wells were emplaced on the delta. One row ran transversely across the delta, about 50 feet from the lake shore, and included wells #1, 3, 5, 6, 7 and 8 (Figure 2). The second row extended longitudinally up-delta from the lake and consisted of wells #2, 3, and 4. Well #3 was included in both rows.

Infiltration.—The discharge of a stream may be determined di-

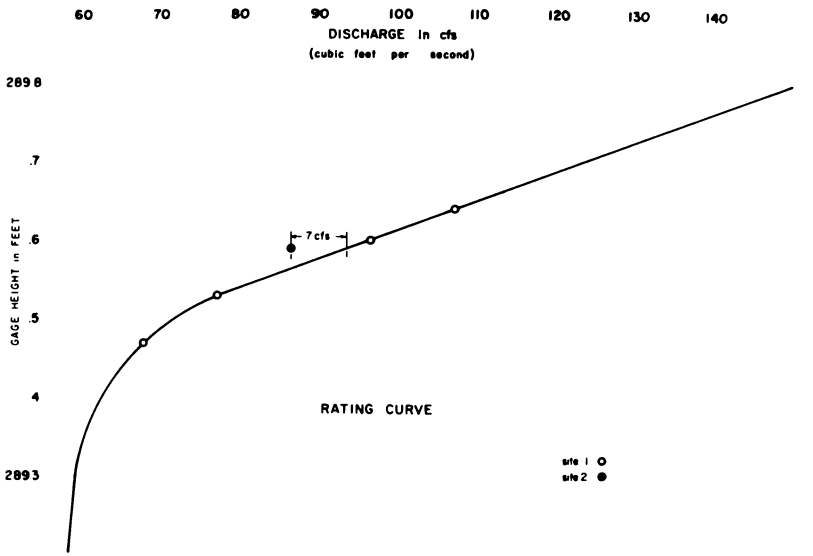


FIGURE 3.—Graph of discharge vs. gage height at the principal measuring site on "Constant Creek."

rectly from the rating curve if the water level of the gage at the rated site is known (Figure 3). The water level at the rated site, during the time that a measurement was being taken 300 feet downstream, indicated a discharge of 93.7 cubic feet per second. The measured discharge at the downstream site was only 86.7 cubic feet per second, which indicated a loss of 7 cubic feet of flow per second in a distance of only 300 feet! This amounts to a very high infiltration rate of 510 gallons per day per square foot of delta surface.

Flooding.—The water table began to rise suddenly on the 8th of July when water began to accumulate against the upper edge of the delta in the tunnels in the toe of the glacier. By evening the pressure head had built up sufficiently to force out the sediments which were plugging the tunnel openings. This sudden release of stored water issued from "Roaring Spring" in the toe of the glacier at the north margin of the delta (Figure 1). "Roaring Spring" gave rise to "Roaring River" which entrained cobbles, boulders, blocks of ice, trees, and other debris along its channel. A general flood followed which inundated most of the delta and all of the ground wells with the exception of #1 and #8 which were located on the less active flanks of the delta. The water table initially began to rise in well #8, nearest the spring, and finally in well #1 on the opposite side of the delta (Figure 4). The early rise in well #8 indicates that the water was building up in the glacier behind the delta and seeping through

the delta sediments as ground water several hours before the river appeared on the surface.

Longitudinal profile.—The longitudinal profile shows the relationship of the ground surface and the lake level to the water table

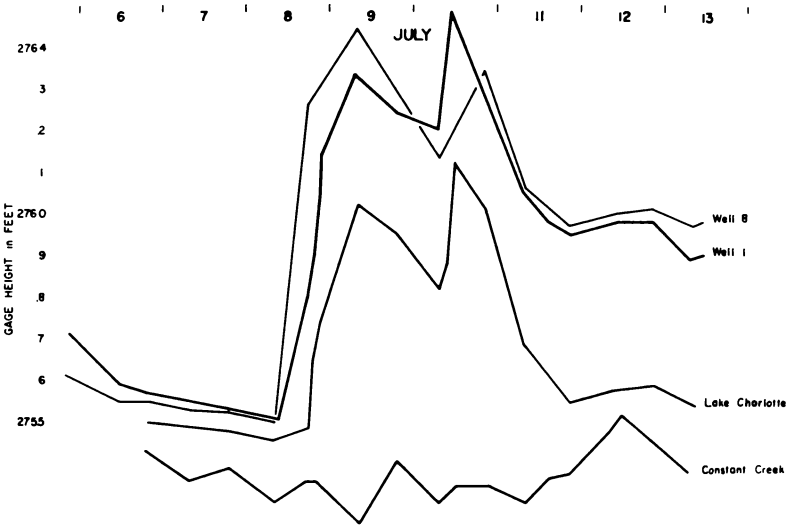


FIGURE 4.—Correlation of hydrographs from selected stations on the Charlotte Lobe Delta (for location see Figure 2).

in the area of the Charlotte Lobe Delta. Profile "A" represents the situation at 0815 hours on July 8th, prior to the flood. Profile "B" shows the situaiton at 2000 hours on July 10th, as the flood waters were receding (Figure 5).

Profile "A" shows only a slight inclination of the water table toward Lake Charlotte which was at 75.06 feet. The water table was at 75.81 feet in well #4, 325 feet from the lake, at 75.64 feet in well #3, 125 feet from the lake, and at 75.48 feet in well #2 only 45 feet from the lake. On July 10th the comparable ground water levels were 77.5 feet, 76.68 feet, and 76.02 feet, respectively, while the lake was at 75.61 feet. The profile continues from well #4 to the edge of "Constant Creek." In a pit 1½ feet from the edge of the stream the water table was 4 feet beneath the surface! The delta surface in the vicinity of the pit was about 2 feet higher on July 10th because of sedimentation during the period of high discharge from the spring.

Transverse profile.—The transverse profile runs from well #1 to well #8. Profile "A" represents the water table at 0815 hours on July 8th prior to the flood, and profile "B" shows the rise which had occurred by July 10th due to the flood (Figure 6).

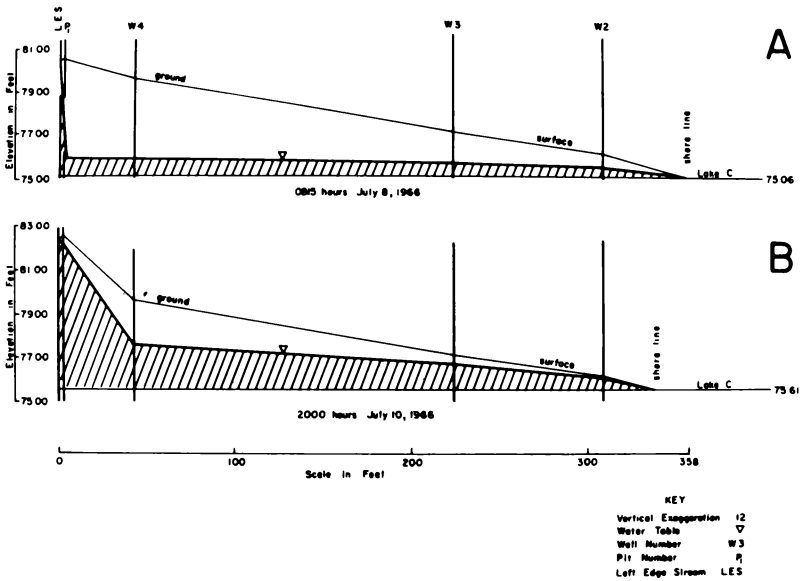


FIGURE 5.—Longitudinal profile of the ground water table in the Charlotte Lobe Delta. Profile "A" represents the situation at 0815 hours on July 8th, 1966, and profile "B" the situation at 2000 hours on July 10th, 1966.

On July 8th the lake level was at 75.06 feet and the water table was nearly flat. "Constant Creek" crossed this profile between wells #6 and #7. The water table was about ½-foot above the lake level along most of this profile which was approximately 50 feet from the shore line. This indicates a gradient of about 1% in the water table on that date.

The lower profile shows the same row of wells just after the flood waters began to recede. The lake level was at 75.61 feet and the water table averaged about one foot higher. This indicated a gradient of about 2% in the water table.

Well #5 was still inundated by a distributary, which accounts for the elevation of the water table above the ground surface in this area. The discharge of streams crossing the delta was low on July 8th and the water table was about 1 foot below the ground surface. Stream discharge was high two days later and the water table was only a few inches below the ground surface.

Temperature correlation.—The hydrograph of "Constant Creek" correlates directly with the thermograph from Miller Lake camp 5 miles away. The only exceptions were on July 11th and 12th when the creek rose due to a light rain (Figure 7). July 6th, 7th, and 9th had high temperature readings with corresponding high discharge

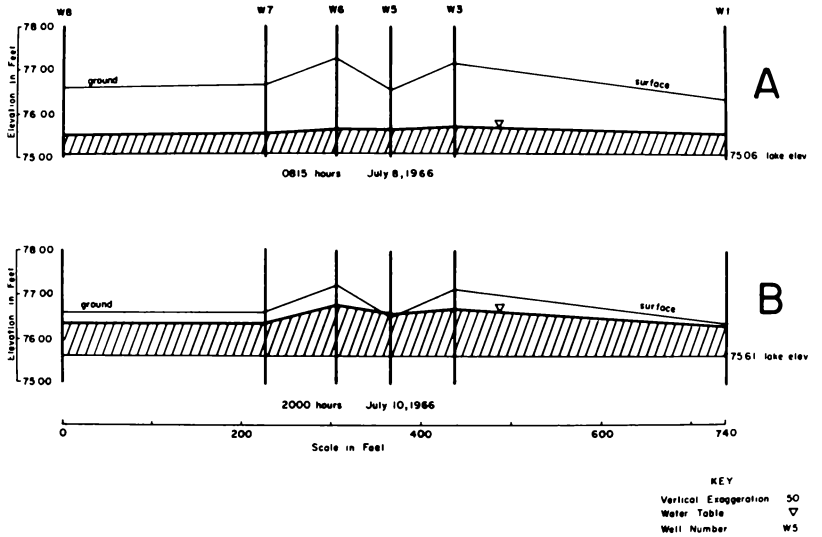


FIGURE 6.—Transverse profile of the ground water table in the Charlotte Lobe Delta. Profile "A" represents the situation ot 0815 hours on July 8th, 1966, and profile "B" the situation at 2000 hours on July 10th, 1966.

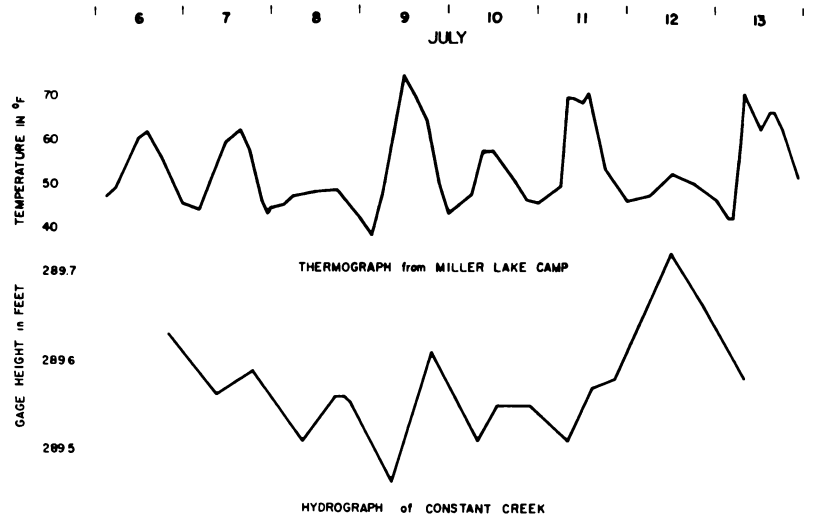


FIGURE 7.—The Correlation of temperature fluctuations with stage gage readings in "Constant Creek" for the period July 6-13, 1966.

in "Constant Creek." July 8th and 10th had low temperature rises due to overcast and "Constant Creek" had a corresponding low increase in flow. The peaks and troughs on the hydrograph lag 8 to 10 hours behind the corresponding peaks and troughs on the thermograph. The lag time is presumed to be a function of the distance between the gage and the drainage source up-glacier.

CONCLUSIONS

1. The delta sediments are highly permeable as demonstrated by the measured infiltration rate of 510 gallons per day per square foot of surface area.

2. The streams traversing the delta are influent; the water level of streams was higher than the adjacent water table.

3. No subglacial connection exists between "Constant Creek" and "Roaring River." Variations of flow in one stream had no affect upon the other.

4. Meltwater was the primary source of flow in "Constant Creek" during the period of observation. This was indicated by the direct correlation of gage height and temperature fluctuations.

5. The water table level is primarily controlled by the volume of discharge in streams which cross the delta. The water levels in the ground wells correlated directly with gage heights in the streams.

ACKNOWLEDGMENTS

This study was sponsored by the National Science Foundation through the Undergraduate Research Participation Program. The cost of transportation and field subsistence was financed as part of a National Science Foundation Grant (GP-4448) awarded to Dr. John R. Reid, Associate Professor of Geology. The main project was the measurement of flow rates and ablation in the Martin River and Sioux Glaciers. The hydrology research was undertaken as a subsidiary project.

I wish to express my thanks to Dr. Reid for his practical suggestions and guidance throughout the entire research program, for his supervision of the compilation and analysis of data, his assistance in the mapping of the delta, and finally in the writing of this paper. I want to thank John R. Tinker, a graduate student, for his assistance in the mapping of the lower delta, and Kent A. Johnson, a geological engineering student, who rendered indispensible assistance in all phases of the field study. I am also indebted to Dr. Lee Clayton for critical reading of the manuscript.

MULTIPLICATION AND TRANSLOCATION OF BARLEY STRIPE MOSAIC VIRUS

Daryl R. Pring[1]

Department of Plant Pathology
North Dakota State University, Fargo, North Dakota

Second Place Winner
A. Rodger Denison Student Research Competition

During the period 1951-1960, barley stripe mosaic virus (BSMV) caused greater losses in yield of barley than did any other plant pathogen (1). In North Dakota, BSMV was present in 93% of 214 fields examined in 1954 (7). Although the prevalence of BSMV has been reduced since that time, the seed-borne nature of the virus presents a pathway by which the disease could again become a major problem.

Very few studies have been reported on the multiplication of cereal viruses, and, to the author's knowledge, no reports have been published on comparisons of the multiplication of strains of cereal viruses in host varieties. Haunold (3) conducted infectivity assays of wheat streak mosaic virus in wheat at 10, 20, and 30 days after inoculation, and found maximum titer at 10 days. Kassanis and Slykhuis (6) found that infectious BSMV multiplied rapidly during the first five days after inoculation, reaching a maximum at 10 days. Kankam and Arny (5) reported that BSMV reached a higher virus titer at 24 C than at 16C.

This investigation was initiated to study the infectivity titer of BSMV as influenced by strains of the virus, barley hosts, and by the first four foliar leaves.

METHODS AND MATERIALS

Four barley hosts were used in the investigations. Three of these varieties, Black Hulless, Moreval, and Dickson, were six row spring barleys (*Hordeum vulgare* (L.) Lam), and one, C.I. 3212-1, was a two row spring barley (*Hordeum distichum* L.). Black Hulless and Dickson were susceptible to the strains used, while Moreval and C. I. 3212-1 were resistant.

Three strains of BSMV were used: a yellow leaf strain (#18), a white leaf strain (#110), and a mosaic strain (type). Moreval and C.I. 3212-1 were immune to type strain. The strains were increased in quantity and lyophillized prior to the initiation of the investigations. At the beginning of each experiment, lyophillized material was increased in Black Hulless through a single transfer before use.

In all experiments concerning the effect of strains and hosts on virus titer, plants from which infectivity assays were made were

[1]NDEA fellow.

grown in a growth chamber at 27± 1.0 C. In experiments designed to determine translocation and multiplication in leaves of barley plants, all plants were grown in a greenhouse at 27± 2.0 C. A 16-hour photoperiod was followed by an eight-hour dark period in all experiments. Light intensity was about 1200 ft c.

About 10 seeds per four inch pot were sown in composted, autoclaved soil, and seven days later the plants were inoculated with BSMV, using a Thayer and Chandler artist's airbrush at 60 psi with about 2% silicon carbide (400 mesh) added to the inoculum. In experiments where more than one strain of virus was used, treatments were randomized within the growth chamber.

The concentration of infectious virus in the plants was determined at 48 hour intervals beginning either 24 or 48 hours after inoculation. On each assay date 30 plants were selected at random from each treatment. The plants were harvested, ground with a mortar and pestle, and the sap was expressed through four layers of cheesecloth. Distilled water was added to make a series of quarter-log dilutions and each dilution was then used to inoculate about 35 Black Hulless indicator plants.

At eight days after inoculation, the percentage infection produced by each dilution was recorded and the data was evaluated according to the method of Brakke (2). The log-log $\left(\frac{100}{\%\text{healthy plants}}\right)$ was plotted against log dilution, and the best straight line with a slope of minus one was drawn through the points. The ID_{63}, or the log of the dilution that resulted in 63% infection of the indicator plants, was then determined.

RESULTS AND DISCUSSION

Multiplication of BSMV as Related to Hosts.—Strains #18, type, and #110 were used in two experiments with each of the four barley varieties to ascertain the relative titer of each strain in each host. These experiments (Figure 1) showed that, regardless of strain, higher levels of infectious virus occurred in Black Hulless than in any of the other varieties. Dickson, also classified as a susceptible variety, had a higher infectivity titer than the two resistant varieties. The differences among Black Hulless, Dickson, and Moreval were significant at the 5% level for most dates. C. I. 3212-1 had a lower virus titer than did Moreval, but the difference was not significant at most dates.

Multiplication of BSMV as Related to Strains.—Of the virus strains studied, the highest infectivity titer occurred in plants inoculated with strain #18, regardless of host (Figure 2). In susceptible varieties, there was a significant difference in the virus titer present throughout most of the period. In resistant varieties the differences were generally significant only during the observed peaks of virus titer at 7 and 13 days after inoculation. Regardless of host, strain

#110 had the lowest infectivity titer. In Black Hulless, strain #110 showed a single peak of infectivity at 9 to 11 days. This strain depleted the leaves of chlorophyll more than other strains, often causing death of the second, third, and fourth foliar leaves emerging above the coleoptile. In many cases the entire plant was killed.

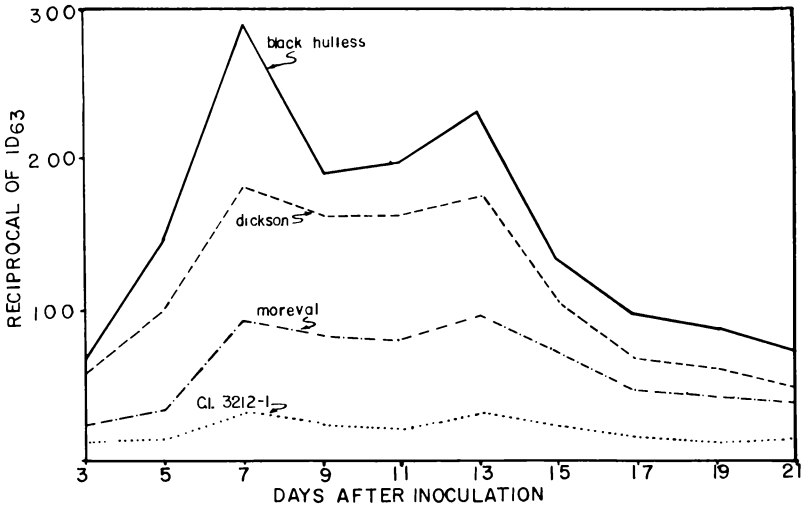


FIGURE 1—The time curve of infectivity of barley stripe mosaic virus as influenced by host variety.

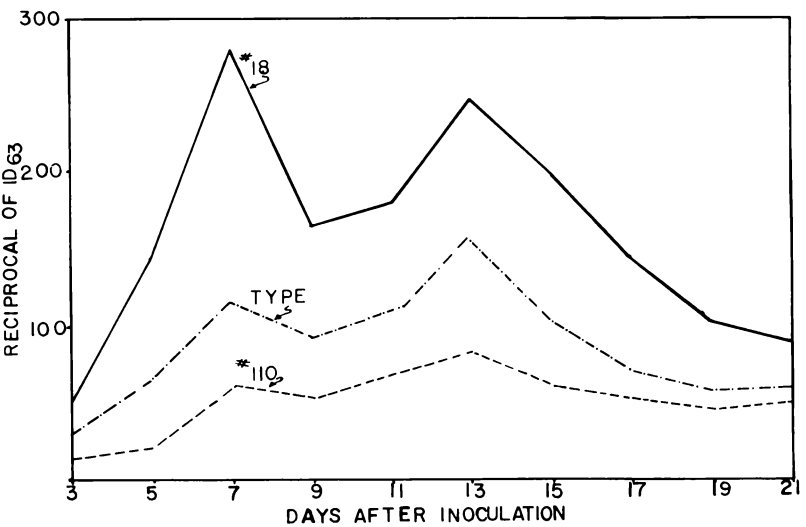


FIGURE 2—The time curve of infectivity of barley stripe mosaic as influenced by strains of the virus.

Multiplication and Translocation of BSMV in Plant Parts.—In nearly all experiments, two peaks of infectivity were observed. It was suggested that these peaks in virus titer were related to growth of the plants, and an experiment was therefore designed to determine virus titer in each developing leaf. Strain #18 in Black Hulless was selected for this purpose because this strain-host combination resulted in the highest virus titer observed in the experiments. Relative increases of virus in each leaf would therefore be easier to detect than in other host-strain combinations. As in all other experiments, plants were inoculated seven days after planting. At that time the first foliar leaf above the coleoptile was fully enlarged, and the second foliar leaf was just beginning to emerge. Twenty-four hours after inoculation, and at 48 hour intervals thereafter, infectivity assays were made. Assays were made only on the first leaf for the first two assays since the other leaves had not developed sufficiently. Leaf two was assayed beginning at 120 hours after inoculation, and leaves three and four were assayed beginning at 7 and 11 days after inoculatoin, respectively. Leaves were about 2.5 cm long when assays were initiated. Results (Figure 3) showed that the first peak of infectious

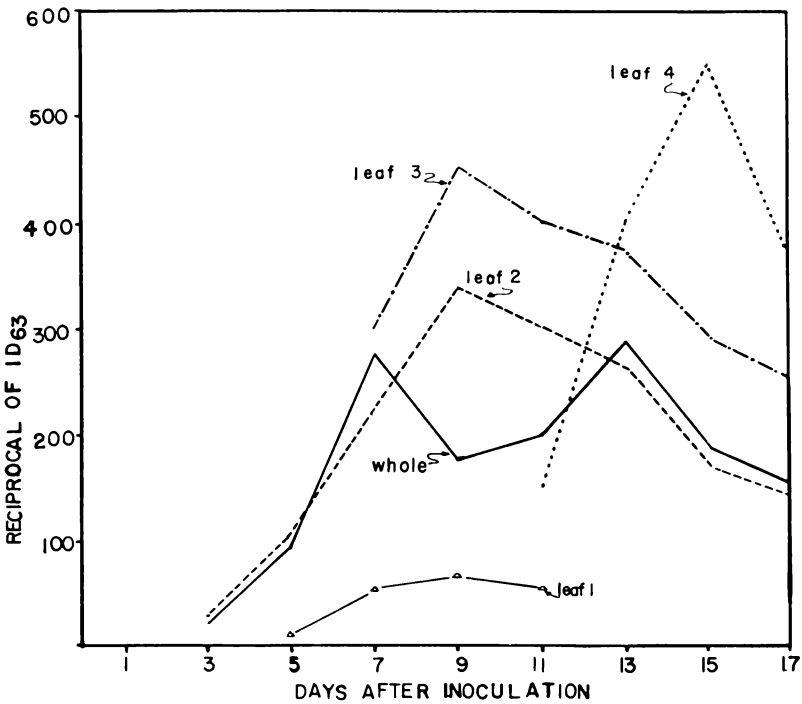


FIGURE 3—Multiplication of strain #18 of barley stripe mosaic virus in individual plant parts.

virus from entire plant assays was due to the initial increase in the second and third foliar leaves. The second peak, at 13 to 15 days after inoculation was due to an extremely high titer in the fourth foliar leaf.

Apparently the virus moved out of the inoculated leaf (leaf one) and began to multiply in leaf two before a detectable quantity of virus was presented in leaf one. This leaf was maximally elongated at the time of inoculation, and hence the net flow of photosynthate would have been out of it towards the growing point. Since viruses move passively, it is hypothesized that the initial virus increase in leaf one was transported out of this leaf *via* the phloem tissue.

The infectivity titer measurements in leaves two, three, and four showed a rapid increase followed by a decline to equilibrium. It was also observed that the degree of chlorosis and necrosis in each leaf was not related to virus titer. Leaf two with a maximum ID_{63} of about 1/300, was chlorotic at emergence, with the basal two-thirds of the leaf eventually showing necrosis. Leaf four, with a maximum titer of about 1/550, showed less chlorosis, and necrotic areas were not present at 20 days after inoculation.

Incubation Time as Related to Titer of Inoculum.—During the experiments, it was observed that plants inoculated with concentrated solutions of BSMV had a shorter time period between inocualtion and appearance of symptoms. Therefore, Black Hulless barley plants were inoculated with a series of dilutions of type strain, and daily counts of infectivity were made. The data (Figure 4) show that when concentrated suspensions of virus were used as inoculum, the incubation period was shorter than that of plants inoculated with dilute suspensions. This effect was probably due to the increased number of infection sites resulting from the increased number of infectious particles. Hooker and Benson (4) observed a similar effect with *Datura tatula L.* infected with potato virus X, but no reports of this effect occurring in the cereal viruses have been published.

CONCLUSIONS

Veldee and Fraenkel-Conrat (8) have suggested that the concentration of infectious virus in a host is more representative of the viability of a strain than are the more subjective observations of the nature of symptoms, usually referred to as "virulence." Using their definition, strain #18 of BSMV was the most virulent of the strains tested. Symptoms of the strains did not always depend on titer, as strain #18 of BSMV reached a higher virus titer than did strain #110, yet resulted in less stunting and in symptoms of a less severe nature than those produced by strain #110.

Single leaf assays also showed that symptom severity was not proportional to virus titer. Leaf four, which had the highest virus titer of the leaves tested, showed only striping symptoms, while other leaves which had a lower virus titer showed severe mottling and chlorosis. Investigations designed to determine the relationship be-

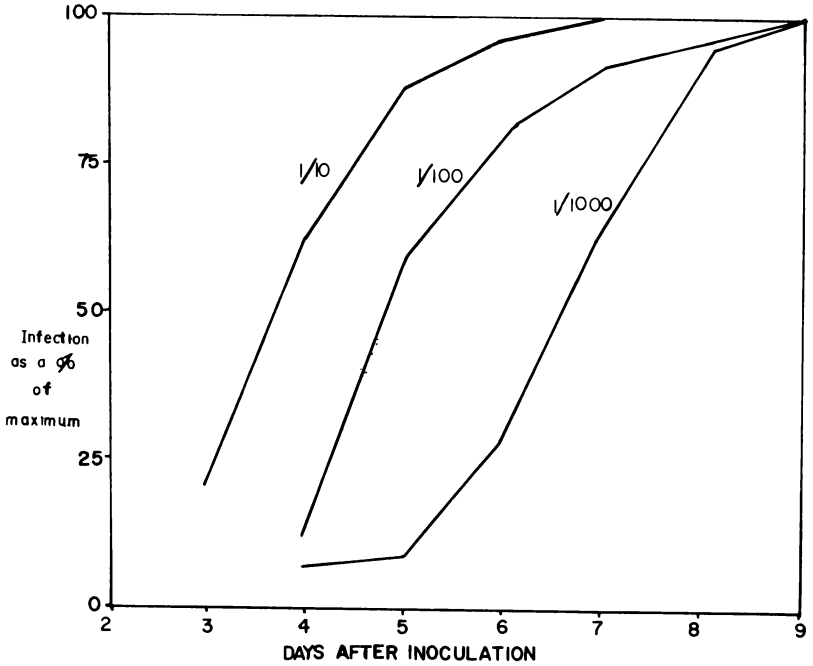


FIGURE 4—Percentage of Black Hulless barley plants showing symptoms at daily intervals after inoculation with three dilutions of type strain of barley stripe mosaic virus.

tween virus titer in each leaf with chlorophyll degradation, ribonuclease levels, and plant growth rates are now in progress.

Comparisons of titer values of the strains in each host showed that a particular strain in a susceptible host often had a 10-fold increase in virus over the titer in a resistant host. This is most applicable with the more virulent strains. A strain that occurs in relatively low titer in a susceptible host only had a two- or three-fold increase over the titer in a resistant host.

It was also observed that regardless of host, the strains were ranked in the same order in each case. A similar situation existed for rank of hosts, regardless of strain. It is interesting to note, however, that type strain had a higher virus titer in the susceptible varieties than did strain #110, yet was unable to infect Moreval or C. I. 3212-1. Strain #110 easily infected these two resistant varieties. Thus the relationship of type strain with Moreval and C. I. 3212-1 is of a different nature than all other situations examined.

LITERATURE CITED

1. Anonymous. 1965. Losses in agriculture. USDA Ag. Handbook No. 291. 120 p.

2. Brakke, M. K. 1958. Properties, assay, and purification of wheat streak mosaic virus. Phytopathology 48:439-445.
3. Haunold, E. 1958. Factors affecting the virus content of cheyenne wheat infected by wheat streak mosaic virus. Phytopathology 48:411-414.
4. Hooker, W. J., and A. P. Benson. 1960. Time of symptom response in *Datura tatula L.* to potato virus X as a function of virus concentration. Virology 10:245-256.
5. Kankam, J. S. Jr., and D. C. Arny. 1960. Effect of temperature on concentration of barley stripe mosaic virus in susceptible and tolerant varieties. Phytopathology 50:631. (Abstr).
6. Kassanis, R., and J. T. Slykhuis. 1959. Some properties of barley stripe mosaic virus. Ann. Appl. Biol. 47: (2) 254-263.
7. Timian, R. G., and W. W. Sisler. 1955. Prevalence, sources of resistance, and inheritance of resistance to barley stripe mosaic virus. Plant Disease Reptr. 39: 550-551.
8. Veldee, S., and H. Fraenkel-Conrat. 1962. The characterization of tobacco mosaic virus strains by their infectivity. Virology 18: 56-63.

A FLOOD-FREQUENCY GRAPH BASED ON TREE-SCAR DATA

Samuel S. Harrison and John R. Reid

Department of Geology

University of North Dakota, Grand Forks, North Dakota

Third Place Winner

A. Rodger Denison Student Research Competition

INTRODUCTION

Many trees with scarred trunks are found growing on the flood plain of the Turtle River in eastern North Dakota. The scars, which range in length from a few inches to several feet, commonly reach a height of 10 to 12 feet above the flood plain in Turtle River State Park, near Arvilla, North Dakota (Figure 1). Because the scars are present only on the upstream side of the trunks and occur at generally accordant heights on adjacent trees, it is apparent that the scars resulted from abrasion during floods (Figures 2 and 3). Theoretically, a study of the scars would reveal their age, which along with their height, might provide sufficient data for construction of a flood-frequency graph for the river. To test the validity of this hypothesis, flood data obtained from the scars were compared with stream-gage records from the U.S. Geological Survey gaging station at Manvel, about 20 miles downstream from the study area (Figure 1). If a usable flood-frequency graph can be obtained from tree-scar data it will provide a means of determining the frequency and depth

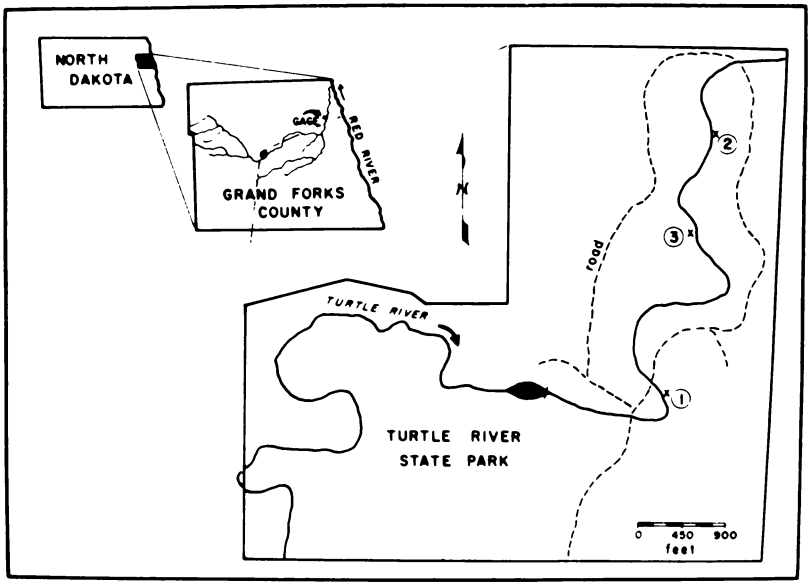


FIGURE 1—Map showing location of study area. Note location of trees collected (shown by numbers) and Manvel gaging station (on county map).

of flood plain inundation on streams where gaging stations are not maintained.

Previous work.—As early as 1848 David Dale Owen (1852, p. 177) noted an abundance of scars on trees bordering the Red River near Grand Forks, North Dakota. A more recent study by Sigafoos (1964) involved analysis of flood-scarred trees near Washington, D.C. He found that flood scars along with other botanical evidence provided a reasonably complete flood record for the Potomac River. Sigafoos, however, worked primarily with fallen and buried trees and shrubs instead of scarred trees as were used exclusively in this study. Furthermore, he did not attempt to construct a flood-frequency graph from the data.

Origin of scars and factors affecting their formation.—The bark on the upstream side of tree trunks is frequently removed by abrasion during floods. Because flooding typically occurs during March or April in this area of North Dakota, most of the abrasion is accomplished by floating ice. Once the trunk is damaged, new wood and bark begin to grow over the scar. The number of annual rings formed subsequent to the scarring thus provides a simple means of dating the flood which inflicted the damage (Sigafoos, 1964, p. 3).

Assuming the abrasion occurred in March or April, rapid growth

FIGURE 2—Ice-scarred trees on the flood plain of the Turtle River in Turtle River Park, eastern North Dakota. Note accordant level of scars on adjacent trees. Tree 1 in foreground. View facing downstream.

during the early part of the ensuing growing season results in a ring of relatively large cells. Growth usually becomes progressively slower throughout the growing season and smaller cells make up a relatively darker layer, thus completing the annual ring. Trees such as oak, ash, hickory, and elm have especialy sharp annual rings due to the great difference in early and late growing season growth rates. Such trees as cherry, maple, and birch, however, have cells of about the same diameter throughout the entire annual ring, making the rings difficult to differentiate. Two elm trees and one cottonwood were studied in detail in this investigation. The annual rings in cottonwood, which is intermediate between the two types described above (Brown, *et al.*, p. 517, 549), were often difficult to distinguish.

Although the scars appear to provide a simple means of dating the floods, several factors were recognized in the course of this study which detract from the accuracy and completeness of the resulting record. For example, scars do not necessarily mark the maximum height of the flood. If, as is often the case, the river ice breaks up

FIGURE 3—Closer view of tree 1 (cottonwood). Exposed scars on this tree provide a record of seven different floods; buried scars date back to about 1909.

before the flood crests, the resulting ice scars may be significantly lower than the flood-crest level. Observation of the March-April, 1966, flood in the study area revealed that some of the new scars were several feet lower than the observed flood crest. In addition, much of the abrasion from the 1966 flood, which was the largest flood on the Turtle River in the past fifteen years (U.S. Geological Survey unpublished gage records), removed only a thin layer of bark and is therefore unlikely to produce distinct scars. The intensity of scarring thus does not necessarily reflect the magnitude of the flood. In addition, not every flood will cause scarring. Summer floods, which occasionally occur in June in this area, lack the debris (ice) of the spring-breakup flood and probably inflict little damage to tree trunks.

It was found that the length of relatively complete flood record obtainable by this method is limited by the healing time of the scars. Scars older than about 25 years were not visible from the outside of the tree trunks and were discovered only when intersected by a random cut. The actual time necessary for a scar to heal completely depends on many factors, including the size of the scar and the extent of subsequent scarring of the same trunk area.

PROCEDURE

Three trees growing on the floodplain of the Turtle River were selected for detailed scar dating. Trees with the most severe scarring were located either on the outside of a sharp bend in the river or only a few feet from the edge of the bank. A cottonwood was cut in January, 1966, and two elms were collected in April, following the spring breakup flood of 1966.

Prior to cutting, the height and size of all external scars on the trees were measured and photographed. The trees were then felled and cut into sections convenient for transporting. In the laboratory additional sections were cut in a search for completely healed scars. The surfaces of all sections were sanded and coated with clear shellac to make the annual rings more distinct.

The age of each scar, and hence the flood which caused it, was determined by counting the number of annual rings that had grown over the scar (Figure 4). Counting was done with the aid of low magnification. Annual rings in the elm trees (trees 2 and 3) were more distinct than in the cottonwood (tree 1). Despite this, the ages obtained for scars on the cottonwood are believed to be within one year of the true ages.

RESULTS AND INTERPRETATIONS

The year and height of individual scars are depicted in Figure 5. Scars of uncertain exact age are shown by the symbol "0". The height of each scar was measured from the top of the scar to the datum. The river surface at about midday on April 30, 1966, was arbitrarily chosen as the datum. The height above the datum of the base of each of the three trees was determined with a hand level.

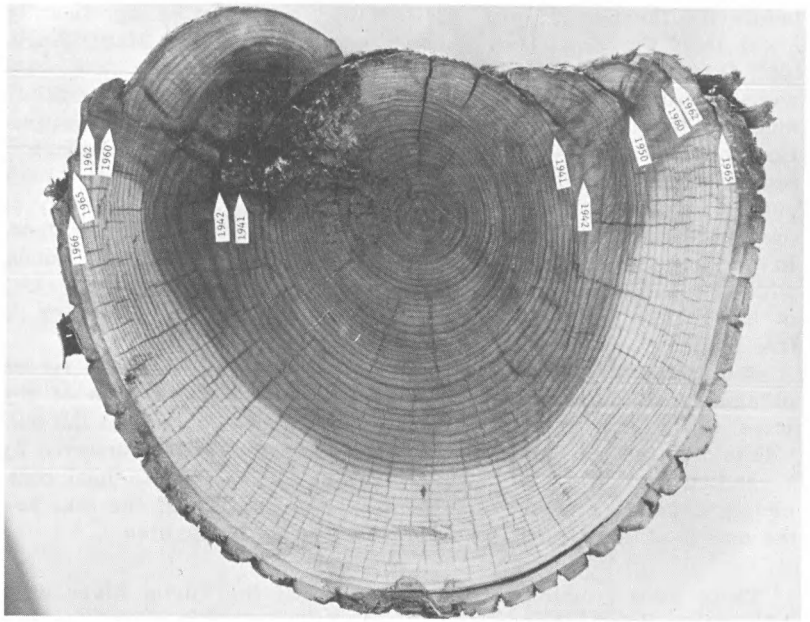


FIGURE 4—Section through tree 3 (elm) showing dates of scars. Flood causing these scars were at least 5 feet above the flood plain and 7½ feet above the datum. Section is 11½ inches wide.

The gage reading for that day at Manvel, about 20 miles downstream, was 6.9 feet. Recorded heights of peak annual crests have been corrected to the April 30 datum by subtracting 6.9 feet from the reported gage readings.

Despite the numerous variables which affect formation and preservation of scars, scars from the same year usually occur on at least two of the three trees studied. It appears that examination of more than three trees would have improved the record only slightly. Of the 21 years of gage records (1946 through 1966) there were 12 floods higher than 7 feet above the datum. Of these, there were only two for which no corresponding scars were found. Only one scar (1953) was found for years having annual floods less than 7 feet above the datum (Figure 5).

Only three scars formed prior to 1941 were found, the oldest having formed in 1909. The relative scarcity of older scars is probably due to a combination of several factors: 1) scars older than about 25 years are completely healed and less likely to be detected, 2) the 1930's were brought years in this area, thus annual floods were relatively small, and 3) of the three scars prior to 1941, two

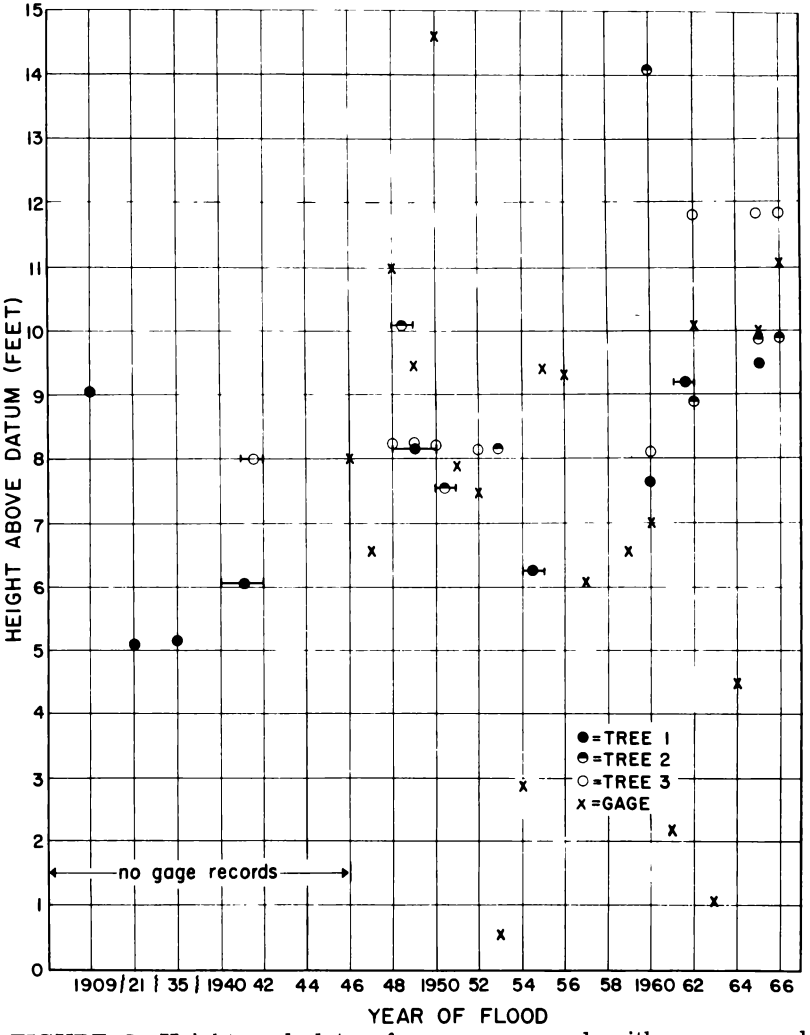


FIGURE 5—Height and date of scars compared with gage-record crests. Scars of uncertain exact age shown by "0".

were less than 5½ feet above the selected datum, which is below the base of tree 2 (as determined by hand level).

The difference in the relative height of scars of the same age on different trees ranges from a few inches (1950) to more than six feet (1960), though most are within two feet of each other. The most likely cause of large discrepancies in scar height is probably local damming by ice and debris.

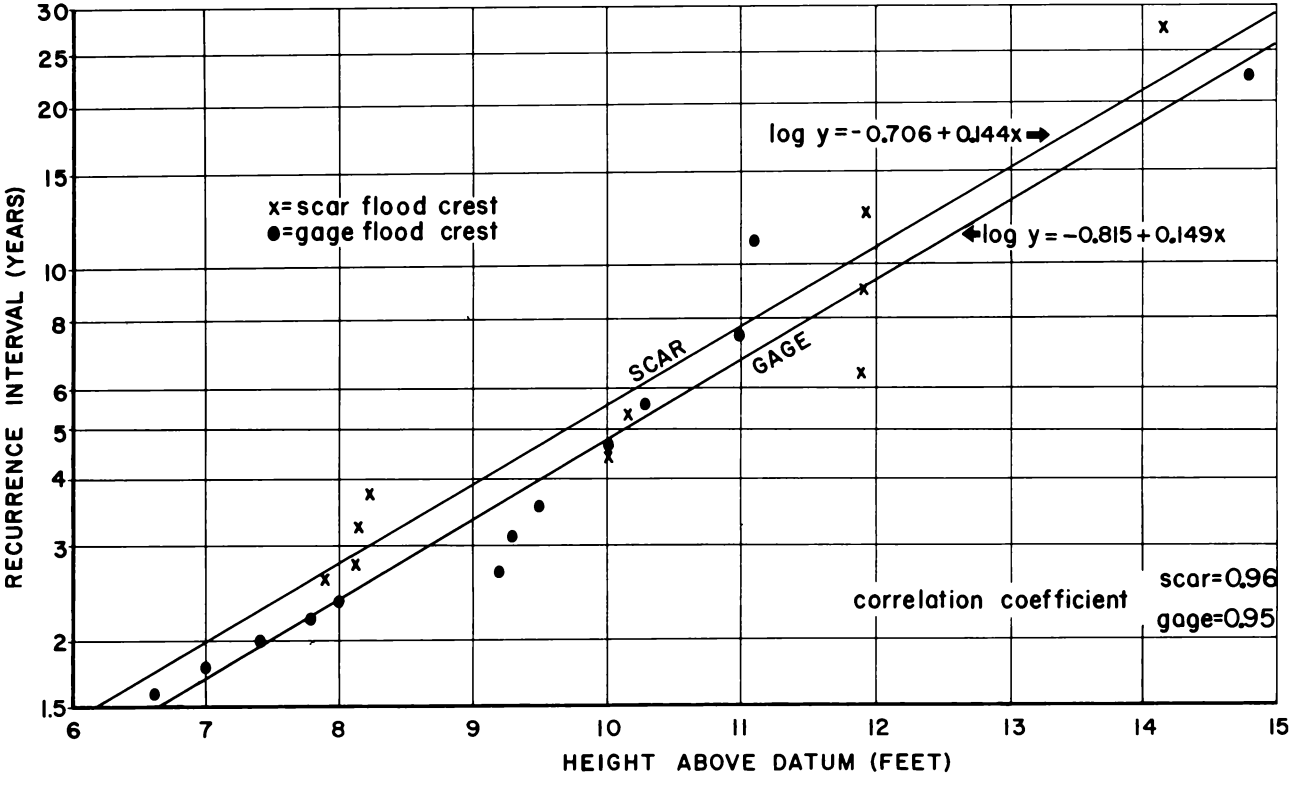


FIGURE 6—Flood-frequency graph for the Turtle River based on scar data and gaging station records.

In most cases the height of individual floods as indicated by scars is within a foot or two of the crest recorded at the gaging station 20 miles downstream. An exception to this is the 1950 flood, the scar for which is about seven feet lower than the reported crest. This difference probably resulted from the fact that the ice in this area broke up several days before the actual crest of the 1950 flood (Q. F. Paulson, Geologist in Charge, U.S. Geological Survey, Bismarck, North Dakota, oral communication). Scars significantly higher than the gage crests can best be attributed to local ice damming within the study area.

In order to construct a flood-frequency graph from these data it was first necessary to obtain a single height for each flood. Where a scar of the same age occurred on more than one tree, the highest scar was used because it is assumed to have been closest to the maximum height of the flood. In constructing the graph (Figure 6), only exposed scars (post-1941) were used because the sampling of completely healed scars is random and therefore not representative. If desired, however, dates from these older (healed) scars could be used to extend the graph if the procedure for plotting historical floods were followed (Dalrymple, 1960, p. 17).

Values for the recurrence interval, which is the average lapsed time between the occurence of two floods equaling or exceeding a given height, were calculated using the U.S. Geological Survey method (Dalrymple, 1960, p. 16).

$$T = \frac{n + 1}{m}$$

where T = recurrence interval in years,
n = number of years of record, and
m = rank of flood (based on height), the highest being 1.

The number of years of record for the scar dates was 25 years; for the gaging station, 21 years. Table I shows the rank and height of scars and gage crests for the period of record of each.

To construct the flood-frequency graph (Figure 6), recurrence interval versus height of the floods was plotted on semi-logarithmic paper with the recurrence interval plotted on the log scale as suggested by Dalrymple (1960, p. 17). Regressions analyses were made using the least-squares method to find the curves which best fit both sets of data. Correlation coefficients of 0.96 and 0.95 for the scar and gage data, respectively, indicate a close fit of the points to each of the curves. The value of the recurrence interval at the origin of the graph was selected as 1.5 years because this recurrence interval theoretically corresponds with the recurrence interval of the bankfull stage of most rivers (Leopold, *et al.*, 1964, p. 319).

The high degree of similarity between the two curves results in nearly idnetical values of flood frequency for the heights given. For instance, at a height of 14½ feet above the datum, the highest flood recorded, the predicted recurrence interval is 20 years for the gage curve and 22 years for the scar curve. It appears, then, that a

TABLE I

RANK OF FLOODS AS INDICATED BY SCARS AND GAGE RECORDS

Year	Rank of Gage Crests	Rank of Scar Crests
1966	2	2
1965	5	4
1964	16	
1963	19	
1962	4	3
1961	18	
1960	12	1
1959	13	
1958	21	
1957	15	
1956	8	
1955	7	12
1954	17	
1953	20	9
1952	11	8
1951	10	11
1950	1	7
1949	6	6
1948	4	5
1947	14	
1946	9	
1941	beginning of gage record	10

reasonably accurate flood-frequency graph can be constructed from tree scars. Accuracy of either of the curves in predicting large infrequent floods (greater than 30 years recurrence interval) is probably poor, however, because the period of record is relatively short.

This graph (Figure 6) differs from the usual flood-frequency graph, which is based on gage data, in that the magnitude of the flood is expressed in height rather than discharge. The type of graph shown here is especially appropriate, however, if one wishes to know the frequency that an area bordering a river will be flooded and to what depths it will be inundated. Hydrologic information of this type is essential for land use and construction planning on flood plains, but has heretofore often been unobtainable in ungaged drainage basins. An additional merit of the scar-dating method tested here is its simplicity. Scars provide easily observable evidence of the height of past floods even to the casual observer.

SUMMARY

Flood-abrasion scars on tree trunks provide visual evidence of the height of past floods. The age and height of scars on three trees

taken from the flood plain of the Turtle River provided sufficient data for construction of a flood-frequency graph based on 25 years of record. The resulting graph is very similar to one based on gage records for the same river. For instance, the predicted height of a 30-year flood as determined on each of the graphs is within one-half foot of each other. From the results of this study it thus appears that a flood-frequency graph based on scar data can provide a useful means of determining the frequency and depth of flood plain inundation along ungaged streams, provided, of course, that the conditions for tree scarring are present.

ACKNOWLEDGMENTS

We wish to express our appreciation to Mr. James R. Kittle, North Dakota Park Service, and Mr. Vernon Thoren, Senior Park Ranger at Turtle River State Park, for allowing us to fell and remove trees from the park. We also thank Dr. Robert L. Nichols of the Tufts University Department of Geology for encouraging us to undertake this study and for bringing to our attention the work by Sigafoos. Several students and faculty members of the University of North Dakota Geology Department provided assistance in the form of physical help and discussions, for which we are also grateful.

REFERENCE CITED

Brown, H. P., Panshin, A. J., and Forsaith, C. C., 1949, Textbook of Wood Technology; Vol. I: McGraw-Hill Book Co., Inc., N. Y., 652 p.

Dalrymple, Tate, 1960, Flood Frequency Analysis: U. S. Geological Survey Water Supply Paper 1543-A, U. S. Govt. Printing Office, Washington, 80 p.

Leopold, L. B., Wolman, M. G., and Miller, J. P., 1964, Fluvial Processes in Geomorphology: W. H. Freeman and Co., San Francisco, 522 p.

Owen, David Dale, 1852, Report of a Geological Survey of Wisconsin, Iowa, and Minnesota; and incidentally of a portion of Nebraska Territory: Lippincott, Grambo and Co., Philadelphia, 638 p., 80 fig.

Sigafoos, Robert S., 1964, Botanical Evidence of Floods and Flood-Plain Deposition: U. S. Geological Survey Professional Paper 485-A, U. S. Govt. Printing Office, Washington, 35 p.

DESIGN OF A HYPERBARIC TEST FACILITY

S. Ehrick, R. E. Thompson, H. L. Dowell, Jr., and T. K. Akers

Departments of Mechanical Engineering;
Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In recent years there has been much emphasis placed on the ability of many to survive in an alien physical environment. Recent

tests have demonstrated that this survival is possible, but little else is known. It is therefore the purpose of this study to determine which parameters and conditions affect animal behavior during other than normal (atmospheric pressure and temperature) conditions. The object of this phase of the investigation is to design and construct a hyperbaric test facility for the evaluation of these parameters using rats.

A facility of this type should provide for (1) residence of up to one year while under continuous pressure, (2) provisions for maintaining up to 450 psi (30 atmospheres) internal pressure, (3) viewing ports so that visual observations are possible, (4) instrumentation so that measurements of test animal conditions can be made. These would include (1) chemical and physical, (2) gastro-intestinal, (3) nervous system, and (4) whole animal parameters. Instrumentation of the environmental system should monitor chemical and physical conditions of the atmosphere, as well as light and sound parameters.

The facility which has been designed and is now under construction consists of two chambers with a connecting passageway in an H-configuration. This passage-way can be closed manually by the operator so that the two chambers are isolated. This allows one chamber to be depressurized and opened so that food and water may be added, test instruments added or removed, or animal waste removed.

The larger of the two chambers is cylindrical in shape and measures 16 inches in diameter by 36 inches in length. It contains two round viewing ports, one 16 inches in diameter located at one end; it serves also as a door. The other is 4 inches in diameter and is positioned so that the animal may be observed while in the passageway. The smaller chamber is also cylindrical in shape but measures 6 inches in diameter and 27 inches in length. It contains one round viewing port which serves as a door.

Each chamber is equipped with a nozzle and blind flange through which electrical and fluid connections for test purposes are made. Other connections such as those for pressurization and ventilation are made through the chamber wall.

INTRODUCTION

This project is, one of the first of its type to be undertaken at this University, and has required the cooperation of the Departments of Physiology and Mechanical Engineering. Basically, this equipment will be used for physiological research and is being designed and constructed by the Department of Mechancial Engineering. This approach to engineering problems is necessary when it is desired to integrate the design of a particular apparatus with the discipline with which it will be used.

DESIGN OF HYPERBARIC TEST FACILITY

The design of the complete apparatus has been divided into two main areas. The first of these was the design of the chamber itself,

exclusive of any support equipment. In the initial concept, the chamber was visualized as a transparent cylinder with metal ends. This would provide maximum visibility. This was found to be an impractical construction due to the internal pressures involved. The present form which is an all-steel construction was then adopted. This allowed the use of fittings typical for high pressure piping systems. These, together with the appropriately sized sections of pipe comprise the body of each of the chambers. Schedule 40 pipe and forged steel fittings are used throughout. These may be welded directly to form a closed tank. The hatch consists of two steel flanges with a acrylic plastic viewing port between them. The acrylic plastic is a permanent part of the outer flange, so that when the bolts are removed, the flange and the acrylic plastic are removed as one unit. This allows the same unit to serve as both a hatch and a viewing port. In the closed position the hatch is sealed with a neoprene ring and is held in place with the flange bolts. To open the hatch, the bolts are removed and the hatch rolled to one side on a track provided for that purpose.

During operation, the chambers are usually isolated from each other. When it is desired to move the animal between chambers, the connecting passageway is opened.

The device which permits opening and closing the passageway at will is simply a gate valve, which has a four inch bore. This size is sufficient to allow the passage of an animal under test. A valve was chosen since it was judged to be the most reliable and simplest device available for the purpose. Reliability of the valve is extremely important since it is the only part which is common to both sections, and its failure would result in the termination of the experiment.

Instrumentation is perhaps the most important phase of a test of this type. Data will be extracted by two methods: (a) information will be telemetered directly from the animal to antennae mounted within the chambers, (b) data may be secured or cue signals given via conductors that pass through blind flanges which consist of round steel ports bolted in place. These may contain any number of through-wall conductors, fluid as well as electrical, and may be replaced with different flanges for subsequent tests. These flanges may have test apparatus or food dispensers mounted directly on them. This would permit, for example, different types of food dispensers for different series of tests.

The atmosphere conditioning system which was not part of the design effort but which will be incorporated in the finished project will be capable of maintaining temperatures from 4°C to 40°C continually as well as controlling humidity and atmosphere gas composition.

The internal ftitings will include food dispensers and various test apparatus previously mentioned, and also such things as a floor grate. This would allow both feces and urine to fall below where

they would drop into a tray and be removed periodically. The floor grate would be electrically insulated from the body of the chamber and have electrical connections outside the chamber. This would be useful in test situations to insure the movement of the animal into the passageway. Lights will also be installed within the chamber. Physiological tests have not been completed as of this date, but all indications are that a standard lamp will suffice in this application. In particular, a 6-watt 120 volt lamp is capable of withstanding a static pressure of over 1000 psi inch with no damage.

The entire structure including chambers, passageway and atmosphere conditioning system will be mounted in a carriage with wheels permitting some mobility. Preliminary work has begun on the chambers and the entire apparatus should be operational before September 1967.

THE SYNTHESIS AND POLAROGRAPHIC ANALYSIS OF SUBSTITUTED 9-BENZALFLUORENES[1]

Charles DeRemer, Doris Rutten and Vernon J. Feil[2]

Department of Chemistry
Jamestown College, Jamestown, North Dakota

INTRODUCTION

Monosubstituted 9-benzalfluorenes are conjugated hydrocarbons having the structure indicated in Figure 1, where X represents various substituents. The purpose of this study is to polarographically determine the ease of reduction of the double bond in a series of substituted 9-benzalfuorenes, and to correlate the half-wave potentials thus obtained with the Hammett σ values of the substituents

Polarography is a form of electrolysis whereby one may determine the potential required to reduce a compound under a given set of conditions. The value of the half-wave potential thus obtained is dependent upon the pH, the buffer system, the supporting electrolyte, the ionic strength, the solvent, and several other factors. However, the halfwave potentials for solutions of the 9-benzalfluorenes were determined under very similar conditions, and therefore the half-wave potentials obtained should be a measure of the relative ease of reduction of the compounds.

DISCUSSION

Several of the 9-benzalfluorenes may be synthesized by reacting the appropriate benzaldehyde with a solution of fluorene and sodium

[1]This study was supported in part by a National Science Foundation Academic Year Extension of the Research Participation Program for College Teachers grant.

[2]Present address, Metabolism and Radiation Research Laboratory, State University Station, Fargo, North Dakota.

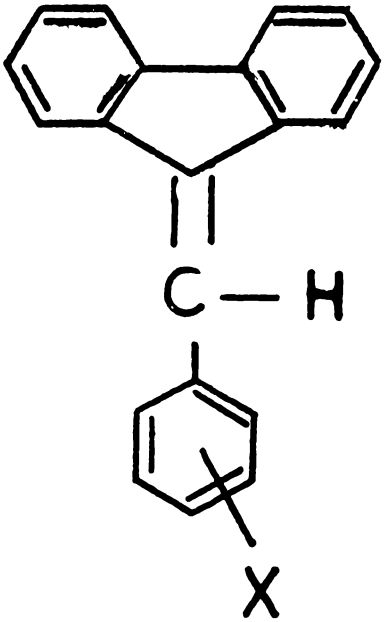


FIGURE 1—

ethoxide in ethanol (1). The o-, *m*-, and *p*-methyl, *m*- and p-chloro, *m*- and *p*-bromo, *p*-methoxy, *m*- and *p*-fluoro, and unsubstituted 9-benzalfluorenes were prepared in this manner, but attempted preparations of the other *ortho* compounds by this procedure were unsuccessful. The *ortho*-substituted 9-cinnamylidenefluorenes were isolated instead. This reaction was reported by Fletcher, Namkung, Dice, and Schaefer, and is thought to involve the initial oxidation of ethanol to acetaldehyde with subsequent condensation to form either 9-ethylideneflurene or an *ortho*-substituted cinnameldehyde or both (2). The 9-ethylidenefluorene might then condense with the benzaldehyde or the *ortho*-substituted cinnamaldehyde with fluorene in the basic media to yield the *ortho*-substituted 9-cinnamylidenefluorene. *p*-Hydroxy-9-benzalfluorene was obtained by demethylation of the corresponding methoxy derivative (3). Preparations of the remaining compounds studied in this paper were effected by exposing the appropriate benzaldehyde to the ylid formed from the salt of 9-bromofluorene and triphenylphosphine (2).

Polarograms of each of the monosubstituted 9-benzalfluorenes were run and the half-wave potentials evaluated. Table I shows the values obtained. Electron-withdrawing substituents should tend to lower the half-wave potential relative to that of 9-benzalfluorene; i.e., increase the ease of reduction by lowering the electron density

TABLE I

X	Our m.p.	Lit. m.p.	E½	Hammett σ	Ref.
-H	77-8	76	1.68	0.000	6, 13
o-CH_3	107-9	109.5	1.71		1
m-CH_3	50-2	oil	1.68	-0.069	10
p-CH_3	99-100	97.5	1.71	-0.170	1
c-Cl	59-60	59.5-60	1.61		2
m-Cl	89-90	90.5	1.605	+0.373	7
p-Cl	149-50	149.5	1.65	+0.226	7
o-Br	83-4	83.5-4.5	1.56		2
m-Br	93-5	92-3	1.61	+0.391	8
p-Br	144-6	144	1.65	+0.232	8
m-F	92-4		1.62	+0.337	
p-F	116-17	120	1.67	+0.062	9
p-OCH_3	128-9	128-9	1.73	+0.268	11
o-NO_2	125-6	126-7	1.44		
			1.88		2
m-NO_2	113-15	115-15.5	1.26		
			1.89		12
p-NO_2	167-8	167	1.46		
			1.81		9
p-OH	108-11	110-12	1.74	-0.357	3

in the region of the double bond, thus decreasing the potential necessary to force electrons into the comparatively electron-rich region. Conversely, electron-donating substituents should increase the half-wave potential. This reasoning is supported by the data in Table 1. Table 1 and Figure 2 also show that the half-wave potentials obtained for the *m*- and *p*-substiutted 9-benzalfluorenes can be correlated with the Hammett σ values for the substituents.

Polarographic behavior of the nitro-substituted compounds was different than the other benzalfuorenes. The polarograms indicate that at least two separate reductions are taking place between 1.4 and 1.9 volts. One explanation for this behavior might be the reduction of the nitro group below 1.4 volts followed by reduction of the double bonds in the resulting mixture.

The half-wave potentials obtained for the three *o*-substituted compounds compare quite well with those obtained for the corresponding *p*-substituted compounds, indicating that reduction of the double bond at the dropping mercury electrode is relatively insentitive to steric hindrance.

In the reaction of fluorene with o-methyl benzaldehyde, both the benzal and the cinnamylidene compounds were isolated. The n.m.r. spectra of the basic benzalfluorene and cinnamylidenefluorene systems show complex patterns in the aromatic region (7-8 δ) and are of no value in assigning structures; however, a comparison of the

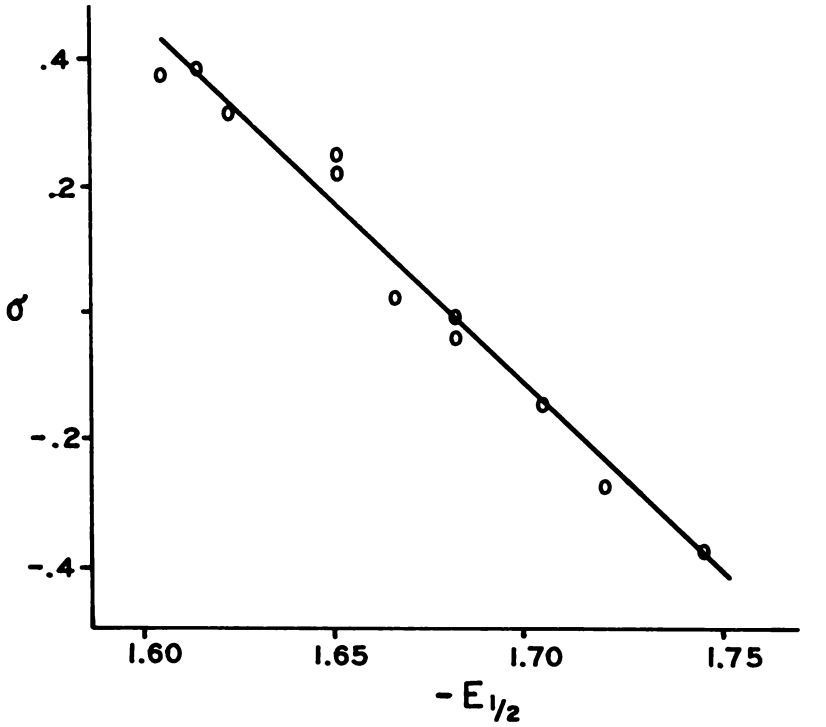


FIGURE 2—Plot of Hammett δ values versus half-wave potentials of *m*- and *p*-substituted 9-benzalfluorenes.

total number of aromatic protons show a 13 to 3 ratio for the benzal compound and a 15 to 3 ratio for the cinnamylidene compound. Also, the polarograms obtained on these compounds quite clearly indicate that more than one double bond is present in the molecule. This appears in the form of a gradual reduction between 1 and 1.9 volts.

EXPERIMENTAL

I. *Appartus.*—The polarographic reductions were accomplished with an Electrochem Auto-Scan Polargraph and a Lab-Line Graphicorder. An H-cell containing a saturated calomel reference electrode and a dropping mercury electrode was used. N.m.r. spectra were obtained in hexadeuterioacetone using tetramethylsilane as an internal standard on a Varian A-60A spectrometer .

II. *Materials.*—Mercury was purified by filtration through a pinhole in filter paper. A layer of ten per cent nitric acid containing mercurous nitrate was then poured over the mercury and filtered air was bubbled through the two layers for three hours, after which

the mercury was washed with distilled water and again filtered through a pinhole in filter paper.

Commercially available fluorene, triphenylphosphine and substituted benzaldehydes were used in the preparation of the 9-benzalfluorenes.

III. *Polarography.*—The utilization of the polarograph on solutions of 0.175 M tetrabutylammonium iodide and 0.001 M 9-benzalfluorenes in seventy-five per cent dioxane-twenty-five per cent water yielded the necessary polarograms. The half-wave potentials were then determined by a graphical method outlined by Delahay (4).

IV. *Synthesis of substituted 9-benzafluorenes.*—
Procedure 1. The *o*-methyl together with the *m*- and *p*-substituted 9-benzalfuorenes were prepared according to the procedure outlined by Sieglitz (1).
Procedure 2. The *o*-bromo, o-, *m*-, and *p*-nitro, and *o*-chloro-9-benzalfluorenes were prepared according to the procedure outlined by Johnson *et. al.* (2).

V. *Demethylation of p-methoxy-9-benzalfluorene.*—p-Hydroxy-9-benzalfluorene was obtained by demethylation of *p*-methoxy-9-benzalfluorene using the method reported by Allen *et. al.* (3).

VI. *Synthesis of 9-bromofluorene.*—9-Bromofluorene was synthesized from fluorene according to the procedure in "Organic Synthesis" (5).

SUMMARY

In summary, it can be said that electron-withdrawing groups tend to increase the ease of reduction, and electron-donating groups tend to make the reduction of substituted 9-benzalfluorenes more difficult. The half-wave potentials of the meta- and para-substituted 9-benzalfluorenes were found to correlate with the Hammett σ value of the substituent.

ACKNOWLEDGEMENT

The authors are indebted to Donald Kubik for his assistance in the completion of this project.

REFERENCES

1. Adolph Sieglitz, Ber. *52*, 1515 (1919).
2 T. Lloyd Fletcher, Mose J. Namkung, John R. Dice, and Sandra K. Schaefer, J. Med. Chem. *8* 347-50.
3. R. E. Allen, F. P. Palopoli, E. L. Schumann, W. C. Day, and V. J. Feil, U. S. Patent 2,891,957.
4. Paul Delahay, *Instrumental Analysis,* 1st ed., p. 88, MacMillan Company, New York (1957).
5. *Organic Synthesis,* John Wiley & Sons, New York, Vol. 23, p. 100.
6. J. Thiele and F. Henle, Ann,. *347,* 290 (1906)
7. Sieglitz, p. 1516.
8. Sieglitz, p. 1235.

9. E. D. Bergmann, Y. Hirshberg, D. Lavie, Y. Sprinzak, and J. Sznuszkowiez, Bull. soc. chim. France *1952*, 703-9
10. G. Defazi, *Beilstein* 5 I 334, II 649.
11. J. Thiele and F. Henle, p. 300.
12. V. M. Ingram, J. Chem. Soc., *1950*, 2318-24
13. Stanley Wowzonek and Joyce Wangfan, J. Am. Chem. Soc. *68*. 2541-2544 (1946).

EXPERIMENTAL MAGNETOTROPIC PROOF FOR THE GEOTROPIC EFFECT IN PLANTS

Robert H. Rotering, Jr.

Department of Biophysics
St. Mary's High School, New England, North Dakota

Winner, Ralph E. Dunbar Award,
North Dakota High School Science Competition

ABSTRACT

Is it true that the geomagnetic field has a definite effect upon plant life? The extent and variations of these effects are debated, therefore, research was conducted to determine the effect, if any, of the geomagnetic force on plant life.

The effects of the geomagnetic forces as well as induced magnetism were studied. Dormant seeds were exposed to an induced magnetic force to determine whether or not a growth difference could be observed after planting. Both types of magnetism were used to see if there is such a phenomena as a magnetotropic effect in root systems. Other experiments were carried on to study the effects of induced magnetism on early plant growth. Studies were also made to determine which duration of pre-seeding exposure is best, if the moisture content of the dormant seeds during exposure would alter the magnetic effect and to see if the angle at which the lines of flux permeated the dormant seed made any difference.

It was found that magnetism produced healthier and faster growth as well as stronger root systems. Seeds that are exposed to an induced magnetic field before germination, store this "super growth" property indefinitely even when removed from the induced magnetic influence. The higher the moisture content of the seed, the greater the effect seems to be. Growth responses were greatest when the lines of flux were permeated parallel to the long axis of the seeds. It was found that the property which speeds growth is stored indefinitely by the seeds. Seeds that are oriented with their long axis parallel to the geomagnetic force also grow better than those which are randomly arranged. Growth was also incited in seeds which appeared dormant in germination tests by the use of induced magnetism.

Growth responses of approximately the same degree and nature were obtained when the magnetic treatment during germination was relative to the geomagnetic lines of force or those of an introduced magnetic field. The hypotheses which have resulted from this research for the effects are; migration of plant auxins caused by magnetism, magnetic interference with the electric fields generated by the plants and the movement and concentration of indoleacetic acid caused by the stress of the magnetic field.

It appears, therefore, that a magnetic field of low intensity may be an effective way to stimulate, excite and increase plant germination and growth.

GLACIERS—"LIVING AND DEAD"

John R. Reid

Department of Geology

University of North Dakota, Grand Forks, North Dakota

Invited Paper

ABSTRACT

Of the approximately 10,000 square miles of dead ice topography in North Dakota, almost 9,000 square miles form the surface of the Missouri Coteau. The rugged terrain, the numerous perched lake plains, and the highly disturbed character of many deposits was explained by the hypothesis that these features and sediments were deposited in direct association with a huge mass of detached ice buried beneath ablation drift.

The Martin River Glacier, south-central Alaska, was selected to test the validity of the hypotheses proposed for these dead-ice features. The ablation till covering the lower 4½ miles of this glacier varies from a fraction of an inch thick 4½ miles from the terminus to 15 to 30 feet thick in the stagnant zone just below the terminus. This drift is constantly sliding and flowing into adjacent depressions, thereby inhibiting ablation of the underlying ice and exposing ice under the higher areas to more rapid ablation.

Drift accumulating in the numerous ice sinkhole lakes on this glacier could, under ideal conditions, become "moulin kames." But, the sediments here contain more silt and clay than most kames, and no kames exist in the recently deglaciated parts of the Martin River Glacier. "Moulin kames," therefore, do not normally form in ice sinkhole lakes, but they may form in similar depressions containing no ponded water. Instead, the meltwater and runoff flow into these depressions and out through the bottom, leaving the coarse sands and gravels behind. The absence of such kames in this area is explained by the very high precipitation here.

When the ice walls of these lakes become stabilized through

slope reduction and by burial beneath six or more feet of ablation till, the lake water may become warm enough to support a successful faunal assemblage. In such ice-walled lakes the complete ablation of the bounding ice may cause the lake sediments to be left high above the surrounding areas where relatively clean ice had previously existed. The ice-walled-lake plains on the Missouri Coteau of North Dakota were produced under similar conditions.

Despite a contrast in glacier type and in the climate of the area, the features found on the Martin River Glacier are probably very similar to the features in the process of formation on the Missouri Coteau between 12,000 and 9,000 years ago.

INTRODUCTION

Purpose.—Approximately 10,000 square miles in North Dakota are characterized by very hummocky, poorly-drained topography. This type of topography is interpreted by geologists to have formed during the melting of a debris-covered mass of ice left behind when the active glacier terminus retreated from those areas into Canada. The question of the validity of this interpretation, as well as the interpretation of the origin of certain unusual features in this area, encouraged a team from the Department of Geology at the University of North Dakota to establish a series of research stations in the area of the Martin River Glacier, south-central Alaska, in order to test the hypotheses on an existing glacier. This paper is an attempt to present the problems and reveal how the Alaskan studies have aided in an understanding of the features in North Dakota.

Location areas.—In North Dakota the principal area of hummocky, poorly-drained topography (known as dead-ice topography) extends from the south-central border in McIntosh County to the northwest corner of the State, in Divide County, where it continues into the Province of Saskatchewan, Canada (Figure 1). The width ranges from approximately 15 miles to almost 70 miles and it is bounded along most of the northern and northeastern margin by a topographic escarpment up to 400 feet high, the Missouri Escarpment. This entire area is known as the Missouri Coteau (Coteau du Missouri).

Other lesser areas of dead-ice topography include the Turtle Mountains, along the north-central border of North Dakota, and the Prairie Coteau (Coteau des Prairies), in the extreme southeastern corner of the State.

The chosen glacial counterpart in Alaska is the Martin River Glacier which is located approximately 60 miles east of the town of Cordova, in south-central Alaska (Figure 2).

Previous work.—As far as can be determined, two geologists working for the U. S. Geological Survey in 1950, were the first to guess that the surface of the Missouri Coteau was shaped during ablation of drift-covered ice. They stated that "the Max Moraine (on the Missouri Coteau) may have been deposited largely by ablation . . . Ablation prevailed, blocks of ice were stranded, and kames

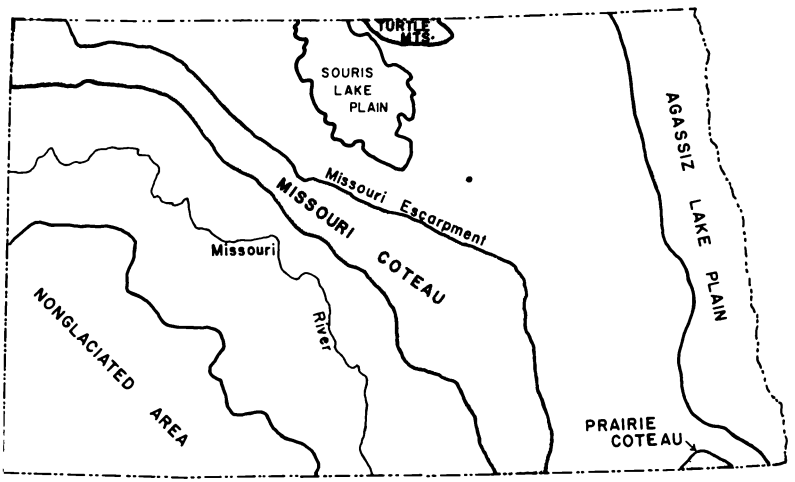


FIGURE 1—Map of North Dakota showing locations of dead-ice moraine (stippled): the Missouri Coteau, the Turtle Mountains, and the Prairie Coteau.

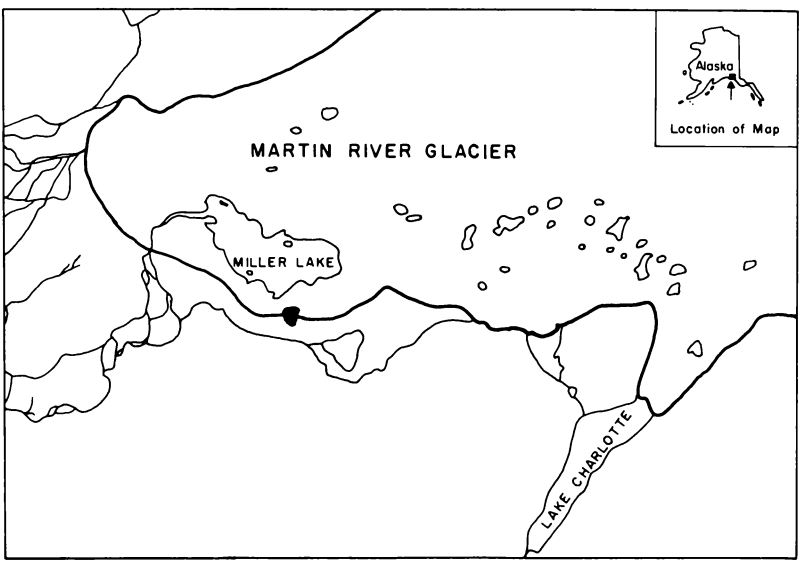


FIGURE 2—Location map of the Martin River Glacier area. The small black area south of Miller Lake is Black Lake.

and eskers, and at least the top portion of the ground moraine were deposited" (Townsend and Jenke, 1951, p. 857). Lemke and Colton (1958, p. 56) later concluded that the surface deposits on the Missouri Coteau "are believed to be stagnation features or 'dead-ice' moraine in many places, rather than distinct end moraines."

But, the most conclusive evidence was presented by Clayton (1962) in his detailed report on dead-ice features in the south-central part of the State. His descriptions and interpretations still stand as the authority for North Dakota dead-ice moraine.

DEAD-ICE FEATURES IN NORTH DAKOTA

Distribution.—The principal area of dead-ice topography in North Dakota is the area of the Missouri Coteau, covering over 9,000 square miles (Figure 1).

Characteristics.—The surface of this area is typically hummocky and pitted with numerous small and large sloughs (Figure 3). Relief

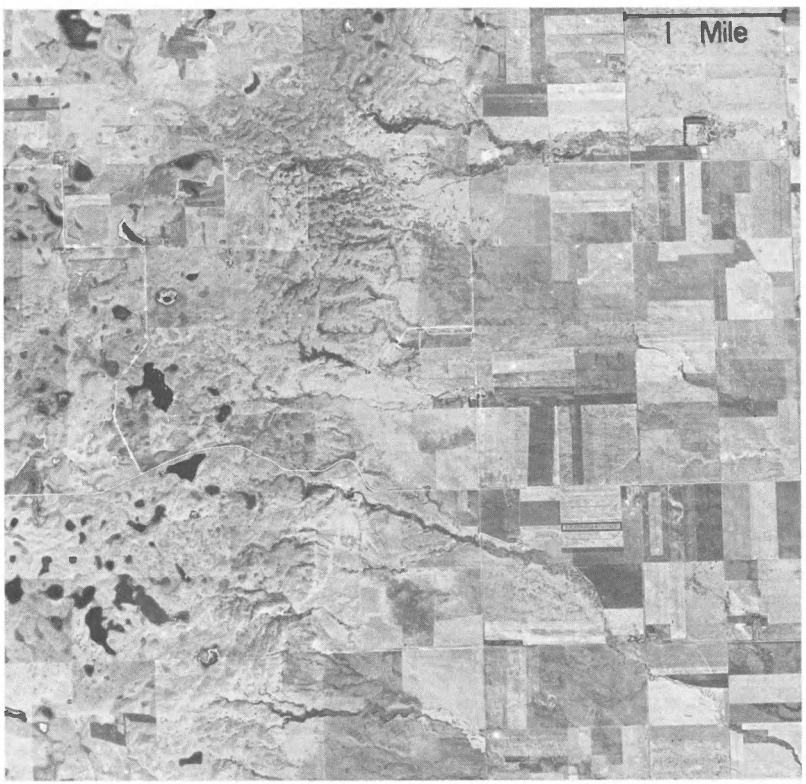


FIGURE 3—Air photo of the Missouri Escarpment showing dead-ice moraine on the left and normal ground moraine on the right. (Photo from Clayton, 1967, Fig. R-9).

probably averages around 70 feet per square mile, but local relief may be as much as 300 feet (Townsend and Jenke, 1951). In most places the surface is underlain by till, but large areas of fluvial sand and gravel and lacustrine clays and silts are very common. Where exposed, the stratified sediments usually display faulted or otherwise disturbed bedding. Large areas, however, contain lacustrine sediments that are essentially undisturbed.

Numerous features found on the Coteau are either rare or completely absent in the rest of the State. Such features include linear disintegration ridges and trenches, circular ridges ("doughnuts") ice-walled-lake plains, and collapse features.

Origin.—The unusual abundance of these features caused early workers to conclude that this entire area was underlain by a mass of ice that had become detached from the late Wisconsin ice sheet as it melted into Canada (Lemke and Colton, 1958; Clayton, 1962). This mass of ice persisted for perhaps 3,000 years after the active ice had left this area because it was buried beneath a mantle of superglacial debris. The debris acted to insulate the ice from solar radiation and from surface waters and rain. But, what was the source of this debris?

As the late Wisconsin ice advanced south and southwestward into North Dakota it ultimately abutted against the Missouri Escarpment, a bedrock escarpment separating the lowlands to the northeast and the higher bedrock surface to the southwest. When the ice was thick enough to override this escarpment the flow became compressive and shearing of the ice occurred. The shearing caused basal sediment to be brought into the ice and as the ice continued to flow over the Coetau it carried the englacial debris with it. The continuous process of ablation slowly lowered the surface of the ice sheet thereby exposing the englacial debris to the surface. As more and more ice melted, the thickness of the superglacial cover increased and the rate of subsequent ablation decreased. At the close of the cycle of glacial activity the relatively clean ice occupying the lowlands to the northeast melted at a much greater rate than the buried ice of the Coteau, and the terminus of the active ice retreated, leaving behind a detached mass of buried ice.

The buried ice melted slowly and differentially. Irregularities in the surface induced mass movement of the superglacial drift causing the sediment to slide and flow into the adjacent depressions, thereby exposing the ice on the higher areas to more rapid melting. In many cases these initially higher areas then became depressions and thus the repository for sediments sliding and flowing from the now higher areas. The process of inversion of the topography continued until all of the buried ice was melted.

Many of the depressions received meltwater from the ablating ice as well as runoff from precipitation. The lakes that resulted were a common feature on the Coteau during the approximately 3000 years after the ice ceased to be active. Early lakes were impounded in the

ice and were both cold and turbid. Later lakes bottomed on the subglacial surface and were protected from the cold ice surrounding the lake by a thick cover of superglicial drift. The water in these lakes was warm enough to support a large population of mollusks and fish, as is evidenced by fossils now found in these sediments. These lakes slowly filled with sediment and when the bordering ice finally melted, the sediments along the margin collapsed. The sediment far enough away from the margins was undisturbed, however, and the sediment was left perched high above the surrounding terrain which had formerly been occupied by ice.

In other areas sands and gravels were deposited on the ice by superglacial streams and rivers. As the underlying ice ablated the sediments eventually came to rest on the subglacial surface, but not before they had been faulted and deformed during differential ablation. Where the buried ice was thick at the time of deposition of the fluvial sediments, the postglacial surface was low. And where the ice was thin, the postglacial surface remained high. The topography has been inverted from what it was either at the time of deposition of the sediments, or, at least, during the last stage of ablation of the dead ice.

Proof for all these statements was lacking in 1960, however. The assumption that fish existed in ice-walled lakes was particularly subject to criticism. The assumption that so large an area as the Coteau was the site of a mass of buried ice that had been left behind the retreating Pleistocene ice sheet was also unusual, to say the least. Prior to 1960 little had been written describing similar featuers elsewhere. Hoppe (1952) described in great detail what he termed "hummocky moraine terrain" in northern Sweden.. Although he was not the first to propose a dead or stagnant ice origin for this area, he did make several important contributions to an understanding of the processes involved. In the United States, Hartshorn (1958) described deposits in Massachusetts which he called "flowtill" and which he concluded had been formed by the flowage of water-saturated till off a stagnant ice mass. Similar deposits and related dead ice features were described by Gravenor (1955) and Gravenor and Kupsch (1959) in western Canada. But, except for one study by Sharp (1949), no study had been made on the relationship of these features to similar features on existing glaciers.

LIVING GLACIER COUNTERPART

General.—In 1962 the National Science Foundation awarded a research grant to the University of North Dakota to initiate a study of the Martin River Glacier in south-central Alaska. The purpose of the research was to examine a glacier that had the appearance of what the ice on the Missouri Coteau was thought to have looked like during late Wisconsin time. The Martin River Glacier was selected because of an extensive drift cover (ablation till) on the lower 4½ miles of the glacier and because of the presence of numerous lakes on the ice.

The glacier is located in south-central Alaska approximately 60 miles due east of the nearest town of Cordova. The glacier originates in the Bagley Ice Field at an elevation of approximately 5000 feet and flows through a low area at the southwest end of the basin. The terminus, 35 miles from this point, is at an elevation of about 400 feet. The lower 15 miles give it the characteristics of an expanded-foot glacier.

Characteristics.—The drift veneer and the superglacial lakes are the most striking features of the Martin River Glacier. The drift covers the entire lower 4½ miles of the glacier surface (Figure 4). It varies from a fraction of an inch in thickness in the upglacial margin of this zone to greater than 15 feet at the terminus (probably it is actually in excess of 30 feet thick here). The particle size ranges from clay to blocks more than 40 feet in diameter. The average particle is probably small pebble in size, but the concentration of larger

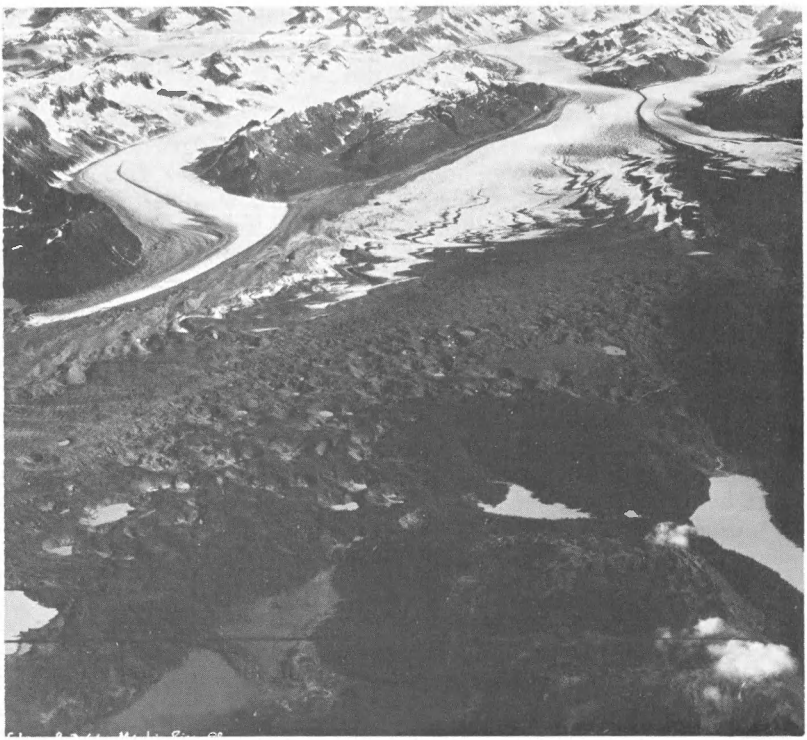


FIGURE 4—Air photo of the Martin River Glacier showing extent of ablation till cover and superglacial lakes. Charlotte Lake is in lower right corner; Miller Lake is in lower left corner. View looking east-northeast. (Photo by Austin Post, Sept. 1966).

particles at the surface is easily misleading; beneath the surface the silts and clays are dominant.

Linear elements visible on the air photos of the glacier were found to represent different lithologies in the drift. Although most of the drift is a mixture of particles of varying lithologies, slate is the sole rock type in some areas, while granodiorite predominates elsewhere.

The variations in lithology of the ablation till are apparent, but are minor when compared to the variations in the relief of the surface. The relief is greatest in the area 1½ to 3 miles from the terminus of the glacier. Here, large depressions characterize the surface. Some, 300 to 700 feet in diameter and up to 350 feet deep, contain 150 feet or more of water (Reid and Clayton, 1962) (Figure 5). Others obviously contained lakes in the very recent past history of the glacier. Lake sediments that settled out of the cold milky meltwater can be found in isolated patches high on the sides of these now-drained depressions. Additional sediment accumulated in the depression as mass movement of ablation till accompanied differential melting of the surface. Continuous sliding of superglacial drift into these de-

FIGURE 5—Ice sinkhole lakes on the Charlotte Lobe of the Martin River Glacier. Charlotte Lake is about 1 mile to the right at the terminus of this lobe. Upper limit of ice here is along the center line of the photo (Aug. 1962).

pressions was a hazard to those working on the slopes or in the lake itself during the four summers on this glacier.

The presence of these depressions, small and large, added to the difficulty of traversing the glacier to carry out glacial studies. But, nearer the terminus, particularly the Charlotte Lobe terminus, another difficulty impeded easy access to the parts of the glacier farther up the valley. Forests of alder, willow, spruce and hemlock growing in the ablation till on the glacier surface were so thick that in most cases a trail had to be cut with a machete. Otherwise, these areas were essentially impassable. Most of these vegetated areas are on relatively stable ice which is flowing at less than 10 feet per year. The ablation till here is usually only 1 to 2 feet thick. One area, however, contained spruce trees 3 feet in circumference and up to 100 feet tall. These 100-year-old trees were growing on only 4½ feet of ablation till. The ice thickness could not be determined, but it exceeded 80 feet, the distance from the upper surface to the bottom of an adjacent depression.

As the ice continues to melt back the ablation till is left unsupported and the till and the trees slide down the slope to the bottom. Most of these trees come to rest either on their sides or upside down with their roots exposed. But, some of them land in an upright position and continue to grow. Most areas in an advanced stage of ablation, however, are characterized by masses of broken dead timber and brush.

The analogy between the development of surface features of the Martin River Glacier and surface features of areas underlain by soluble rock, such as limestone, has been made by Clayton (1964).

Studies.—The research projects that have been carried out in the area of the Martin River Glacier include the following:

1. Detailed topographic mapping of selected areas of the glacier.
2. Chemical and sedimentological analysis of the superglacial and proglacial lakes and comparison with analyses of nearby nonglacial lakes.
3. Botanical and zoological investigation in these same environments.
4. Evaluation of the lithology of the ablation till.
5. Dendrochronological studies of spruce trees in the superglacial, proglacial, and nonglacial environments.
6. Ice movement determinations at selected areas of the glacier.
7. Ablation rate studies and the relation of these rates to meteorological data collected at the camp weather stations.
8. Relationship of partical size and drift thickness to the rates of ablation.

Results.—A clearer understanding of the dead-ice features of North Dakota is a major result of the four summers spent in the area of the Martin River Glacier. The most important ideas concern the

origin of kames, ice-walled-lake plains, and the insulated thickness relationships of ablation till. Each of these is discussed in detail below.

Moulin Kames: Kames are usually defined as conical hills of outwash sands and gravels deposited in depressions in a glacier or as an alluvial cone deposited against a steep ice face at the terminus of a glacier. Examination of superglacial lakes (called ice sinkhole lakes by Reid and Clayton, 1962) has supported the belief that most moulin kames are formed in such depressions. The ice sinkhole depressions on the Martin River Glacier are up to 700 feet in diameter and up to 350 feet deep (Figure 5).

A constant hazard of working in these areas is the continuous washing, sliding and rolling of ablation till into the depressions. Because most of the larger depressions contain lakes, the depressions are also receiving glacier flour which settles out of the milky water. If these depressions were to fill and the ice were to ablate the cone of sediments might be inverted and a moulin kame might result.

But, there is some question as to whether all or even a majority of the ice sinkhole lakes originate through the enlargement of moulins. Two additional mechanisms appear to be worthy of consideration for the origin of the Martin River Glacier depressions: First, some may result from the enlargement of a scarp made by the typically meandering superglacial meltwater streams that are so common on the more active ice immediately upglacier from the area of the ice sinkhole lakes. If the scarp faces south it will receive more direct radiation from the sun than the surrounding level surface and subsequent ablation will widen and deepen the depression. Surface waters will collect here and add to the deepening process through transfer of heat energy. This process will slow down and probably even cease during the colder winter months, but if the depressions are deep and wide enough the water will not freeze solid. Probably the winter ice cover on the typical ice sinkhole in this area is less than 3 to 5 feet thick. (The average January temperature at the nearest U.S. Weather Bureau station at Cordova, 60 miles to the west, is between 0 and -10°C.)

A second possible mechanism of formation of ice sinkhole depressions is by differential ablation of the glacier surface due to an uneven cover of ablation till. The thinly veneered surfaces will ablate at a considerably higher rate than the surfaces covered by a thicker veneer. Once a depression is formed it will increase in size. The relatively warm meltwater and the rainwater will cause additional melting, and the ablation till sliding into the depression will leave a coating of silt and clay on the surface of the ice walls, thereby increasing the radiation absorption and the rate of melting.

If these mechanisms are as important as it is believed, the kames that might be formed upon complete ablation of the ice should not be called moulin kames.

Examination of kames in North Dakota and elsewhere reveals

that sand and gravel is the characteristic lithology; finer sediments are uncommon. But, on the Martin River Glacier fine sediments are an important and even a major constituent of the ice sinkhole lake sediments. There are several possible explanations for this descrepancy: 1) The North Dakota ice sinkholes may not have contained lakes. Water flowing into these depressions may have drained through the bottom carrying away the silts and clays in suspension, leaving the coarser sediments behind. 2) Or, fine sediments may not have been present in the ablation till in North Dakota. This, however, is not the case. The bedrock of North Dakota is characteristically high in silt and clay content.

Another problem is the fact that no kames have been found in the deglaciated parts of the Martin River Glacier area. This is explained by the difference in climates of the two areas. North Dakota, during the waning stages of glaciation, 12,000 years ago, probably had a climate that was less continental than presently. Whereas annual precipitation now is between 15 and 20 inches, it probably was between 35 and 40 inches then. Summers were undoubtedly cooler, but winters were probably slightly warmer because of the effect of the topography produced by the mass of glacier ice. In contrast to this, the average annual precipitation on the lower areas of the Martin River Glacier is in excess of 100 inches. The destruction of isolated mounds by running water, solifluction and mud and earthflow is major here and the probability that kames could remain after deglaciation is exceedingly small.

Ice-walled-lake plains: A fairly common feature in the dead-ice moraine of the Missouri Coteau is the ice-walled-lake plain (Clayton, 1962, p. 39). These plains are identical or very similar to other features called "moraine plateaus" (Hoppe, 1952, p. 5), "dead-ice plateaux" (Stalker, 1960, p. 5), "prairie mounds" (Gravenor, 1955, p. 475), and "perched lacustrine plains" (Winters, 1963, p. 40).

In North Dakota these plains are usually flat-topped mounds up to 12 square miles in area, 15 to 100 feet high, and underlain by as much as 50 or 100 feet of lake sediments (Clayton, 1962, p. 40). Some, however, are composed largely of till (e.g., "moraine plateaus" of Hoppe, 1952, p. 5). Most of these features are less than 2 square miles in area and 40 feet high, and all of these, regardless of their appellation, probably formed under similar circumstances, in ice-walled lakes. Those that are now primarily lacustrine plains probably formed either in large ice-walled lakes, where the ice wall constituted only a small portion of the total area, or where the surrounding ice slopes had become stabilized by reduction or by growth of vegetation.

The large lakes on the Martin River Glacier as well as the smaller ones beyond the present terminus are such ice-walled lakes; they do not have an ice bottom. Although Miller Lake, at the terminus of the main lobe (Figure 2), does have at least a partial ice bottom,

it can be used as an example of what ice-walled-lake plains would look like upon complete ablation of the confining ice. This lake presently receives an average of about 50 million gallons per day of extremely milky meltwater and runoff during the summer season. The rate of accumulation of silts and clays on the bottom may be several centimeters per week (as determined by sediment traps on the bottom) (Callender, 1964, p. 125). But, in addition to true lacustrine sediment, an important contributor is the superglacial drift on the surface of the surrounding ice. Ablation of the confining ice wall causes the drift to fall and slide into the lake as it is undercut. Masses of the ice wall also frequently break loose and slide and tumble into the lake, spilling the drift upon contact with the water. The resulting sediment on the floor of the lake has all the characteristics of till even though it was deposited in a lake environment.

Farther away from the active terminus there are lakes that probably are at least 75% ice-walled. One such lake, Black Lake (Figure 2), has an abundant fauna including gastropods, arthropods, and beavers. The water is clear, not milky, and appreciably warmer than the ice sinkhole lakes. The sediments that are presently accumulating in this lake are largely organic. This lake is obviously more stable than the ice sinkhole lakes and is more nearly identical in characteristics to the late phase of the ice-walled lakes in North Dakota. The resultant ice-walled-lake plains in North Dakota often contain abundant gastropods, pelecypods, and ostracods (Tuthill, 1967). At least some of pelecypods required the presence of fish, since their glochidia are parasitic on fish. But, no fish remains have yet been found in ice-walled-lake plains of North Dakota. The presence of this faunal assemblage, however, implies that the water must have been warm enough to permit successful propagation.

The explanation for the successful propagation in Black Lake is therefore important. The depth of the ice at the one pit excavated to determine the minimum thickness of ablation till in Black Lake was 5 feet, 11 inches. Presumably, the ablation till is thicker in most other parts of the lake. This single site was selected because the slope here appeared to be more active, presumably reflecting a minimum till cover. The conclusion that can be drawn is that only a thin till cover is necessary to permit the waters of an ice-walled lake to become warm enough to permit propagation of fauna.

Insulation of ice by drift: The spreading of soot or other dark sediment on an ice surface has long been used by the Chinese to increase the rate of glacier and snow melting during the seasons when increased water is needed for agricultural purposes (Ice, 1960, p. 10). The dark particles absorb more radiation than the snow and ice, and melting is increased. But, if the layer of particles is too thick, it acts as an insulator and inhibits melting. The critical thickness depends on factors such as the color, composition, size and the degree of packing of the particles, as well as the amount of radiation received.

On the Martin River Glacier close attention was paid to finding absolute measurements of this critical thickness. The best examples were found associated with two features: medial moraines and dirt cones. Invariably, the medial moraines on the glacier are ridges standing 70 to 90 feet above the surrounding relatively clean ice. The drift comprising these ridges, however, is only one to two particles thick! That is, the ridge may have one single boulder resting on the ice surface and protecting the ice beneath it from the rapid ablation occurring elsewhere. When the particle size is smaller, there may be several on top of one another inhibiting the ablation. The average thickness on these high moraine ridges is probably less than one foot!

Small dirt cones are easier to measure precisely. These cones are formed when sand washes into small moulins less than a few feet deep and one foot in diameter. Upon ablation of the ice, only the bottom of the moulin is protected by the sand and this becames the peak of a cone, the result of complete inversion of topography. Most of the cones observed were in the area along the upper limits of ablation till cover, approximately 4½ miles from the main terminus. Such cones were typically about one foot high with a slope angle of 30 to 35°. The sand veneer, composed largely of quartz and feldspar Grains, was always less than one inch and usually less than one-half an inch thick!

The conclusion is that for sand particles, only a thin cover is necessary to inhibit ablation. For larger particles, where there is more space between the particles, a cover in excess of about six inches is necessary.

The ice on the Missouri Coteau 12,000 to 9,000 years ago was buried beneath an ablation till cover as much as 20 feet thick in places. The resulting features must have been very similar to those found on the Martin River Glacier today.

Conclusions.—The investigation of the Martin River Glacier in south-central Alaska has permitted a deeper appreciation of the origin and characteristics of the dead-ice topography on the Missouri Coteau of North Dakota. Ice-walled-lake plains, kames, and the typically irregular topography on the Coteau can best be explained by the former existence of a detached mass of glacial ice buried beneath a cover of ablation till. Mass movement of this till controlled the rate of ablation of the underlying ice and upon complete ablation of the ice the sediments assumed the form determined by the last stage of ablation. Presently high areas were produced through accumulation of sediments in depressions in the ice surface; low areas reflect the former presence of thicker ice just prior to final ablation. Inversion of topography is the single most important consideration necessary for a complete understanding of dead-ice features.

Continued studies in areas of dead-ice topography in North Dakota will benefit greatly from this investigation of an active glacier.

ACKNOWLEDGMENTS

Acknowledgements.—Most of this study is a result of work carried out on the Martin River and Sioux Glaciers in south-central Alaska. The research was supported by National Science Foundation grants (G22016 and GP-4448) during the summers of 1962, 1963, 1965, and 1966. Without this support much of the present glacial interpretations for North Dakota would still be merely hypotheses.

I wish also to acknowledge the excellent cooperation rendered by the other members of the field parties, especially Kirth Erickson and Frank Schulte, each of whom accompanied me during two of the field seasons. Their enthusiasm, their willingness to work under conditions that were often adverse, and their patience in working with me have not gone unnoticed.

REFERENCES

1. Callender, E., 1964, The physical limnology and sedimentology of Miller Lake, Martin River Glacier, south-central Alaska: unpubl. thesis, University of North Dakota
2. Clayton, Lee, 1967, Road log, Glacial geology of the Missouri Coteau and adjacent area: N. Dak. Geol. Surv., Misc, Ser. 30
3. ————————, 1964, Karst topography on stagnant glaciers: Jour. Glaciology, v. 5, no. 37, p. 107-112
4. ————————, 1962, Glacial geology of Logan and McIntosh Counties, North Dakota: N. Dak. Geol. Surv. Bull. 37, 84p.
5. Gravenor, C. P., 1955. The origin and significance of prairie mounds: Am. Jour. Sci., v. 253, p. 475-481
6. ————————, and W. O. Kupsch, 1959, Ice-disintegration features in western Canada: Jour. Geology, v. 67, p. 48-64
7. Hartshorn, J. H., 1958, Flowtill in southeastern Massachusetts: Geol. Soc. Am. Bull., v. 69, p. 477-482
8. Hoppe, Gunnar, 1952, Hummocky moraine areas, with special reference to the interior of Norrbotten: Geog. Annaler, v. 34, p. 1-71
9. Ice, Jan. 1960, no. 5, p. 10
10. Lemke, R. W. and R. B. Colton, 1958, Summary of the Pleistocene geology of North Dakota: *in* Guidebook, Midwestern Friends of the Pleistocene; N. Dak. Geol. Surv., Misc. Ser. 10, p. 41-57
11. Reid, J. R. and Lee Clayton, 1963, Observations of rapid water-level fluctuations in ice sink-hole lakes, Martin River Glacier, Alaska: Jour. Glaciology, v. 4, no. 35, p. 650-652
12. Sharp, R. P., 1949, Studies of superglacial debris on valley glaciers: Am. Jour. Sci., v. 247, p. 289-315
13. Stalker, A. M., 1960, Ice-pressed drift forms and associated deposits in Alberta: Canada Geol. Surv. Bull. 57, 38p.
14. Townsend, R. C. and A. L. Jenke, 1951, The problem of the origin of the Max Moraine of North Dakota and Canada: Am. Jour. Sci., v. 249, p. 842-857

15. Tuthill, S. J., 1967, Late Pleistocene mollusca on the Missouri Coteau District, North Dakota: *in* Glacial geology of the Missouri Coteau and adjacent areas: N. Dak. Geol. Survey, Misc. Ser. 30, p. 73-80

16. Winters, H. A., 1963, Geology and ground water resources of Stutsman County, North Dakota: N. Dak. Geol. Surv. Bull. 41, pt. I, 84p.

TRANSDUCER TO MEASURE VERY LOW VELOCITY OF AIR[1]

Richard L. Witz, Allen F. Butchbaker and Howard J. Gunnarson[2]

Agricultural Engineering Department
North Dakota State University, Fargo, North Dakota

Air movement in a swine house appeared to affect meat production in nutrition trails at the North Dakota Agricultural Experiment Station. Commercial units suitable for measuring velocities below 5 miles per hour, the range necessary if differences in air movement on opposite sides of the barn were to be determined, were not available.

Attempts at correlating air velocity with millivolt signals from therocouples measuring temperature rise across a heat source in a tube were unsuccessful. A survey of the literature indicated that hot wire anemometers probably would not measure with accuracy the low air velocities required. Also, it was considered more important to obtain magnitudes of the air velocity than the direction; the hot wire anemometers are characteristically directional.

The very small mass increasing the speed of response and high sensitivity due to a large change in resistance with change in temperature of thermistors indicated their possible use. A circuit for measuring thermal conductivity was suggester by Fenwal Electronics (1). This circuit was a bridge circuit using two thermistors, similar to the one shown in Figure 1. It had been used for measuring (A) thermal conductivity of various gases and gas mixtures, (B) gas pressures

[1]This paper is based upon research conducted under North Dakota Project Hatch 4-14, "Swine Housing Environment," and is a contributing project to North Central Regional Propject NC-72. Paper originally presented at 1966 Winter Meeting of American Society or Agricultural Engineers, in Chicago, as Paper No. 66-834. Permission granted for printing by North Dakota Academy of Science.

[2]Authors are Professor, Associate Professor, and former Graduate Research Assistant, Agricultural Engineering Department, North Dakota State University, Fargo, North Dakota.

under vacuum conditions, (C) as a flow meter for either gas or liquid in a pipe, and (D) as a temperature differential thermometer.

Sanford (2) had found the effect of ambient temperatures the main limitation to thermistors. He used a single thermistor in a bridge circuit and concluded that the constant resistance circuit with a feed-

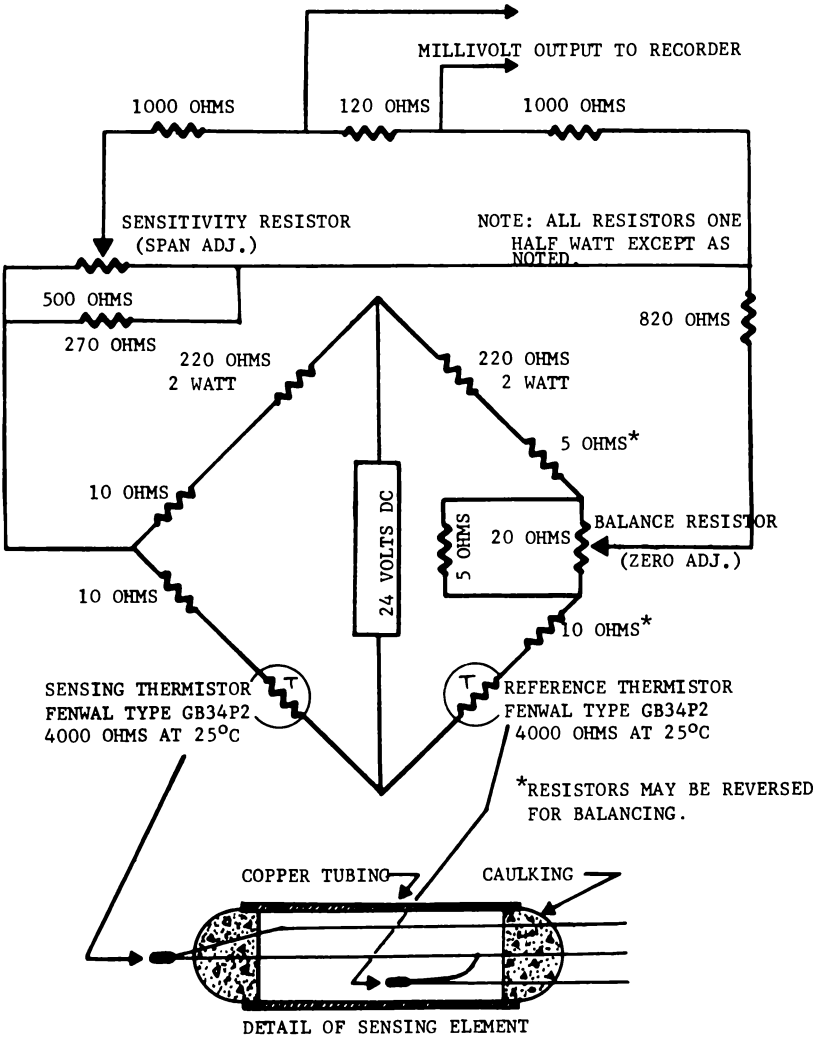


Figure 1. Thermistor Circuit Used for Measuring Low Air Velocities (0-352 feet per minute)

back amplifier offered the best possibility. Another limitation was the direction error, up to 19 per cent, due to the geometrical asymmetry of the thermistor. He did not use velocities below five miles per hour because "the calibration of the tunnel was uncertain in this range."

By using two thermistors, one enclosed in a sealed cavity and the other exposed to the air stream, it was possible to compensate for the efect of the ambient temperature. The air flow past the exposed thermistor reduced the temperature. This reduced temperature increased the resistance of the thermistor and produced an unbalanced condition in the bridge. This unbalanced condition produced a voltage on the output of the bridge.

The sensing unit is shown in Figure 1. To get maximum sensitivity, the first units tried were with an uncoated bead thermistor. This proved to be too sensitive for the multipoint recorder, and the bead was not rugged enough to be handled easily. This was replaced with a glass probe thermistor, Fenwal No. GB 34P2, which gave more stability to the recorder while still maintaining adequate sensitivity, additional rigidity for handling, and a much better geometric symmetry. Rotating the thermistor with respect to direction of air flow gave errors due to geometric asymmetry of three to ten per cent with the several thermistors tested.

Variation of ambient temperature from 40 to 80°F affected reading by less than 10 per cent. Further studies are needed at various temperatures, but at the relatively constant temperatures encountered in the swine unit, the error would be somewhat less than 10 per cent.

A major problem was drifting of the signal as received by the recorder, caused by the balancing voltage of the recorder feeding back into teh bridge circuit of the transducer. This was remedied by increasing the impedance of the transducer circuit. Resistors in the series parallel circuit were selected for maximum electrical separation between transducer and recorder without excessiv loss of signal to the recorder. The transducer circuit is shown in Figure 1.

Calibration was accomplished by mounting the transducer on a rotating arm at the constant peripheral velocity. To simplify connections between the transducer and the recorder, the recorder and DC power supply were mounted on a revolving platform. A vertical shaft was extended to a bearing mounted on the ceiling for rigidity, and a horizontal arm attached to the vertical shaft. Two or more sensing units may be calibrated at the same time. The velocity of the sensing units were controlled by a variable speed drive and by varying the distance the units were mounted from the vertical shaft. Rotation could be adjusted from 2.5 to 16 revolutions per minute with air velocity adjustable between 10 and 500 feet per minute. The 115 volt AC power and ground were supplied to the rotating shaft by the use of three commutator rings.

Procedure for the initial circuit calibration was as follows:

1. Transducers were connected to the power supply for 12 hours to reach thermal equilibrium.
2. Sensing thermistor was covered with a plastic bag over a protective screen and allowed to reach equilibrium, which took about 2 hours.
3. The balance resistor was adjusted until the voltage output was zero.
4. The plastic bag was removed and the transducer mounted on the revolving arm and revolved at a peripheral velocity of 352 feet per minute. The sensitivity resistor was adjusted to an output of 4 millivolts.
5. Intermediate points were plotted using several transducer units to develop a composite curve. Additional units were checked against this composite curve.

Since unmatched thermistors and 20 per cent tolerance resistors were used, it was expected that it would be necessary to use a correction curve for each transducer unit. This did not prove necessary, and the composite curve is shown in Table I.

TABLE I

AIR VELOCITY AS MEASURED BY MILLIVOLT OUTPUT OF THE TRANSDUCER

Output (Millivolts)	Air Velocity (Feet per minute)
0.0	0.0
0.5	14.3
1.0	38.6
1.5	71.4
2.0	107.1
2.5	152.8
3.0	207.1
3.5	271.4
4.0	352.0

A check indicated units followed the composite curve to within 2.5 per cent at mid range. A calibrated rule was developed for use in reading temperatures directly from the chart of the multipoint recorder.

Recording of the voltages indicating the air velocity was made with a standard multipoint thermocouple recorder modified to receive both thermocouple and 0 to 4 millivolt inputs. This combination of thermocouple and millivolt inputs is available on newer recording units. The full scale of a standard 0 to 10 millivolt range recorder could be utilized by changing the 120 ohm resistor (see Figure 1) across the leads to the recorder, to 300 ohms and reducing one of the adjacent 1,000 ohm resistors to 820 ohms. Other voltage outputs could similarly be obtained.

The current flow for each transducer unit was about 172 milliampers or 86 milliamperes through each thermistor under balanced conditions. Voltage across the thermistor of 2.6 volts indicated an internal resistance of 30.2 ohms. Based upon manufacurers' tables, the thermistor temperature was 430°F. This was below the manufacturer's recommended maximum of 600°F.

The regulated DC power supply was a Sola No. 281024-1, 24-volt, 6-ampere unit. This could provide power for up to about 30 units. It proved to be very well corrected for fluctuations in the 120 volt AC power input, but was not well corrected for variations in load on the output. This made it necessary to maintain a constant load on the output. The load selected was 4.5 amperes which resulted in an output voltage of 24.1 volts.

While results with this transducer unit were satisfactory, improvements could be made in the design by using matched thermistors, precision resistors, and trimmer-type potentiometers. The sensing element mounted by itself in the air stream with the rest of the unit on a panel near the recorder would reduce turbulence of the air stream, simplify adjustments, and minimize moisture problems. Using a plastic bag to obtain the zero adjustment may be questionable, but other methods such as embedding in insulation also seem unsatisfactory. An alternative would be to use 0.4 and 4.0 mph for major adjusting point, and omit the zero adjustment. The circuit could also be modified to produce a linear output.

REFERENCES

1. Fenwal Electronics, Inc. Thermistor Manual, Publication No. EMC-5, 1962.
2. Sanford, E. R., "A Wind-Tunnel Investigation of the Limitations of Thermistor Anemometry," Journal of Meteorology, **8**:182-190, 1951.

THE USE OF FIBERGLASS IN PREPARING NATURAL MODELS OF THE SIMPLE AND RUMINANT STOMACH

John W. Goertel and I. A. Schipper

Department of Veterinary Science
North Dakota State University, Fargo, North Dakota

INTRODUCTION

In 1961 Kitchell *et al.* (1) devised a fiberglass technique for the preparation of natural models of the ruminant stomach. It has been found in our investigations that this same procedure can be applied to that of preparing natural models of the simple stomach. This paper is written for the purpose of presenting this procedure as well as to stress certain methods which are believed to be of major importance in the construction of natural models of the rumiannt stomach as well as the simple stomach.

METHODS AND MATERIALS

The simple stomachs utilized in this investigation were obtained from swine that were fasted on a liquid diet for approximately twenty-four hours prior to slaughter. This facilitated the removal of the gastric contents, which was accomplished by running luke-warm water into the stomach via the esophagus and flushing the water and ingestia out through the duodenum. The stomach was massaged throughout this process to aid in the removal of the gastric contents. Once the stomach was free of gastric contents it was placed in a vat containing a solution of 99% isopropyl alcohol. Approximately one gallon of alcohol was pumped into the stomach. After five days the alcohol was drained and replaced by a fresh solution, for a total fixation period of ten days. At the conclusion of the fixation period the stomach was drained and suspended on cheese cloth, and inflated by a constant air flow into the esophagus. The degree of distention was regulated by a hemostat placed on the duodenum. The stomach is inflated in such a manner that the stomach walls are taut but not overstretched. The total drying process took approximately two weeks.

When the stomach was dry the specimen was covered with fiberglass. The procedure outlined by Kitchell (1) for preparing and covering the specimen with fiberglass was followed. This procedure is as follows:

1. When the stomach is dry, the exterior surface is roughened with a steel brush to assure a better bond between it and the fiberglass.
2. Mix the resin and hardener in a beaker in half-pint quantities just before use.
3. Three by five inch pieces of fiberglass mat are saturated with resin and applied to the external surface of one side of the stomach. Occasionally one will want to use smaller pieces of fiberglass mat. A small brush is used to smooth it on and work out all air pubbles from beneath the mat. (Acetone may be used to clean the brush, etc.).
4. One side is allowed to dry before the other side is covered.
5. When the surface is dry, the surface is sanded with rough sandpaper. Three to five additional coats of resin are applied. We suggest sanding the stomach after each layer has dried to assure a smoother surface.
6. If the stomch is to be opened, resin should be applied to the cut edges in order to prevent any separation of the stomach wall from the surrounding fiberglass.

DISCUSSION

The ruminant specimens which were used in the construction of natural models in our laboratory were obtained from a slaughtering plant, thus the specifications listed by Kitchell *et al.* (1) in order to

obtain an ideal stomach specimen for the construction of natural models was not at our disposal.

In obtaining stomach specimens from a slaughtering plant, the best results in the construction of natural models are obtained from those stomach specimens which exhibit the following specifications:

1. The stomach appears to be thin-walled.
2. There should be minimal amounts of fat on the stomach wall, around the blood vessels, and in the ruminal grooves.
3. The ingestia appears to be mostly liquid in nature.

Care should be taken during the slaughter to avoid cutting or tearing through the gastric wall. This same caution should be observed in removing fat that may be on the stomach wall, around the blood vessels and in the ruminal groove.

If specimens are not dried properly there is a tendency for the stomach to shrink within the fiberglass coat. However, the drying period will vary with the age of the animal from which the specimen was made.

SUMMARY

Natural models of the simple stomach were prepared utilizing the procedure of Kitchell *et al.* (1). Certain methods for the preparation of natural models of the ruminant and simple stomach are stressed in order to obtain good specimens.

BIBLIOGRAPHY

1. Kitchell, R. L., Turnbull, J., Nordine, R. A., and Edgell, S. C., 1961, Fiberglass Techniques for Preparation of Natural Models of the Ruminant Stomach: J. Amer. Vet. Med. Assoc., 138: 6, p. 329-331.

TECHNIQUES IN PREPARING MULTICOLORED CORROSION SPECIMENS WITH NEOPRENE LATEX[1]

John W. Goertel and I. A. Schipper

Department of Veterinary Science

North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota

INTRODUCTION

Multicolored corrosion specimens of bovine kidneys and a mammary gland were prepared utilizing Neopreme latex as the injection mass. The lactiferous ducts and the circulatory system of the mammary glands, as well as the circulatory and excretory systems of the kidney, were demonstrated by the addition of color pigments to the latex prior to injection. This paper summarizes the procedures

[1]Published with the approval of the Director of the North Dakota Agricultural Experiment Station in the Proceedings of the North Dakota Academy of Science.

utilized by other investigators (1, 2) and the authors for the preparation of multicolor corrosion specimens.

METHODS AND MATERIALS

Kidneys.—Kidneys were obtained from slaughtered animals with care being exercised to assure sufficient amounts of perirenal tissue and long lengths of the renal artery, renal vein, and ureter.

Procedure utilized in preparation of kidneys for injection.—The following procedures were utilized to prepare the specimens for injection of latex:

1. The vascular system was irrigated with tap water at room temperature by inserting a cannula into the renal artery. The tap water was allowed to flow through the specimen until the escaping fluid was grossly clear of blood. The apparatus utilized for the irrigation of the vascular system is based on a description by Narat (3).
2. Upon the exclusion of blood from the specimen, air was allowed to flow through the specimen.
3. The specimen was then ready to be injected with colored latex.
4. In order to obtain a multicolored vascular system, the renal artery and its tributaries were injected with red colored latex (Watchung Red B, No. RW-635P) while the renal vein and its tributaries were injected with blue colored latex (Monastral Blue B, No. BW-372P). The renal pelvis was injected with yellow colored latex (Dalamar Yellow, No. YW-718P) by way of the ureter.
5. The renal artery and the renal vein and their tributaries were injected with their respective colors simultaneously. This was performed by connecting the two flasks containing the blue and red colored latex to the same pressure source. Latex from the two flasks was allowed to flow to the end of the tubing, after which the glass cannulae, previously inserted into the renal artery and the renal vein, were connected. The hemostats were then removed from the tubing of both flasks. Latex was allowed to flow approximately three to four minutes, after which time, the artery and the vein were tied off. The cannulae were then withdrawn. The renal pelvis was then injected with yellow colored latex by means of the ureter.
6. Following the injection of latex the specimen was placed in a tightly covered container containing concentrated hydrochloric acid. After approximately twelve hours the tissue should have been digested. The hydrochloric acid was poured off and saved for repeated use. Tap water was allowed to flow into the container at a slow rate. This washed the specimen free of digested tissue and residue of hydrochloric acid. The corrosion specimen can be placed in clear water if so desired. To prevent growth

of mold the specimen should be stored in 0.3% Dowicide solution.(4).

Mammary gland.—The mamary gland was secured fresh from a slaughtered animal. The procedure utilized to inject the vascular system, with the arteries being red and the veins being blue, is the same as the steps utilized in preparation of the kidney. For the injection of the mammary gland with liquid latex the general principles of Bechtel and McLeod (2) were followed, with modifications made regarding the use of glacial acetic acid as an irrigation solution.

The step in which Bechtel and McLeod (2) used glacial acetic acid as an irrigation solution to rinse the udder was omitted.

DISCUSSION

The procedure utilized to prepare a multicolored corrosion specimen was in many ways similar to the method of Leib (1). However, we found that the process of Leib, by which water was allowed to flow through the specimen for eight to eighteen hours did not improve the quality of the specimen. It was found that by obtaining specimens from animals upon slaughter the amount of blood found within the specimen was limited. By processing the specimen immediately with warm tap water, approximately fifteen to twenty minutes was needed to secure a blood free specimen. It was felt that the amount of edema would be limited. This would be far superior to that procedure where Leib washed the specimen for eight to eighteen hours or until pale gray. Upon washing the specimen Leib suggested that the specimen be placed in an icebox for six or seven hours and at the time of injection the specimen be warmed for approximately one hour. Our results indicated that a specimen which is processed immediately following slaughter and irrigation with tap water is far superior to that of Leib's procedure.

In step 2 of our procedure air was pushed through the specimen in order to eliminate as much water as possible. The latex itself should be placed at room temperature for at least an hour prior to injection. During the process of injection of latex, leakage will occasionally occur. These can be eliminated by the use of a hemostat, and more successfully, by placing a pledget of cotton soaked in either alcohol or 4% acetic acid at the site of leakage.

In preparing the corrosion specimen of the mammary gland we indicated in the procedure that the step by which Bechtel and McLeod utilized glacial acetic acid as an irrigation solution was omitted. Although Bechtel and McLeod allowed the glacial acetic acid to drain, it is certain, in our estimation, that there will be a residue of this acid in the tissue with which it has come in contact. Our experiences have indicated that glacial acetic acid itself will cause the latex to coagulate. Thus, if latex is injected into a vessel or cavity previously impregnated with glacial acetic acid, coagulation will take place and consequently block the passages. The incidence

of such a process would definiely be encountered when small cavities and vessels are injected.

The corrosion specimens of the mammary gland can be stored in the same manner as the kidney: however, due to the fact that our multicolored corrosion specimens were to be utilized as visual aids for teaching they were embedded in Turtox Embedding Plastic (5).

SUMMARY

A procedure is described whereby multicolored corrosion specimens of kidneys and a bovine mammary gland were prepared. The success in obtaining ideal specimens utilizing our procedure or that of Leib, and Bechtel and McLeod will depend to a certain degree on the technique of the individual. Multicolored corrosion specimens embedded in plastic serve as an ideal teaching aid in that they are permanent and unbreakable.

BIBLIOGRAPHY

1. Leib, Ethyl, 1940. Demonstration of Vascular Tree with Neopreme: J. Tech. Methods, 90: 48, pp. 48-56.
2. Bechtel, H. E., and McLeod, W. M., 1945. Latex in the Preparation of Corrosions of Mammary Glands. Amer. J. of Vet. Research, Vol. 6, 18, pp. 17-20.
3. Narat, J. K., Loef, J. A. and Narat, M., 1953. On the Preparation of Multicolored Corrosion Specimens; Anat. Rec., 64: 155, pp. 155-160.
4. Gamble, D. L., 1939. Liquid Latex as an Injection Mass for Blood-Vessels. Science, 90: 520.
5. General Biological Supply House: Turtox Service Dept., 8200 South Hoyne Ave., Chicago, Ill.

SOME CHEMICAL CHARACTERISTICS OF AEOLIAN DEPOSITS OF SNOW-SOIL ON PRAIRIE WETLANDS

V. A. Adomaitis, H. A. Kantrud and J. A. Shoesmith

Fish and Wildlife Service, U.S. Department of the Interior
Jamestown, North Dakota 58401

INTRODUCTION

Broad ecological studies are necessary to properly describe the prairie wetlands of North Dakota. An important contributory factor assisting the biological evaluation of these habitats for migratory waterfowl is the chemical characterization of soils and waters (Cook, 1964). A close natural relationship exists between wetland ecology and geochemistry. The movement and distribution of many chemical elements and compounds in nature is related to the biogeochemical processes in waters, soils, plants and animals (Goldschmidt, 1958). There has been speculation about the type of matter transported during winter storms in the prairie region, and its effect on marshes

or other areas where snow is trapped. The ultimate result is the development of environments which are beneficial or deleterious to waterfowl and other wildlife.

METHODS

The severe blizzard of March 2 to 5, 1966 afforded the opportunity to collect information on the deposition of snow-soil in northern prairie wetlands. Snow samples were collected from snow drifts on frozen wetlands on March 9, 1966. The collection sites were located in the Drift Plain and Missouri Coteau physiographic districts of North Dakota in a rectangular area including ranges 63 to 67 of township 139 North. The elevations ranged from approximately 1500 on the east end to about 1800 feet on the west end.

Although the relative ability of different wetlands to trap wind-blown snow and soil depends on the plant associations present, sampling of aeolian deposits was possible in all of the different types of vegetation. The type of land-use proctice surrounding the sample sites is shown in Table I; the original chronological order was renumbered to conform to the logical order as used in the following tables. Snow classification was determined by visual inspection.

TABLE I

DESCRIPTION OF SAMPLING SITES

Sites	Land Use Practice	Snow Classification
1-2, 7-8, 10-12.	100% plowed	Dirty
3-5, 6, 9.	100% cultivated	Dirty
13, 27.	80% pasture, 20% cultivated	Clean
16-25.	100% pasture	Clean
14.	Highway and cultivated	Clean
15.	50% hayland, 50% cultivated	Clean
28.	80% hayland, 20% cultivated	Clean
26, 29.	100% hayland	Clean

Pyrex 500 ml Erlenmeyer flasks were filled to the snow capacity and stoppered with Pyrex glass wool and beakers. They were then stored at 5° C for six months with four similar flasks filled with distilled water that served as storage controls. The chemical analyses of the melted snow were determined by titration and colorimetry following a modified procedure based on the manual of Standard Methods (A.P.H.A., 1965).

RESULTS

The individual chemical components that were determined are listed in Table II and Table III; Table IV is a summary. The storage and chemical analytical procedure blank was found to be zero parts per million. All the other results are also expressed in parts per million except for the hydrogen ion concentration.

TABLE II

CHEMICAL CHARACTERISTICS IN P.P.M. OF DIRTY SNOW

Site	pH	Total Alkalinity	Total Hardness	Calcium Hardness	Magnesium Hardness	Total Phosphate	Sulfate	Nitrogen, Nitrate	Silica
1.	7.9	30	30	20	10	0.50	8.0	1.8	3.4
2.	—	60	70	50	20	0.40	9.0	2.8	8.4
3.	8.3	180	195	140	55	0.61	7.0	4.2	15.1
4.	7.9	40	30	30	0	0.25	6.0	0.8	4.0
5.	7.7	65	65	40	25	0.35	7.0	2.8	9.6
6.	8.2	130	135	90	45	0.40	5.5	4.0	11.2
7.	8.4	105	110	80	30	0.35	6.0	4.4	10.8
8.	—	75	80	60	20	0.30	5.0	2.6	6.4
9.	7.9	20	30	20	10	0.30	5.0	0.9	4.8
10.	8.2	55	60	50	10	0.25	3.0	2.2	4.0
11.	8.3	60	60	50	10	0.80	3.0	1.1	5.4
12.	8.3	70	85	70	15	0.30	4.0	2.0	7.0

TABLE III

CHEMICAL CHARACTERISTICS IN P.P.M. OF CLEAN SNOW

Site	pH	Total Alkalinity	Total Hardness	Calcium Hardness	Magnesium Hardness	Total Phosphate	Sulfate	Nitrogen, Nitrate	Silica
13.	7.8	50	50	45	5	0.20	3.0	0.4	3.4
14.	7.8	30	25	20	5	0.40	6.0	1.2	3.4
15.	8.5	60	60	55	5	0.40	2.0	0.9	3.0
16.	8.0	30	30	20	10	0.20	7.0	1.1	3.0
17.	7.8	50	50	45	5	0.20	4.0	0.6	4.0
18.	7.8	50	50	40	10	0.21	10.1	1.3	4.2
19.	8.2	45	50	40	10	0.20	4.0	0.7	3.2
20.	7.9	30	30	30	0	0.50	4.0	0.8	2.9
21.	8.4	85	90	70	20	0.30	12.0	0.8	8.4
22.	8.1	50	50	40	10	0.30	4.0	1.2	5.8
23.	8.2	70	80	60	2	0.35	5.0	2.2	7.2
24.	8.4	50	45	40	5	0.25	4.0	1.1	4.6
25.	—	45	50	40	10	0.20	7.0	1.1	5.0
26.	7.9	20	20	10	10	0.21	3.0	0.2	2.4
27,	8.0	35	45	30	15	0.10	3.0	1.0	3.8
28.	7.6	15	15	20	5	0.18	2.0	0.3	2.5
29.	7.5	15	10	10	0	0.10	3.0	0	1.3

TABLE IV

SUMMARY OF CHEMICAL CHARACTERISTICS IN P.P.M. OF SNOW SAMPLES

Sites	pH	Total Alkalinity	Total Hardness	Calcium Hardness	Magnesium Hardness	Total Phosphate	Sulfate	Nitrogen, Nitrate	Silica
1-12									
Av.	8.1	74	79	58	21	0.40	5.7	2.5	7.5
Range	7.7-8.4	20-180	30-195	20-140	0-30	0.3-0.8	3-9	1.1-4.2	3.4-11.2
13-29									
Av.	7.9	43	44	36	7	0.25	4.9	0.9	4.0
Range	7.5-8.5	15-85	10-90	10-70	0-20	0.1-0.5	3-12	0-2.2	1.3-8.4

DISCUSSION

The chemical characteristics of the aeolian mixture of snow and soil on prairie wetlands indicated that surrounding fields without vegetation yielded nearly twice as much deposit into the prairie potholes as fields with vegetation. Total alkalinity was 1.7 times as high in the former case. Total hardness was 1.8, calcium hardness 1.6, magnesium hardness 3.0, and phosphates 1.6, sulfates were 1.2 and silica 1.9.

The comparison of nitrate nitrogen levels for clean and dirty snow shows the latter to be 2.8 times as high. These ratios imply that fair amounts of top soil travel with the snow during high winter winds. A corollary to these results is that the prevention of wind erosion in winter on the northern prairie also extends the topographical life of waterfowl breeding habitats.

SUMMARY

The chemical characteristics of aeolian snow-soil indicate that northern prairie wetlands without surrounding vegetation receive nearly twice as much deposit in winter, and thus have a shorter topographical life when compared to wetlands surrounded by vegetation.

We wish to thank Director Harvey K. Nelson for his encouragement.

REFERENCES

American Public Health Association 1965. Standard methods for the examination of water and waste water, 12th ed. A.P.H.A., New York, N. Y., 769 p.

Cook, A. H. 1964. Better living for ducks—through chemistry, J. P. Linduska ed., Waterfowl Tomorrow. U. S. Department of Interior, Washington, D. C. p. 569-578.

Goldschmidt, V. M. 1958. Geochemistry. Oxford University Press, London, 730 p.

MORPHOLOGICAL LAWS

John C. Hudson[1]

Department of Geography

University of North Dakota, Grand Forks, North Dakota

In the search for orderliness in physical systems ranging from a microscopic to a universal scale, there are several different types of laws that may be desired, depending on the point of view of the scientist. One type of inquiry may seek laws that concentrate on the physical-chemical composition of the objects, another may seek relationships between events in time, while still another may seek laws governing the spatial structure of phenomena (23). This distinction among types of laws is quite apart from a categorization recognizing the difference between process laws and cross-section laws (2). Instead, it is based more upon the level of abstraction which the researcher is seeking.

Spatial or form laws are generally quite abstract, usually broad in scope, and hence explain the configuration of many phenomena. Spatial properties are of interest in all sciences, from celestial mechanics to crystallography. In the last several decades, especially, they have been extensively developed in the social sciences. Spatial economics (20), human ecology (15), geography (4) and psychology (17) have all developed laws of form.

The geographer Schaefer (22) was aware of the importance of form laws as the basis for understanding spatial arrangements on the earth. He gave the name *morphological laws* to this class of generalizations concerning the relationships between terrestrial locations. Theoretical geography, that branch of geography which studies the properties of morphological laws, has much to learn and something to teach scientists in many fields, each investigating different phenomena, but with an eye for discovering general principles of form, or structure, or morphology.

This is the very essence of morphological laws. They are broad in scope because they disregard the non-spatial nature of the phenomena involved. They are concerned with abstract properties—those capable of being expressed geometrically, hence, on a map. Spatial distributions on the earth are lawful. Their maps are instances of mor-

[1]The author was Assistant Professor of Geography at the University of North Dakota, and is currently at the University of Wisconsin.

phological laws. Just as business cycles are instances of economic laws, and tornadoes are instances of meteorological laws, so are town locations instances of morphological laws.

Perhaps the best way to appreciate the nature of morphological laws is through some examples which illustrate how a single principle explains the form of systems having radically different non-spatial properties. Four morphological laws; concentric zones, diffusion, competition and gradients will be cited and illustrated with examples from various disciplines.

Concentric zones is the arrangement of objects which results when phenomena of different competitive ability try to get as close to a point as possible. Land use zones in the city are an example (5). The optimum spot to locate a business in the city is at its center. Assuming that the city has a compact shape, the point which is closest in the aggregate to the city's population is downtown, but it is here where land is most costly. As a result, different land users compete for central location, those benefiting less from a downtown location taking more land farther from city center, those less willing to accept a more remote location crowding into smaller more expensive locations closer to downtown (21).

Tree rings are another example. The plant morphologist might interpret these rings as the result of the tree trying to stay as close

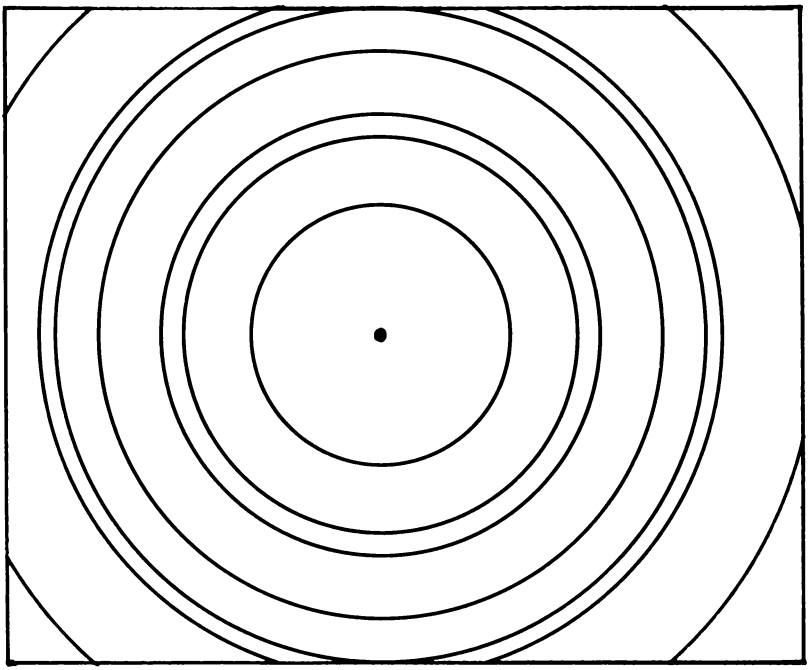


FIGURE 1—Concentric zones.

to itself as possible as it grows outwards. Cities grow in exactly the same way, with suburban development hugging the outside of the older growth.

A most significant property of morphological laws has just been illustrated. This is, that morphological laws almost always involve extemum problems. They describe optimum patterns with respect to certain spatial optimizing criteria. Usually the quantity to be minimized is distance, hence the optimum forms are compact, regular and symmetrical.

A most important concentric zone model is the classical Thunen land use schema in spatial economics (24). Thunen, a nineteenth century German scholar, deduced the location of various types of agricultural production around a single city located in a uniform environment. Land use rings develop due to the differing competitive ability of various land use types, to the geometry of the model and to its concomitant extremum problem: Minimize transportation costs between farms and the city.

The second morphological law, *diffusion*, is the pattern resulting from a phenomena spreading away from a source and gradually building up a spatial distribution through some transmittal network.

The diagram might illustrate the pattern produced by a plant reproducing by means of rhizomes. The lines could be removed, and

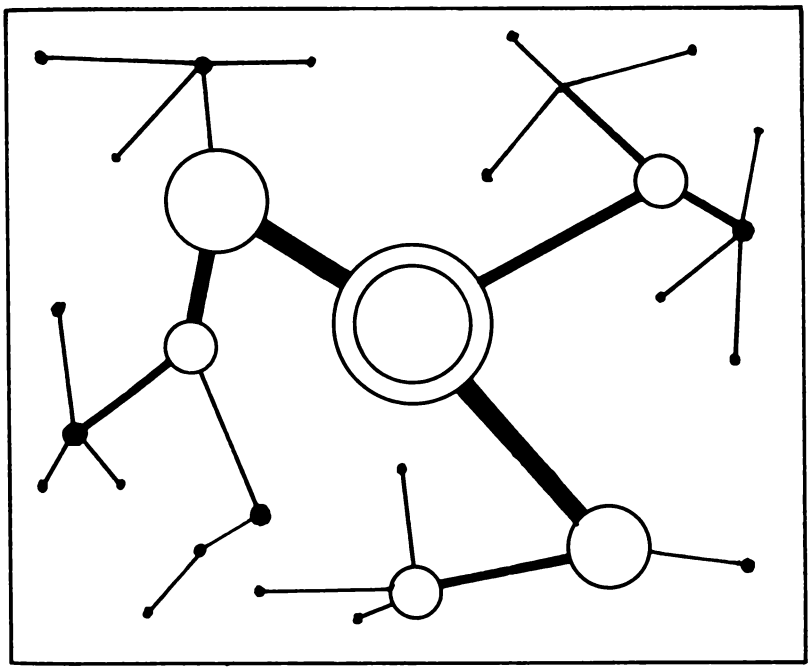


FIGURE 2—Diffusion.

the essential feature of the distribution would remain—that of a clustered growth, with nearby areas being affected before more remote areas. Such a process is found in plant reproductive processes including wind dispersal of seeds (29) and is documented in trap censuses made after small insects are released from a central point(6).

Sociologists and geographers have studied diffusion extensively. At the human scale, the phenomena of migration has many of the same properties (13). The preponderance of moves that people make are of a short distance. Longer moves are more infrequent. The Swedish geographer, Hägerstrand, has termed this phenomena of clustered growth resulting from diffusion, the *neighborhood effect* (14). This title is reminiscent of how a rumor spreads through a population, with neighbors telling neighbors, or how an epidemic might spread. The term "neighborhood effect" is the key to the spatial distinction of the morphology of diffusion, that which produces spatial clustering of growth.

The transmittal network in the diffusion process may or may not be visible. In the case of transportation arteries in the spread of settlement into an unoccupied area (7), and for plants reproducing by rhizomes (16), the network is visible. In the case of epidemic spread (1) the routes are not visible. The networks may consist of one-way channels, as in these examples, or two way channels, as is characteristic of the feedback process found in the flow of information between actual and potential migrants (3). In the process of innovation diffusion both the sender and the receiver of the message may be affected. The channels of the transmittal network characteristically organize themselves hierarchically, with channels near the source of the phenomena carrying the greater flow; those connecting more recent additions to the distribution commonly carrying a smaller volume. An identical statement may be made for the structure and flow of rivers. The river spreads by means of its small tributaries, but its greatest volume is found near its mouth.

A third morphological law is the pattern resulting from interacting objects in a bounded space trying to get as far from each other as possible, and is termed *competition.* The growth of a forest is an example. Each tree needs a certain amount of area around it from which it gathers nourishment from the soil. Clearly, two trees cannot grow too close to each other. Assuming that the soil is of uniform fertility, the optimal pattern for the trees would be to locate as far from each other as possible so as to make the maximum use of the available food supply. Small trees growing in the shade of, hence too close to, larger trees, are eliminated from the population. Competition for space produces a regular pattern of tree locations, with approximately equal distances between nearest neighbors. The pattern which makes the best use of the space is that resulting from the closest packing of convex two-dimensional do-

mains: the honeycomb, with a hexagonal lattice of points, describing the set of center points of the areas (10).

Replacing trees with towns and substituting purchasing power for soil nutrients, we may state another example of the same morphological law, the optimum spacing of towns servicing a rural landscape. Towns, like trees, repel each other. They locate far apart in

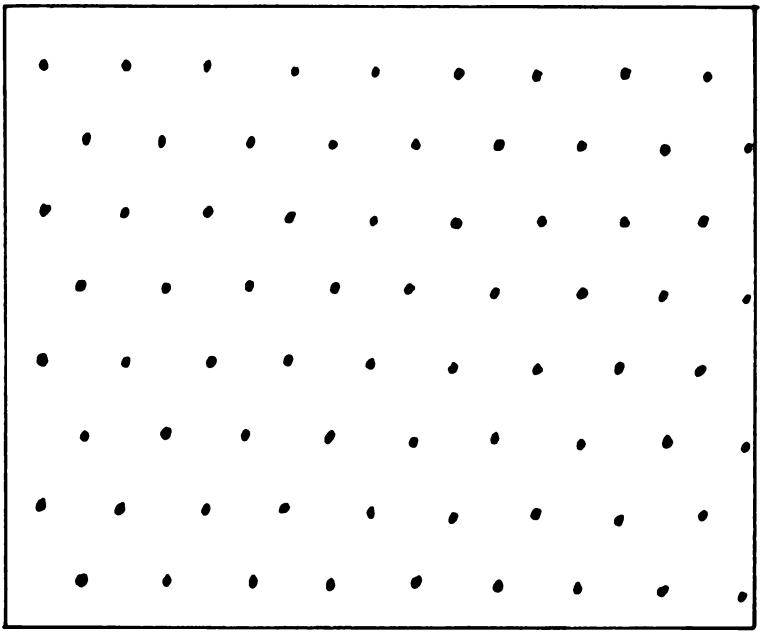


FIGURE 3—Competition.

order to extract the maximum amount of purchases from their hinterlands, leaving no areas unsupplied. The optimum spacing is hexagonal. This law is the basis of central place theory, one of geography's most important deductive structures (8).

The extremum problem involved in competition is to locate points farthest from each other, or, closest to the area. Farms regularly dispersed on the landscape may be interpreted as the result of farmers trying to get as close to their land as possible, thus locating farthest from each other.

In biology, *competition* is often overshadowed by diffusion, hence regular distributions such as those produced by *competition* are less frequent. Exceptions that may be found in the literature are trees stands of homogeneous age (18), prairie dog burrows (9), and of course, the honeycomb itself.

Finally, let us consider the law of *gradients* on a surface. *Gradients* are geodesics, or paths of minimum movement (26). The extremum

problem in this case is easily seen as one of minimizing cost, time or effort in traversing a path on a surface. The gradient of a circle is its radius. On a sphere it is a great circle, which maps as a curved line on a Mercator map of the sphere (25). Following a gradient, or steepest slope, is the most efficient way of climbing to the summit of a surface (28). Indeed, one will never actually reach the peak of a mountain unless the gradient is followed.

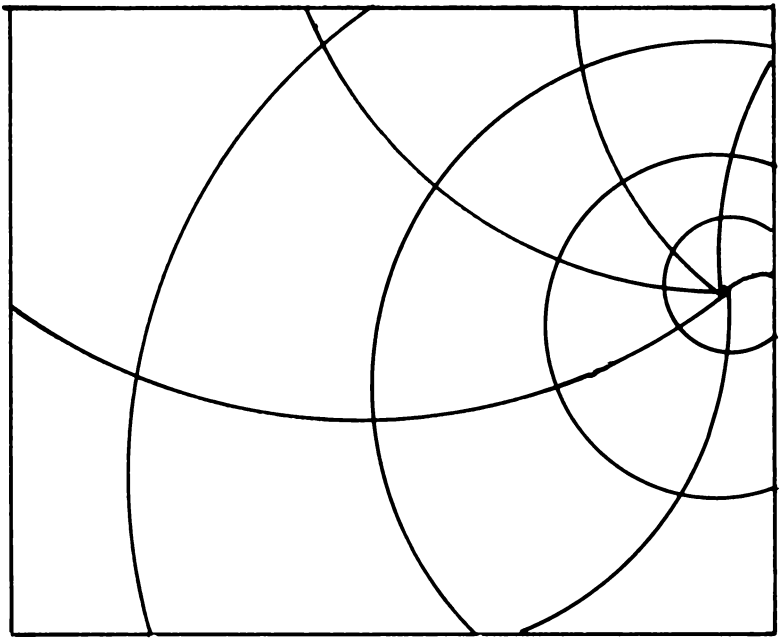


FIGURE 4—Gradients.

The geomorphologist has long used the concept of gradients, for that is exactly what is meant by land surface slope. This quantity is conventionally measured orthogonal to the contour lines. In general, any surface may be decomposed into two sets of orthogonal lines: Gradients, those lines following the direction of steepest slope of the surface, and the dual set of lines, the so-called contour lines or isopleths. Furthermore, slope is the first derivative of elevation, and thus gradients are the first derivatives of contour lines. The two sets of lines are related by a simple rule.

Experiments in animal behavior have found that animals obey these principles. Animals climb up the steepest gradient of surfaces, (12), barnacles orient themselves to water currents (11), and photo-positive animals orient themselves to obtain maximum illumination on their eyes, as they move toward light sources, hence following the gradient of light intensity defined by the inverse-square law (19).

If a map of the acquistion cost of land over some area of the earth is conceptualized as a surface, the elevation of which at each point is proportional to land cost, then finding the steepest gradient between two points on this surface determines the cheapest highway to build between these two points, assuming that all other costs are constant (27).

This discussion of morphological laws has shown that many physically unlike systems may be quite similar, spatially. It is of the utmost importance that similarities between spatial properties found in these phenomena are not simply analogues of one another, but instead are instances of very general laws of morphology. From these, highly generic principles may be inferred which explain the spatial properties. It was stated that these principles underlying morphological laws are often in the form of extremum problems, asking to maximize or minimize some geometric property of a map. All sciences can expect to learn from studying and seeking morphological laws.

REFERENCES

1. Bailey, Norman T. J. *The Mathematical Theory of Epidemics.* (New York: Hafner Publishing Co., 1957).
2. Bergmann, Gustav. *Philosophy of Science.* (Madison: University of Wisconsin Press, 1958).
3. Brown, Lawrence A. *Models for Spatial Diffusion Research: A Review.* (Geography Branch, Office of Naval Research, Task No. 389-140, Contract Nonr 1228(33). Department of Geography, Northwestern University; Evanston, Illinois, June, 1965.
4. Bunge, William. *Theoretical Geography,* 2nd ed. (Lund, Sweden: C. W. K. Gleerup, 1966).
5. Burgess, Ernest. "The Growth of the City," in *The City,* ed. Robert Park, Ernest W. Burgess and Roderick D. McKenzie. (Chicago: University of Chicago Press, 1925).
6. Burla, A. B. Da Cunha, Cavalcanti, A. G., Dobzhansky, Theodore, and Pavan, C. "Population Density and Dispersal in Brazilian *drosophilia willistonia. Ecology* 31 (1950), 393-404.
7. Bylund, Erik. "Theoretical Considerations Regarding the Distribution of Settlement in Inner Northern Sweden." *Geografiska Annaler,* 62(1960), 225-231.
8. Christaller, Walter. *Central Places in Southern Germany.* (trans. Carlisle W. Baskin, Englewood Cliffs, N. J.: Prentice-Hall, 1966).
9. Clark, P. J. "Grouping in Spatial Distributions," *Science,* 123 (1956) 373-374.
10. Coxeter, H.S.M. *Introduction to Geometry.* (New York: John Wiley, 1961).
11. Crisp, D. J. and Stubbing, H. G. "The Orientation of Barnacles to Water Currents," *Journal of Animal Ecology,* 26(1957) 179-196.

12. Fraenkel, Gottfried S. and Gunn, Donald L. *The Orientation of Animals: Kineses, Taxes and Compass Reactions.* (Oxford: Clarendon Press, 1940).
13. Hägerstrand, Torsten. "Migration and Area," in David Hannerberg, Torsten Hägerstrand and Brune Odeving, eds., *Migration in Sweden, A Symposium.* (Lund, Sweden: C.W.K. Gleerup, 1957).
14. Hägerstrand, Torsten. "A Monte-Carlo Approach to Diffusion." *European Journal of Sociology,* 6(1956) 43-67.
15. Hawley, Amos. *Human Ecology.* (New York: Ronald Press, 1950.)
16. Kershaw, Kenneth A. *Quantitative and Dynamic Ecology.* (New York: American Elsevier Publishing Co., 1964).
17. Hull, Clark. *A Behavior System.* (New York: John Wiley, 1952).
18. Laessle, Albert M. "Spacing and Competition in Natural Stands of Sand Pine," *Ecology,* 46(1965) 65-72.
19. Ludwig, W. "Seitensteitigkeit Niederer Tiere. II—Menotaxis," *Z. Wissenschaft Zool.,* 146(1934) 193-235.
20. Losch, August. *The Economics of Location.* (trans. Wolfgang Stolper, New Haven: Yale University Press, 1954).
21. Muth, Richard F. "The Spatial Structure of the Housing Market," *Papers and Proceeding of the Regional Science Association* 7 (1961), 207-220.
22. Schaefer, Fred K. "Exceptionalism in Geography: A Methodological Examination," *Annals of the Association of American Geographers,* 43(1953), 226-249.
23. Thompson, D. Arcy W. *On Growth and Form.,* 2 vols. (Cambridge: Cambridge University Press, 1917).
24. von Thunen, J. H. *Der Isolierte Staat in Beziehung auf Landwirtschaft and Nationalokomie.* (Hamburg, 1826).
25. Tobler, Waldo R. *Map Transformations of Geographic Space.* (Unpublished Ph.D. dissertation, University of Washington, 1961).
26. Tobler, Waldo R. "D'Arcy Thompson and the Analysis of Growth and Form, *Papers of the Michigan Academy of Arts, Letters and Science,* 48(1963) 385-390.
27. Warntz, William. *A Note on Surface and Paths and Applications to Geographical Problems.* (Michigan Inter-University Community of Mathematical Geographers, Discussion Paper No. 6, 1965).
28. Wilde, Douglass J. *Optimum Seeking Methods.* (Englewood Cliffs, New Jersey: Prentice-Hall, 1964).
29. Wolfenbarger, D. O. "Dispersion of Small Organisms: Distance Dispersion Rates of Bacteria, Spores, Seed, Pollen and Insects; Incidence Rates of Diseases and Injuries," *American Midland Naturalist.* 35(1946) 1-152.

PHOTOSYNTHETIC PRODUCTION AND ENERGY CONVERSION IN SPIRITWOOD LAKE, NORTH DAKOTA

G. W. Comita and E. G. Wolf[1]

Department of Zoology
North Dakota State University, Fargo, North Dakota

INTRODUCTION

A limnological investigation of Spiritwood Lake, North Dakota was conducted during the period November 1965, to November 1966. This lake is located 17 miles north-northeast of Jamestown in Stutsman County in the James River drainage basin, and is an end reservoir for its specific drainage area. It is oriented with its longest axis in a northwest-southeast direction. The shore line is composed of sand and gravel to a depth of about 15 feet; the remainder of the bottom is mud, delimited by the dashed line in Figure 1. The region has an average of 17.76 inches annual rainfall.

The lake has a maximum depth of 14.5 m with an average depth of 8.0 (Figure 1). The volume is 22,796,800 m^3, of which 92 per cent is above the 10 m level.

MATERIALS AND METHODS

Samples were taken on Monday and Wednesday from 6 June to 7 September 1966 and on alternating Saturdays during the remainder of the year. A three liter Van Dorn water sampler was used for obtaining all chemical and phytoplankton samples. The following chemical determinations were made: alkalinity, ammonia, nitrite, nitrate, total and reactive phosphates, total dissolved solids dissolved oxygen, acetone extracts of chlorophylls and carotenoids. In addition the following physical characteristics were determined: transparency, water temperature, air temperature at the lake surface and continuous readings of solar and sky radiation with a pyrheliometer at the lake. Weekly measurements of light and dark bottle photosynthesis and respiration were made over a 48-hour period. Zooplankton hauls were made twice a week with a modified Clark-Bumpus plankton sampler (Comita and Comita, 1957) using a #20 net.

Standards Methods (1955) was used where appropriate but most analyses followed were from Strickland and Parsons (1965). Identification of the algae and protozoa were made from the taxonomic keys in Edmondson's Ward and Wipple (1959), Patrick and Reimer (1966), and Smith (1950).

---

[1]Present address: Department of Biological Sciences, University of Idaho, Moscow, Idaho.

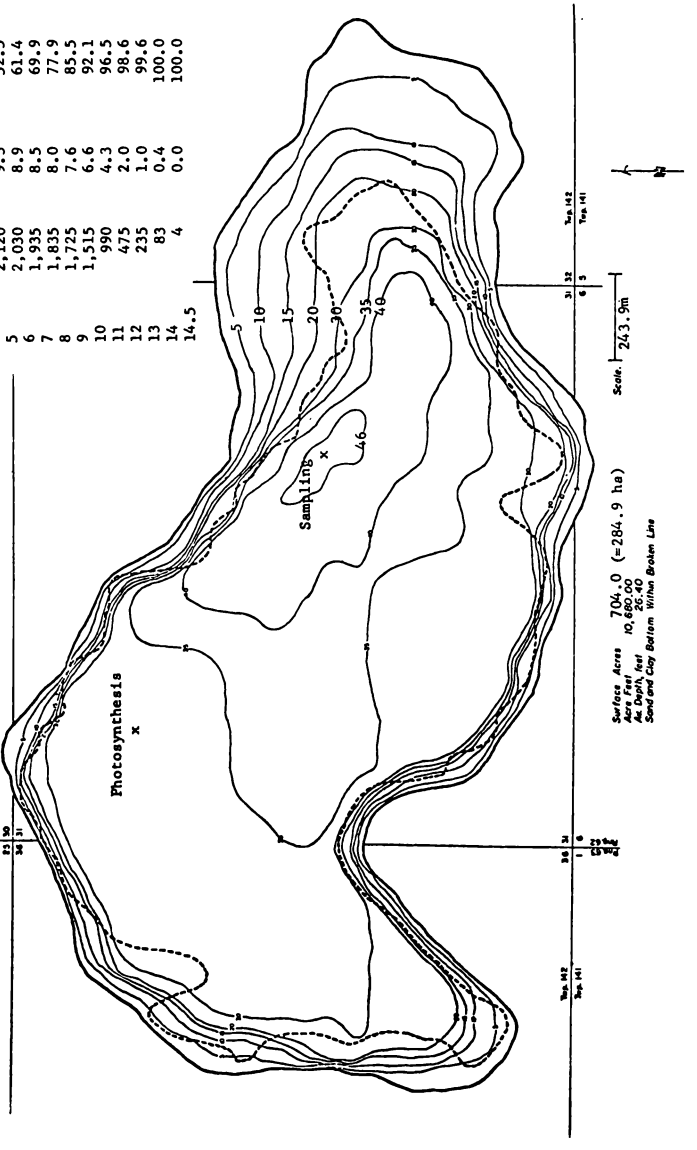


FIGURE 1—Morphometry of Spiritwood Lake, North Dakota.

RESULTS AND DISCUSSION

Spiritwood Lake is an end reservoir and is therefore accumulating dissolved solids. The average concentration at the time of this investigation was about 2150 mg l^{-1}. A concentration of 2600 mg l^{-1} was measured in mid-February (Figure 2) as the result of maximum ice cover. A minimum of 1300 mg l^{-1} was recorded in mid-April, occurring just after run-off and ice melt.

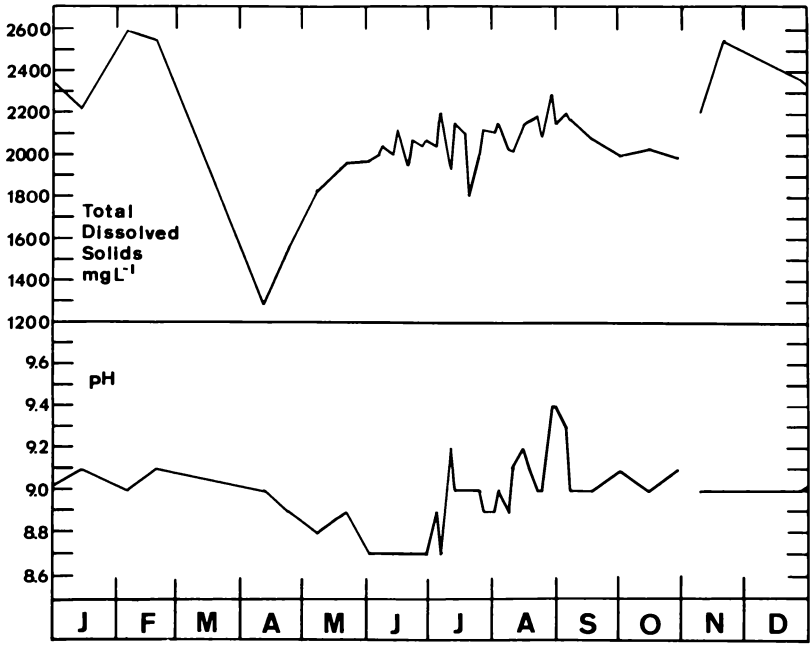


FIGURE 2—Total dissolved solids and pH measurements at the surface (0m) 9 November, 1965 to 29 October, 1966.

The pH values for Spiritwood fluctuated between 8 at the bottom and 9.4 at the surface. During the summer months chemical stratification occurred between seven meters and ten meters. Two relatively sharp peaks (Figure 2) were apparent in the epilimnion, one of 9.2 on 11 July and another of 9.4 on 29 and 31 of August. These were more or less abrupt and correspond to those shown by the transparency data (Figure 4). At the 14 meter level there was a constant drop in the pH throughout the summer with a minimum of 8.0 occurring in August.

The lake was anaerobic at the bottom until the 11th of April when turnover brought about a rise in nitrate at all levels. It became anaerobic again below 10 meters from 6 June to 17 September

1966. As expected, during the summer the nitrate in the epilimnion remained close to zero. A nitrate maximum (averaging 8 mg-at N l^{-1}) was noted following the fall turnover in November.

Phosphate fluctuations were similar to those observed in the nitrogen series. The soluble or reactive phosphates were reduced to zero in the epilimnion on 20 June at the onset of the second bloom. This condition remained until 22 August when again traces of phosphate were recorded in the epilimnion, but as the third bloom increased (31 August) the phosphate concentration was again reduced to zero. In the anaerobic part of the hypolimnion there was a continual build up of reactive phosphate until the fall turnover began on 17 September.

On 20 July, dissolved oxygen in the hypolimnion was not measurable by the routine Winkler method. At that time the average depth of the hypolimnion was about 1.98 meters. Dissolved oxygen was maximal on 7 May 66, 14.08 mg l^{-1}, at the same depth. Appropriate computations demonstrate that the total oxygen loss for the period was 2.788 mg cm^{-2}, yielding a daily oxygen deficit of 0.037 mg cm^{-2} day^{-1}. In Hutchinson's (1957) treatment this is a mesotrophic lake with respect to hypolimnetic oxygen deficit.

Maximum solar and sky radiation (Figure 3, panel E), measured at the lake, occurred from the end of May to mid-July. Total radiation (Table II) for the year amounted to 86,018 cal cm^{-2}; 35 percent (30,848 cal cm^{-2}) of this was available during the summer sampling period (6 June to 7 September).

When the investigation was begun on 9 November 65, the lake was homothermous at 6°C. Freezing occurred about 6 December and the lake remained ice covered for a 4-month period, until 11 April. The maximum ice depth noted was 0.84 meters (33 inches) in mid-February. Depth and volume of the lake permitted some warming from the bottom during the winter.

The heating cycle was evident by late May and a definite themocline was first noted on 6 June (Figure 4); from 22 June to 30 August the thermocline was well developed, ranging from 4 to 9 meters. The maximum temperature in the epilimnion was 26°C and this occurred during mid-July; a period of cooling was noted during August when the temperature dropped to 19°C on the 22nd. This was followed by a final warming period when the temperature rose to 21°C on 31 August.

The transparency measurements (Figure 4) indicated the euphotic zone had developed to a maximum depth of 7.4 meters on 22 January and to a minimum depth of 1.6 meters on 29 August. Figure 4 shows four definite transparency minima occurring during the spring, summer and fall. The 7 May and 15 October minima are produced in response to the spring and fall turnovers. Although an algal bloom

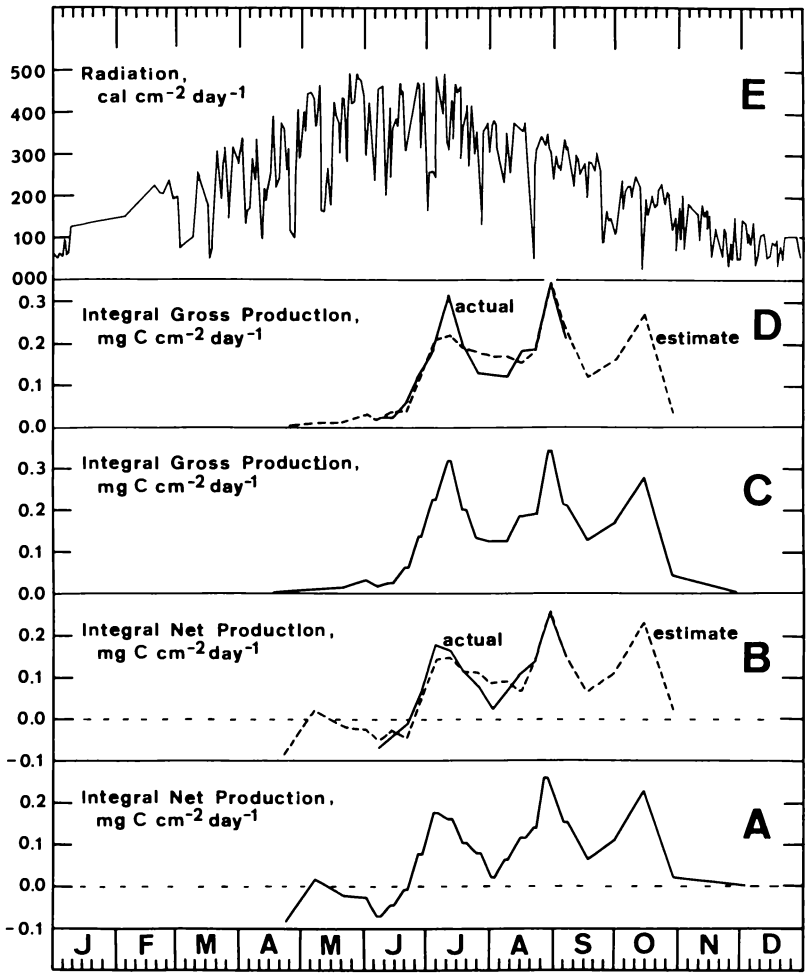


FIGURE 3—Solar and sky radiation measured at the lake (panel E). Integral gross and integral net production as measured (actual) and estimated values. In panels A and C, daily values from 6 June to 7 September are based on measurements made over a 48-hour period plotted for the two days in succession. For the remainder of the year these curves are joined to curves derived from estimates. In panels B and D are shown the mean measurements, solid line, for the period, 6 June to 7 September, along with the estimate, dashed line. The sampling year is from 9 November, 1965 to 29 October, 1966.

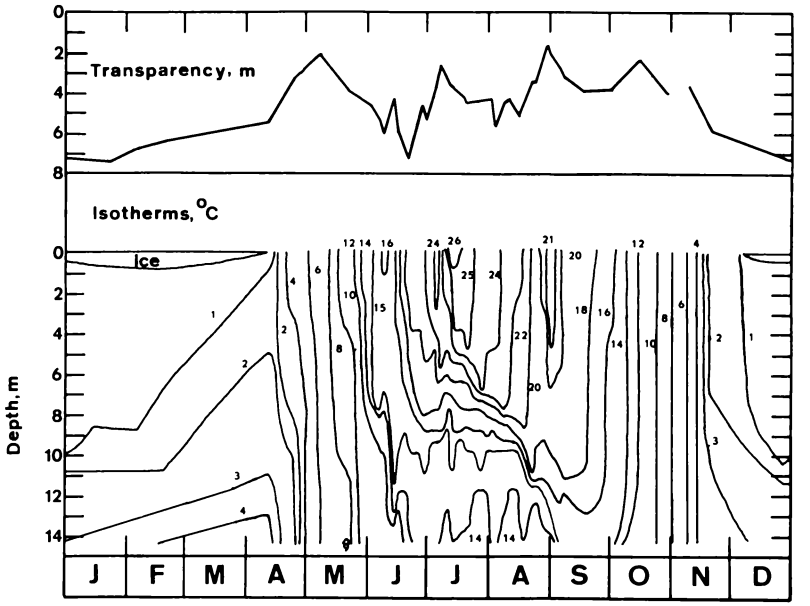


FIGURE 4—Seasonal variations of temperature and transparency in Spiritwood Lake, North Dakota, 9 November, 1965 to 29 October, 1966.

did occur during the spring turnover, it is probable that the magnitude of this minimum is enhanced by turbid conditions resulting from the mixing that occurs during the turnover and run off. The minima of 6 July, 29 August and 15 October are more definitely caused by high plankton populations.

Phytoplankton counts (Table I) were made covering the period 11 April, 1966 to 29 October 1966. Early in the period the Chrysophyceae (35.3 per cent) and Bacillariophyceae (52 per cent) made up 87.3 per cent of the volume of the algal population. On the 13 July the algal composition on a volume basis changed radically and was composed of 97.3 per cent Cyanophyceae, most of which was *Lyngbya sp.* This gave way to *Gomphosphaerium sp.* which comprised 60.2 per cent of the total volume by the 31 August; of the remaining cell volumes, 35.2 per cent was *Stephanodiscus sp.*

During the late summer period, 3 August to 31 August, *Chaetoceros sp.* was present in trace amounts. Total dissolved solids during this period ranged from about 2150 mg l^{-1} early in the month to 2275 mg l^{-1} at month's end, and this is the period of maximum salt concentration for the summer. *Chaetoceros elmorei* has been reported from Lake Lenore, Washington (Anderson 1958) and inland saline waters of Algeria (as *Chaetoceros sp.*, Beadle 1943).

TABLE I

TAXONOMIC COMPOSITION, PER CENT VOLUME AND PER CENT NUMBER OF CELLS FOR FOUR PHYTOPLANKTON BLOOMS

	7 May				13 July				31 August				15 October			
	Per cent Volume		Per cent Number		Per cent Volume		Per cent Number		Per cent Volume		Per cent Number		Per cent Volume		Per cent Number	
	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$
Cyanophyceae																
Lyngbya sp.					99.4-76.2	92.2	99.9-22.2	66.9	13.5-0.0	4.2	2.0-0.0	0.6	Trace		Trace	
Gomphosphaerium sp.					22.1-0.0	5.3	72.1-0.0	28.5	82.5-2.6	60.2	99.6-95.5	98.1	64.9-49.7	59.2	99.8-99.6	99.7
Gleotrichia sp.					Trace		Trace									
Anabaena sp.					Trace		Trace		Trace							
Chlorophyceae																
Characium sp.	14.4-1.4	7.3	51.9-8.7	30.3	Trace		2.2-0.1	1.1	Trace		Trace		Trace		Trace	
Spirogyra sp.	Trace		Trace						Trace		Trace		3.5-1.5	2.6	Trace	
Staurastrum sp.	Trace		Trace										Trace		Trace	
Westella sp.					Trace		4.3-0.0	2.5								
Microspora sp.													Trace		Trace	
Miscellaneous													1.5-0.9	1.2	Trace	
Chrysophyceae																
Miscellaneous	51.9-19.5	35.3	76.3-43.1	59.7									Trace		Trace	
Bacillariophyceae																
Stephanodiscus sp.	29.5-0.0	19.7	Trace		8.32-0.0	2.2	Trace		97.4-14.5	35.2	4.0-0.0	0.8	45.0-30.8	36.6	Trace	
Chaetoceros sp.									Trace		Trace					
Miscellaneous	44.7-24.2	32.3	13.7-4.1	8.5	Trace		Trace		Trace		Trace		Trace		Trace	

TABLE I—Taxonomic composition, per cent volume and per cent number of cells for the four phytoplankton blooms.

The peak of the final phytoplankton bloom was on 15 October. This bloom was composed mostly of *Gomphosphaerium sp.* (52.9 per cent), and *Stephanodiscus sp.* (36.6 per cent).

Chlorophyceae and *Euglenophyceae* were present in every bloom, but *Chlorophyceae* were present in significant amounts only in the first bloom, *Euglenophyceae* were never present in any significant quantities.

Production values for the summer period are computed from *in situ* measurements in the lake. The daily gross production values showed two pulses in phytoplankton activity, the first was apparent on 23 June and developed to a maximum of 3.2 mg O_2 l^{-1} day^{-1} at the one meter level on 11-13 July and ended on 18 July. Thus the duration time between rise in the bloom until climax was about four times as long as that required for the bloom to disintegrate. This appears to be the same for the second summer bloom which began about the 17 August. This bloom increased to a peak of 3.5 O_2 l^{-1} day^{-1} at the one meter level on 29-31 August and by the 5 September it had decreased rather sharply.

After integrating all levels measured the maximum rate of carbon fixation (Figure 3, panel C), 0.345 mg C cm^{-2} day^{-1}, occurred on 29-31 August, and a minimum of 0.020 mg C cm^{-2} day^{-1} was recorded on 68 June. Integrating all the values obtained during the period, 6 June-7 September, an integral gross production of 12.4 mg C cm^{-2} or 111.1 cal cm^{-2} was obtained (Table II). The values for the remainder of the year (8 September to 5 June) are estimations derived from multiple regression equations (Table III) using transparency, integrated cell volume and the one meter water temperatures as independent variables. Integral gross production for the year was 159.2 cal cm^{-2} or 0.19 per cent of total isolation.

Integral phytoplankton respiration (Table II) amounted to approximately 21.2 per cent (23.6 cal cm^{-2}) of the integral gross production for the sampling period, yielding a mean P-to-R ratio of 5.03 (Figure 5). Integral phytoplankton respiration was 18.6 per cent of integral gross production for the period 9 November, 1965 to 3 December, 1965 and 23 April, 1966 to 29 October, 1966, when 43.9 cal cm^{-2} were respired by phytoplankton.

These computations demonstrate that 76.3 per cent (121.3 cal cm^{-2}) of the energy of integral gross production (158.9 cal cm^{-2}) on an 8.3 month (9 November, 1965 to 3 December, 1965 and 23 April, 1966 to 29 October, 1966) basis is stored for further use by herbivores and bacteria. Integral phytoplankton net production (Figure 3, panel A) during the 8.3 months was 0.20 per cent of the total isolation. The net storage for the summer sampling period is 0.28 per cent (87.5 cal cm^{-2}), of the total energy available at the surface of the lake.

REGRESSION RELATIONSHIPS

Nine independent variables (Figures 3, panel E; 4 and 6) were

TABLE II

ENERGY TRANSFORMATIONS AS CALORIES CM^{-2} AND AS PER CENT OF SOLAR AND SKY RADIATION

Sampling Period	Solar and Sky Radiation cal cm^{-2}	Int. Gross Production cal cm^{-2}	Int. Community Respiration cal cm^{-2}	Int. Phyto. Respiration cal cm^{-2}	Int. Net Production cal cm^{-2}	Int. Phyto. Net Production cal cm^{-2}
1	2	3	4	5	6	7
9 Nov 65 to 29 Oct 66	86,017.5	(159.2)**				
6 June 66 to 7 Sept 66	30,847.5	111.07*	52.44*	23.6	58.63	87.5
9 Nov 65 to 3 Dec 65 and 23 Apr 66 to 29 Oct 66	63,212.5	(158.89)	(83.60)	(37.6)	(75.29)	(121.3)

	EFFICIENCES, PER CENT OF SOLAR AND SKY RADIATION		
9 Nov 65 to 29 Oct 66	(0.185)		
6 June 66 to 7 Sept 66	0.360	0.19	0.28
9 Nov 65 to 3 Dec 65 and 23 Apr 66 to 29 Oct 66	(0.251)	(0.12)	(0.20)

*direct measurement; **(calculated)

TABLE II—Energy transformation as calories cm-2 and as per cent of solar and sky radiation. The entries in parentheses are estimates based on the multiple linear regression equation. The entries not enclosed in parentheses are based on *in situ* measurements.

TABLE III

MULTIPLE LINEAR REGRESSION EQUATIONS AND PERTINENT STATISTICS

		S.E. of Dep. Var.	R	F	F_{imp}	Variable	S.E. of Coef.	t-test	Partial Correlation coef-(r)
	Integral Gross Production (Y_{igp}; mgC cm^{-2} day^{-1})								
1.	Y_{igp} = 0.420 - 0.0605 (S)	0.0576	0.811	23.079		S	0.0126	4.804	0.811
2.	Y_{igp} = 0.187 - 0.0554 (S) + 0.00988 (T^oC)	0.0499	0.874	17.858	4.981	S	0.0111	4.972	0.776
						T^oC	0.00443	2.232	0.402
3.	Y_{igp} = -0.0173 - 0.0305 (S) + 0.0132 (T^oC) + 0.000339 (C.V.)	0.0445	0.911	16.206	3.802	S	0.0162	1.889	0.576
						T^oC	0.00431	3.070	0.466
						C.V.	0.000174	1.950	0.530
4.	Y_{igp} = -0.126 - 0.0288 (S) + 0.0124 (T^oC) + 0.000388 (C.V.) + 0.000334 (I_o)	0.0454	0.917	11.867	0.634	S	0.0166	1.736	0.560
						T^oC	0.00452	2.741	0.450
						C.V.	0.000188	2.069	0.567
						I_o	0.000420	0.796	0.518
	Integral Net Production (Y_{inp}; mgC cm^{-2} day^{-1})								
5.	Y_{inp} = 0.362 - 0.0639 (S)	0.0464	0.876	39.582		S	0.0101	6.291	0.876
6.	Y_{inp} = 0.170 - 0.0597 (S) + 0.00814 (T^oC)	0.0398	0.918	29.540	5.303	S	0.00889	6.709	0.847
						T^oC	0.00353	2.303	0.354
7.	Y_{inp} = -0.0197 - 0.0382 (S) + 0.0111 (T^oC) + 0.00357 (Chl *a*)	0.0355	0.942	26.076	3.849	S	0.0135	2.832	0.679
						T^oC	0.00350	3.182	0.415
						Chl *a*	0.00182	1.962	0.505
8.	Y_{inp} = 0.0315 - 0.0379 (S) + 0.00948 (T^oC) + 0.00414 (Chl *a*) -0.00319 (Ast. Carot.)	0.0357	0.947	19.528	0.873	S	0.0136	2.789	0.674
						T^oC	0.00395	2.401	0.383
						Chl *a*	0.00193	2.145	0.543

TABLE III—Multiple linear regression equations and pertinent statistics. T°C is the temperature in degrees Celsius at one meter. C. V. is the integrated cell volume as μ^3 100 ml^{-1}. Chl *a* is the integrated chlorophyll *a* as $mg^{m^{-2}}$. I_0 is incident solar and sky radiation. Ast. Carot., the integrated astacin carotenoids in Specific Pigment Units m^{-2}. S, the Secchi disk measurements as meters.

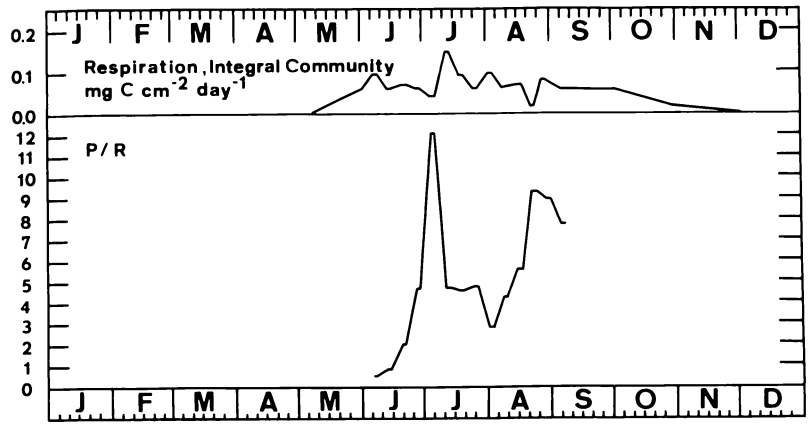


FIGURE 5—Community respiration as integrated daily values, upper panel. P-to-R ratios based on respiration values corrected for bacterial and animal respiration.

examined for regression relationships with integral gross and integral net production. For integral gross production (Table III) transparency (Secchi disk, S) alone provided the most reliable regression relationship. In descending order of acceptability were water temperature (at one meter, T°C), integrated cell volume (C.V.) and incident radiation (I_0). Regression equations involving transparency alone and transparency with the addition of the other three independent variables were computed. Equation number 4 involving incident radiation did not significantly explain the variation of the dependent variable (integral gross production) over that involving the other three independent variables (equation number 3).

For the estimation of integral net production, transparency and water temperature at one meter again provided the most acceptable relationship. The greatest decrease in unexplained variation of the dependent variable (integral net production) was provided by the addition of the chlorophyll *a* measurements (equation 7). The very close approximation to the measured values of both integrated gross and integrated net production is apparent in Figure 3, panels B and D, when equations 4 and 7 are solved.

A study was made of the linear correlations (Table IV A and B) between all variables measured. Of 406 correlations examined 98 were significant of the one per cent level. Most of the significant variables are obviously related. Some are only secondarily related through another of more immediate relation.

A nonsignificant correlation between integral gross production

and solar and sky radiation (Table IV A, Column a) was obtained. The only significant correlation (5 per cent level) obtained for all variables with solar and sky radiation was that with integrated total chlorophyll (Table IV A, Column f).

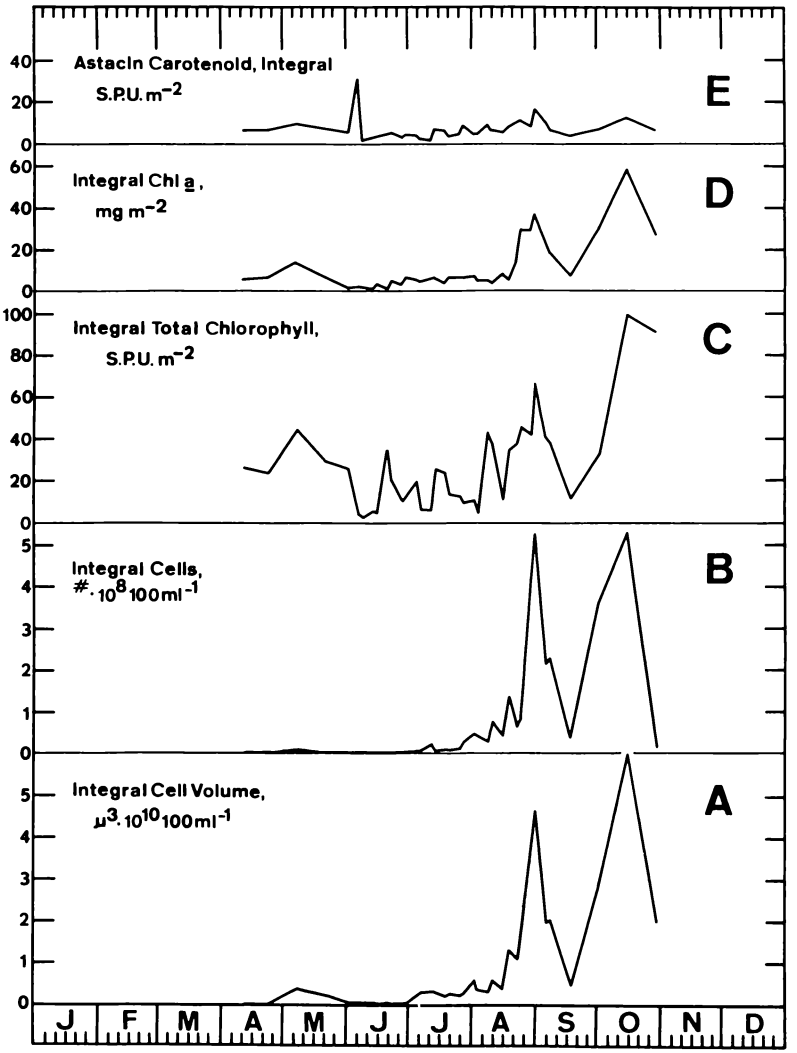


FIGURE 6—Integrated daily cell number (panel B) and corresponding cell volumes (panel A). Plant pigments: integrated chlorophyll *a* (panel D), integrated total chlorophyll (panel C) and integrated astacin carotenoids (panel E).

TABLE IV A

LINEAR CORRELATIONS OF CHEMICAL AND PHYSICAL DATA

		Int. Gross Product ($mgC\ cm^{-2}$)	Int. Net Product ($mgC\ cm^{-2}$)	Int. Chl *a* ($mg\ m^{-2}$)	Int. Astacin Carot. ($SPU\ m^{-2}$)	Int. Total Cells ($\#100\ ml^{-1}$)	Int. Total Chl. ($SPU\ m^{-2}$)	Int. Cell Volume ($\mu^3\ 100\ ml^{-1}$)	Secchi disk (m)	Water Temp. at 1 m (^oC)	pH at 1 m	Phth Alk. at 1 m (mgl^{-1})
		a	b	c	d	e	f	g	h	i	j	k
1.	Solar and Sky Radiat. ($cal\ cm^{-2}$)	0.02	-0.08	-0.45	0.12	-0.34	-0.57	-0.39	0.14	0.33	-0.12	0.24
2.	M.O. Alk at 1 m (mgl^{-1})	-0.72	-0.69	-0.31	0.17	-0.28	0.27	0.30	0.60	-0.83	-0.67	-0.77
3.	Phth Alk at 1 m (mgl^{-1})	0.65	0.60	0.21	-0.24	0.31	0.30	0.28	0.47	0.71	0.52	
4.	pH at 1 m	0.76	0.72	0.74	0.35	0.79	0.67	0.80	-0.71	0.26		
5.	Water Temp. at 1 m(^oC)	0.50	0.45	-0.12	-0.50	-0.11	-0.02	0.11	-0.20			
6.	Secchi disk (m)	-0.83	-0.88	-0.76	-0.21	-0.69	-0.54	-0.75				
7.	Int. Cell Vol.($\mu^3\ 100\ ml^{-1}$)	0.65	0.70	0.96	0.48	0.98	0.79					
8.	Int. Total Chl. ($SPU\ m^{-2}$)	0.52	0.61	0.81	0.36	0.75						
9.	Int. Total Cells ($\#100\ ml^{-1}$)	0.61	0.64	0.90	0.46							
10.	Int. Astacin Carot. ($SPU\ m^{-2}$)	0.00	0.01	0.15								
11.	Int. Chl *a* ($mg\ m^{-2}$)	0.64	0.71									
12.	Int. Net Prod. ($mgC\ cm^{-2}$)	0.96										

TABLE IV A—Linear correlations of chemical and physical measurements; $r_{.05} = 0.50$, $r_{.01} = 0.62$, d.f = 14.

TABLE IV B

LINEAR CORRELATIONS OF CHEMICAL AND PHYSICAL DATA

		Total Alk at 1 m	NO_2 at 1 m	NO_3 at 1 m	NH_3 at 1 m	Reactive PO_4 at 1 m	Total PO_4 at 1 m	Chl *a* at 1 m	Chl *c* at 1 m	Int. Chl *c*	Non Astacin Carot. at 1 m
		(mgl^{-1})	($\mu g\text{-at } Nl^{-1}$)	($\mu g\text{-at } Nl^{-1}$)	($\mu g\text{-at } Nl^{-1}$)	($\mu g\text{-at } Pl^{-1}$)	($\mu g\text{-at } Pl^{-1}$)	($mg\ m^{-3}$)	($SPU\ m^{-3}$)	($SPU\ m^{-2}$)	($SPU\ m^{-3}$)
		a	b	c	d	e	f	g	h	i	j
1	M.O. Alk at 1 m (mgl^{-1})	0.97	0.50	0.79	0.70	0.37	0.30	-0.20	0.05	-0.14	-0.33
2	Phth. Alk at 1 m (mgl^{-1})	-0.60	-0.30	0.70	-0.60	-0.55	-0.21	0.16	0.00	0.23	0.18
3.	pH at 1 m	-0.64	-0.66	-0.61	-0.49	-0.13	0.29	0.70	0.28	0.42	0.77
4	Water Temp. at 1 m (^{o}C)	-0.79	-0.25	-0.65	-0.55	-0.55	-0.61	-0.20	-0.19	0.03	-0.11
5	Secchi disk (m)	0.58	0.62	0.70	0.45	0.07	-0.35	-0.70	-0.29	-0.21	-0.68
6	Int. Cell Vol. (μ^3 100 ml^{-1})	-0.28	-0.60	-0.45	-0.15	-0.01	0.51	0.96	0.57	0.45	0.89
7	Int. Total Chl. ($SPU\ m^{-2}$)	-0.23	-0.33	-0.63	0.03	0.01	0.51	0.83	0.86	0.89	0.75
8	Int. Total Cells (#100 ml^{-1})	-0.24	-0.61	-0.38	-0.19	-0.12	0.42	0.91	0.53	0.42	0.80
9	Int. Astacin Carot ($SPU\ m^{-2}$)	0.13	0.00	0.03	-0.24	0.70	0.50	0.50	0.23	0.19	0.46
10	Int. Chl *a* ($mg\ m^{-2}$)	-0.31	-0.57	-0.50	-0.08	0.13	0.58	0.99	0.62	0.45	0.93
11	Int. Net Prod. ($mgC\ cm^{-2}$)	-0.65	-0.52	-0.83	-0.35	-0.25	0.25	0.67	0.38	0.35	0.68
12	Int. Gross Prod. ($mgC\ cm^{-2}$)	-0.67	-0.57	-0.79	-0.44	-0.25	0.26	0.60	0.26	0.26	0.64

TABLE IV B—Linear correlations of chemical and physical measurements in addition to those of Table IV A; $r_{.05} = 0.50$, $r_{.01} = 0.62$, d.f. = 14.

SUMMARY

Photosynthetic production was measured in Spiritwood Lake, North Dakota by the light and dark bottle method during the summer period from 6 June to 7 September 1966. Estimates of integral gross and integral net production for the period 9 November 1965 to 6 June, 1966 and from 7 September, 1966 to 29 October, 1966 were made from related variables measured simultaneously. The multiple linear regression equations involving the most significant of these variables are: $Y_{1gp} = 0.0132\ (T°C) + 0.000339\ (C.V.) - 0.0305\ (S) - 0.0173$, $Y_{1np} = 0.0111\ (T°C) + 0.00357\ (Chl.\ a) - 0.0382\ (S) - 0.0197$.

Integral gross production for the summer period based upon *in situ* measurements was 111.1 cal cm^{-2}, for an efficiency of 0.36 per cent. Integral gross production for the entire year, which includes the estimates based on other variables measured, was 159.2 cal cm^{-2}, for an efficiency of 0.19 per cent.

Integral phytoplankton respiration for the summer period was 23.6 cal cm^{-2} or 21.2 per cent of integral gross production, yielding a mean P-to-R ratio of 5.03. Integral phytoplankton respiration, estimated for the other 5.3 months (9 November, 1965 to December, 1965, 23 April, 1966 to 6 June, 1966 and from 7 September, 1966 to 9 November, 1966 was 13.1 cal cm^{-2} or 27.4 per cent of integral gross production.

Integral phytoplankton net production for the summer period was 87.5 cal cm^{-2} for 0.28 per cent efficiency. For the other 5.3 months integral phytoplankton net production was estimated to be 33.8 cal cm^{-2}. This yielded an integral phytoplankton net production of 121.3 cal cm^{-2} or 0.20 per cent of the available incident radiation (63,212.5 cal cm^{-2}) for the 8.3-month period.

ACKNOWLEDGEMENTS

This work was supported in part by the North Dakota Water Resources Research Institute with funds provided by the U. S. Department of Interior, Office of Water Resources Research under P. L. 88-379; in part by the North Dakota State Game and Fish Department, Russell Stuart, Commissioner, Dale Henegar, Chief of Fisheries Division, and in part by the North Dakota Institute for Regional Studies. We are grateful to Stanley Johnson for his efforts in the field as well as in the laboratory and the staff at Spiritwood Lake Fish Hatchery for their cooperation.

REFERENCES

Anderson, G. C. 1958. Seasonal Characterisics of Two Saline Lakes in Washington, Limnol. and Oceanogr., 3-51-68.

A.P.H.A., Inc., 1955. Standard Methods for the examination of water, sewage and industrial wastes. Tenth edition. New York, 522 pp.

Beadle, L. C. 1943. An ecological survey of some inland saline waters of Algeria. J. Linn. Soc. Zool., 41:218-242.

Comita, G. W. and J. J. Comita, 1957. The internal distribution patterns of a Calanoid copepod population, and a description of a modified Clarke-Bumpus Plankton Sampler. Limnol. & Oceanogr., 2:321-332.

Drouet, Francis. Ward and Whipple, Ed. W. T. Edmondson, 1959. Fresh Water Biology, 2nd edition. John Wiley & Sons, Inc. New York, p. 171-189.

Hutchinson, G. E., 1957. A Treatise on Limnology Vol. I. John Wiley & Sons, Inc., New York, 1015 pp.

Patrick, Ruth and C. W. Reimer, 1966. The Diatoms of the United States Vol. I. Monographs of the Academy of Natural Sciences of Philadelphia No. 13, 688 pp.

Smith, G. M. 1950. The Fresh Water Algae of the United States, 2nd edition. McGraw Hill, New York, 719 pp.

Strickland, J. D. H. and T. R. Parsons, 1965. A Manual of Sea Water Analysis. Fisheries Research Board of Canada Bulletin No 125, 2nd edition, 203 pp.

Thompson, R. H. Ward and Whipple, Ed. W. T. Edmondson, 1959. Fresh Water Biology, 2nd edition. John Wiley & Sons, Inc. New York, p. 115-170.

Young, R. T. 1924. The Life of Devils Lake, North Dakota. Publ. N. Dak. Biol. Stat. p. 71.

KARYOTYPE OF THE WHITE-TAILED JACK RABBIT

S. M. Jalal, T. R. James and R. W. Seabloom

Department of Biology
University of North Dakota, Grand Forks, North Dakota

With the development of improved techniques for the handling of mammalian chromosomes, an increasing mass of karyological information is rapidly accumulating in this area. While certain mammalian groups, notably man, domestic animals, and rodents have been studied extensively, karyological information of the lagomorphs remains fragmentary and often erroneous. The diploid chromosome number of the domestic rabbit (*Oryctolagus cuniculus*) has been reported variously as 22, 44, and 44 to 54, with 44 generally being accepted as the correct diploid number (Makino, 1951; Ray and Williams, 1966). According to Makino (1951) *Lepus formosus* and *Lepus gichiganus ainu* both have a diploid chromosome number of 48. The chromosome numbers and karyotypes of North American leporids presently remain unreported. The purpose of our investigation was

to establish the chromosome number and the karyotype of the white-tailed jack rabbit (*Lepus townsendii campanius* Hollister, 1915).

MATERIALS AND METHODS

The karyologcal study was conducted on eight animals collected in Hettinger, Adams, and Grand Forks counties of North Dakota between March and December, 1966. Seven animals were collected by shooting, while one animal was obtained by caesarian section and raised to captivity to an age of 207 days.

Standard peripheral blood leucocyte cultures and bone marrow preparations (Yunis, 1965) were employed in the investigation. Blood samples were obtained by cardiac puncture or by using the bleeding technique described by Nace and Spradlin (1962). Bone marrow samples were extracted from the radius, tibia and femur of each animal, in order to determine the best source of actively dividing cells. Samples were processed immediately, following standard procedures and slides prepared by air-dried technique (Rothfels and Siminovitch, 1958). Slide preparations were stained with 2 per cent acetic orcein, giemsa blood stain, or with carbol fuchsin, following the procedure outlined by Carr and Walker (1952). Wherever possible, 10 or more well-spread C-metaphase cells were analyzed from each animal, in order to determine the chromosome number and to etablish a standard karyotype for *L. t. campanius*.

RESULTS AND DISCUSSION

Blood leucocyte and bone marrow techniques both provided satisfactory C-metaphase cells. However, bone marrow preparation, because of its adaptability to field investigatons, proved to be more satisfactory.

Among the three sites selected, the femur appears to be the best source of metaphase cells. Examination of tibial preparations revealed only an occasional metaphase cell, while no dividing cells were found in preparations from the radius.

Carbol fuchsin was observed to stain the chromosomes more intensely than the other stains used. It produces sharply delineated chromosomes, of the quality necessary for photographic work.

On the basis of the examination of 84 C-metaphase cells, from seven animals, the diploid chromosome number of *L. t. campanius* is determined to be 48, with 23 pairs of autosomes and one pair of sex chromosomes (Table I). Countable metaphase cells were not obtained from one animal included in the study. This was attributed to an unavoidable lapse of time between death and treatment. Some deviating counts of 46, 47, and 49 were recorded. These deviations can be attributed to difficulties in counting overlapping chromosomes and to inherent problems in preparation and staining. The preponderance of counts of 48 chromosomes and the representative photographic karyotypes (Figure 1) confirm the diploid chromosome number to be 48.

TABLE I

SOMATIC CHROMOSOME NUMBERS FOR LEPUS TOWNSENDII CAMPANIUS

Animal	Sex	Chromosome Count				Mode
		46	47	48	49	
1	male	2	2	8	1	48
2	female		3	13	2	48
3	female	1	1	7	1	48
4	female	4	4	6		48
5	male	1	1	8		48
6	male	2	2	4	1	48
7	male		2	7	1	48
8	female		No Data			

The karyotype of *L. t. campanius* is represented in five groups, from A to E (Figure 1), based upon the morphological characteristics of the chromosomes. The first, Group A, consists of the five longest pairs of chromosomes with submedian centromeres. The 10 chromosomes in Group B are somewhat shorter than those of Group A and are characterized by having metacentric to sub-metacentric centromeres. There are six pairs of chromosomes in Group C, all of which are shorter than those of Group B. The chromosomes in this group are sub-telocentric to almost acrocentric. Group D is characterized by four pairs of sub-metacentric chromosomes which are shorter than those of Group C. The shortest sub-metacentric to acrocentric chromosomes belong to Group E. A total of three pairs of chromosomes are classified in this group.

The sex chromosomes are distinguished as being X-X in females and X-Y in males. The X chromosome is of medium size and sub-metacentric, like the chromosomes of Group C. The Y chromosome appears to be acrocentric and is the smallest one found in *L. t. campanius*. Using these morphological characteristics as criteria, we have associated the Y chromosome with Group E.

The morphological characteristics of the chromosomes have been represented in the idiogram (Figure 2). The chromosome lengths range from 1.5 to 6.5 microns, in the male. Chromosomes 1, 9, and 13 are almost metacentric, and the Y appears to be acrocentric. All the other chromosomes are submetacentric with variable centric positions.

A few polyploid cells were observed, including several tetraploids and one cell which appears to be an octaploid (Figure 3). It is presumed that a certain proportion of polyploid cells can arise

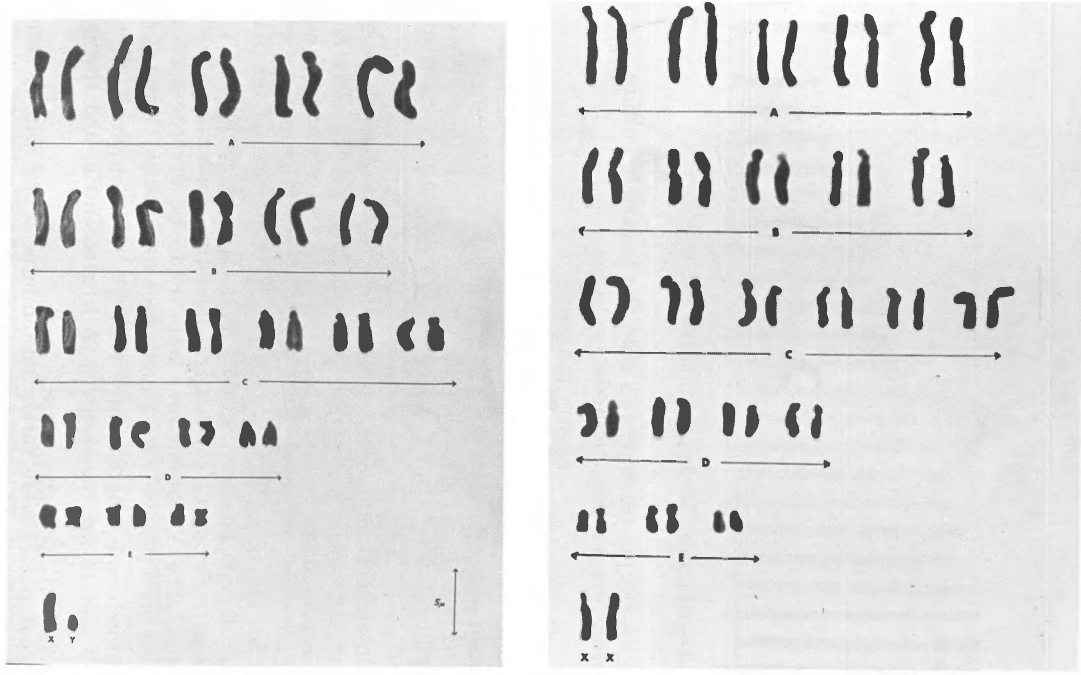


FIGURE 1—a. Karyotype of *Lepus townsendii campanius* male (left). b. Karyotype of *Lepus townsendii campanius* female (right).

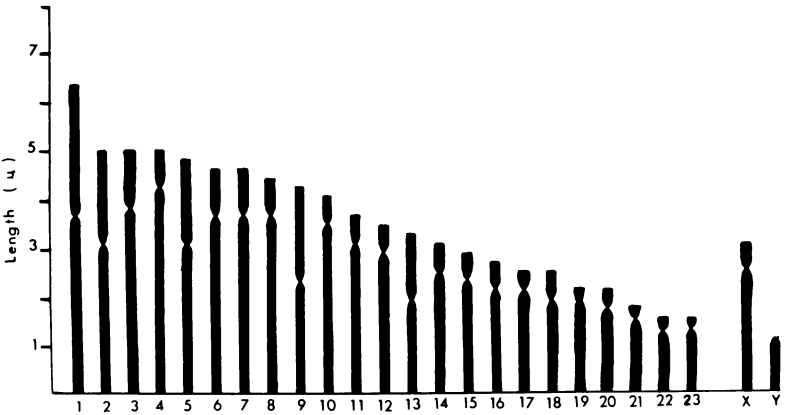


FIGURE 2—Idiogram of *Lepus townsendii campanius.*

due to the colchicine treatment in both the leucocyte culture and the bone marrow preparation methods.

SUMMARY

Leucocyte culture and bone marrow preparations were employed to determine the chromosome number and to establish a standard karyotype in the white-tailed jack rabbit (*L. t. campanius*). The femur appears to be a better source of dividing cells than either the tibia or radius. Carbol fuchsin was the most satisfactory stain among those used.

The diploid chromosome number was determined to be 48, with 23 pairs of autosomes and a pair of sex chromosomes. The karyotype was represented by five groups according to the morphological similarities of the chromosomes. The chromosome lengths were represented in an ascending order, with Group A possessing the largest and Group E the smallest chromosomes. The X chromosome was associated with Group C, since it is a medium length submetacentric chromosome. The Y chromosome appears to be acrocentric and is the smallest chromosome. We have associated it with Group E.

The chromosome lengths range from 1.5 to 6.5 microns. Chromosomes 1, 9 and 13 are metacentric; Y is acrocentric, and the others are sub-metacentric.

Tetraploid nuclei were rarely encountered along with a solitary, apparent octoploid cell. These were presumed to be due to the colchicine treatment.

ACKNOWLEDGEMENTS

Appreciation is expressed to Dr. T. C. Hsu, M.D., Anderson Hospital and Tumor Institute, Houston, Texas, and Mrs. Jean Saumur, Department of Pathology, University of North Dakota, for advice on

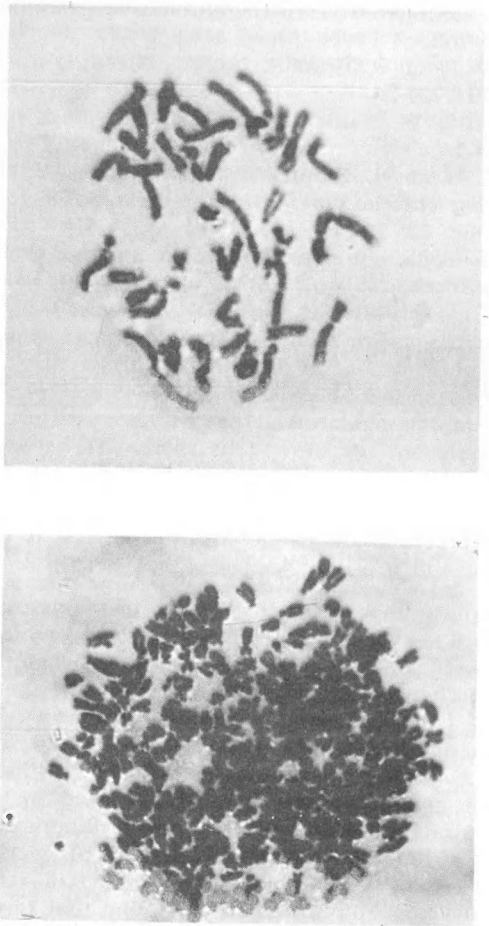


FIGURE 3—
a. A normal cell at C-metaphase with 48 chromosomes
b. A polyploid cell at C-metaphase at octaploid or near octaploid level .

chromosome preparations; Mr. Jack Samuelson, North Dakota Game and Fish Department, gave valuable assistance in collecting animals for this investigation. A partial support from the University of North Dakota, Faculty Research Grant (No. 4422-80) is also gratefully acknowledged.

LITERATURE CITED

1. Carr, D. H. and J. E. Walker. 1952. Carbol fuchsin as a stain for human chromosomes. Stain Technol. **27**: 233-255.

2. Makino, S. 1951. An atlas of the chromosome number in animals. Iowa State College Press, Ames, Iowa p 267.
3. Nace, G. W. and P. Spradlin. 1962. Bleeding rabbits. Turtox News, **40** (1): 26-29.
4. Ray, M. and T. W. Williams. 1966. Karyotype of rabbit chromosomes from leucocyte cultures. Can. J. Genet. Cytol. **8**: 393-397.
5. Rothfels, K. H. and L. Siminovitch. 1958. An air drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technol. **33**: 73-77.
6. Yunis, J. J. 1965. Human chromosome methodology. Academic Press, New York. 258 p.

THE EFFECT OF SULFUR AND PEAT MOSS ON THE SURVIVAL OF PONDEROSA PINE IN ALKALINE SOIL

Larry L. Jensen and Howard L. Bissonnette

Department of Plant Pathology
North Dakota State University, Fargo, North Dakota

INTRODUCTION

Recent attempts to increase the growth of ponderosa pine (*Pinus ponderosa* Dougl.) with soil amendments of mycorrhizal fungi containing soil, has shown that initial survival was poor in alkaline soils (pH 7.5 to 8.5). This paper describes experiments designed to increase the survival of ponderosa pine by reducing the soil alkalinity to a pH between 4.5 and 6.5.

Stoeckler (4) was the first to recognize the value of reducing the soil pH for growing pines when he reported that sulfuric acid or aluminum sulfate would reduce the pH of alkaline soil adequately for the growth of pine trees. However, the soil pH would not remain acidic for more than a year or two before again returning to its original pH. Stoeckler and Arneman (3) found that flowers of sulfur would reduce adequately the soil pH, and its effects would last up to 6 years. Further studies by McComb (2) and Dale, McComb, and Loomis (1) have shown the value of reducing the soil pH to obtain good development of mycorhizal roots and subsequently good growth of pines.

METHODS AND MATERIALS

Experiments to determine if sulfur and acid peat moss (pH 3) would reduce the pH of alkaline soil and to determine the extent to which they reduced the soil pH were set up in the greenhouse. Three hundred and fifty grams of Fargo Bearden soil was amended with sulfur, sulfur-peat moss and peat moss at rates of 0, 2, 4, 8, 16, 32, 64, and 128 grams. Each treatment was replicated 5 times. Fargo Bearden soil has considerable amount of clay, a pH of 7.5 to 8.5 and

is typical of the Red River Valley. The amended soil was placed in six-inch glazed pots which were randomized on greenhouse benches. The pots were watered at regular intervals for the duration of the experiment. The soil treatments used in this experiment were not sterilized because the soil microflora must be present to reduce the siol pH. One-gram soil samples were taken from each pot after 120 and 180 days for pH measurements. Distilled water was added to the sample to make the pH determinations which were made with a Beckman pH meter.

The effects of surfur and peat moss on the survival of ponderosa pine were tested in field plots on the North Dakota State University Agronomy Seed Farm near Casselton, North Dakota. Combinations of sulfur, peat moss and sulfur-peat moss were used as soil amendments. These plots were planted with ponderosa pine seedlings having either mycorrhizal roots or sterile roots. Special steps were taken to grow the sterile trees from seed so as to obtain trees without mycorrhizal roots. The seeds first were surface sterilized with 35 per cent hydrogen peroxide as described by Trappe (5), and germinated on steam sterilized perlite. After germination the seedlings were transplanted into 5-inch glazed pots of soil that had been sterilized with gas (ethylene oxide). The potted seedlings were grown in the greenhouse and watered at regular intervals for 10 months. In May, 1966, the seedlings were transplanted into the field plots. At this time the seedlings had true needles and were about 6 inches high. Before planting, the roots of each plant were carefully examined for mycorrhizae; if fungi were found, the seedlings were not used.

Trees with mycorrhizal roots were obtained from the North Dakota State Nursery, Towner, North Dakota. The trees were classified as 2-2 seedling stock and were about 7 to 10 inches high with well developed mycorrhizal roots.

The soil amendments were applied at the following rates: sulfur (40 lb./acre), acid peat moss (70 lb./acre), sulfur (40 lb./acre) plus acid peat moss (70 lb./acre), a no-amendment plot served as a check. Each treatment was applied with a fertilizer spreader and worked into the ground with a roto-tiller. Each treatment was replicated 3 times with 8 trees per replication in the plot with sterile trees and 30 trees per replication in the plot with trees having mycorrhizal roots. The trees were planted in holes 6 inches deep by 6 inches in diameter and spaced 6 feet apart. After planting, each tree was watered with approximately 3 to 4 gallons of water.

RESULTS AND CONCLUSIONS

The pH of the peat moss-soil mixture changed little after 120 and 180 days from the pH at the start of the experiment (Table I). Only at rates of 64 grams of peat moss per 350 grams of soil, or higher, would peat moss adequately reduce the soil pH to a suitable

TABLE I

THE pH OF 350 GRAMS OF SOIL AMENDED WITH 0, 2, 4, 8, 16, 32, 64, AND 128 GRAMS OF PEAT MOSS AT THE START OF THE EXPERIMENT AND AFTER 120 AND 180 DAYS.

Rate of peat moss in grams per 350 grams of soil	pH at the start	pH after 120 days	pH after 180 days
0	7.47	7.12	7.40
2	7.47	7.04	7.09
4	7.00	7.05	7.33
8	6.90	7.21	7.10
16	6.80	6.94	7.06
32	6.40	6.93	6.35
64	6.30	6.05	6.20
128	5.90	5.52	5.71

range of 4.5 to 6.5 for growing pine trees. After 120 and 180 days the pH of the sulfur-soil mixture was reduced favorably at rates of 4 grams of sulfur per 350 grams of soil or higher (Table II).

TABLE II

THE pH OF 350 GRAMS OF SOIL AMENDED WITH 0, 2, 4, 8, 16, 32, 64, AND 128 GRAMS OF FLOWERS OF SULFUR, AT THE START OF THE EXPERIMENT AND AFTER 120 AND 180 DAYS.

Rate of sulfur in grams per 350 grams of soil	pH at the start	pH after 120 days	pH after 180 days
0	7.20	7.19	7.30
2	7.09	6.92	6.98
4	6.90	6.50	6.38
8	6.53	5.44	4.78
16	6.12	4.62	2.82
32	5.18	2.55	2.24
64	5.16	2.04	2.26
128	5.00	1.99	2.16

However, after 180 days at rates of 16 grams of sulfur per 350 grams of soil the soil pH was reduced below pH 4.5, which is not conducive to good growth of pines. Data from these experiments indicated that sulfur was superior to acid peat moss for reducing the pH of alkaline soil. These experiments point out the necessity for a critical evaluation of the rate of sulfur necessary to maintain the soil pH for long periods of time. Sulfur applied at too high a rate,

after a period of time, continued to reduce the soil pH below a desirable point.

The effect of sulfur and peat moss on the survival of ponderosa pine was determined by amending field soil with sulfur, acid peat moss, and sulfur plus acid peat moss. Trees with mycorrhizal and without mycorrhizal roots were used as test plants.

Trees without mycorrhizal roots at the time of planting survived poorly when compared with trees having mycorrhizal roots. Only 4.4 per cent of the trees without mycorrhizal roots survived the first year. Seventy-nine per cent of the trees with mycorrhizal roots survived the first year. The trees with mycorrhizal roots planted in soil treatments of sulfur or sulfur plus acid peat moss survived significantly better than the untreated check (Tables III and IV).

TABLE III

THE MEAN SURVIVAL OF PONDEROSA PINE TREES PLANTED IN SOIL AMENDED WITH SULFUR, ACID PEAT MOSS, SULFUR PLUS ACID MOSS, AND NO AMENDMENT.

	Replications				
Treatment	1	2	3	Total	Mean
Sulfur	29	28	29	86	28.6
Peat Moss	20	23	20	63	21.0
Sulfur-peat moss	30	30	30	90	30.0
No treatment	15	11	18	44	14.6
Total	94	92	97	283	—

TABLE IV

ANALYSIS OF VARIANCE OF DATA GIVEN IN TABLE III.

Source of Variation	Degree of Freedom	Sum of Sources	Mean Squares	F
Replications	2	50		
Treatments	3	449	149	22.9**
Error	6	39	6.5	
Total	11	538		

** Exceeds the 1 per cent level of significance

This indicated that sulfur either alone or combined with peat moss was a desirable soil amendment to increase the survival of ponderosa pine seedlings planted in alkaline soil.

DISCUSSION

Data presented here have shown that sulfur was superior to acid peat moss for reducing the pH of alkaline soil. However, peat moss because of its other favorable properties should not be disregarded

as a possible soil amendment. Peat moss when mixed with soil increases the water-holding capacity of the soil and improves the physical texture.

The survival of ponderosa pine with mycorrhizal roots was found to be increased significantly by treating the soil with sulfur or sulfur-acid peat moss. Sulfur and peat moss treatments did not effectively influence the survival of ponderosa pine without mycorrhizal roots. This indicated that the presence of mycorrhizal roots along with an acid pH is necessary for optimum survival of ponderosa pine in the alkaline field soil. The acidified soil allowed better development of the mycorrhizal fungi enabling the plant to better absorb nutrients from the soil.

Little difference in survival was noted between the soil treated with sulfur and the soil treated with sulfur-peat moss. Differences may not be apparent between these two treatments in the short time allowed for this experiment. Effects may be noted later when the peat moss has had time to decompose in the soil. When peat moss decomposes it releases organic materials into the soil, which could stimulate the growth of the mycorrhizal fungi as well as the growth of the tree.

LITERATURE CITED

1. Dale, J., A. L. McComb, and W. E. Loomis. 1955. Chlorosis, mycorrhizal and the growth of pines on a high-lime soil. Forest Sci. 11:66-75.
2. McComb, A. L. 1943. Mycorrhizae and phosphorus nutrition of pine seedlings in a prairie soil nursery. Iowa Agr. Exp. Sta. Res. Bull. 314:582-612.
3. Stoeckler, J. H. and H. F. Arneman. 1960. Fertilizers in forestry, p. 127 to 195 *In* A. G. Norman (ed.) Advances in Agronomy. Academic Press, New York.
4. Stoeckler, J. H. and P. E. Stabaugh. 1965. Conifer nursery practice in the prairie-plains. U. S. Dep. Agr. Handbook. 279. 93p.
5. Trappe, J. M. 1961. Strong hydrogen peroxide for sterilizing coats of tree seeds and stimulating germination. J. Forestry 59:828-829.

UPTAKE OF GLYCINE AND PHENYALANINE BY SOME FRESH WATER INVERTEBRATES

Richard Armstrong and Richard A. Tubb[1]

Department of Biology

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

The main objective of this study was to determine whether certain aquatic invertebrates have the ability to remove disolved organic matter from dilute solutions of their habitats. A parallel objective was to assess the ecological significance of whatever uptake could be demonstrated. If aquatic metazoans aquire high energy substances by directly removing decomposition products from the habitat they are shortcircuiting the cyclic trophic system classically postulated for aquatic environments.

There has been much controversy over the ability of aquatic invertebrates to directly utilize dissolved organic matter as an energy source. Puttner (1901) postulated that zooplankton utilize dissolved organic matter as an energy source. Krough (1931) and Ruttner (1963) disagreed with Puttner's calculations and stated that no anatomical modifications existed that would indicate an adaptation for the uptake of dissolved organics from dilute solutions.

Other workers have demonstrated the ability of invertebrates to directly utilize dissolved organic materials. Brazda and Rice (1940) found that oxygen consumption of *Tubifex tubifex* was increased by the presence of glucose, and Stephens and Schinske (1961) demonstrated the ability to remove amino acids from solution is widespread in marine animals. Later work by Stephens (1962) showed that the solitary coral *Fungia scutaria* is capable of transporting glucose-C^{14} inward across the body wall and that the rate of glucose uptake was linear at concentrations likely to be found in natural sea water (0 to 20mg/l. *F. scutaria* was equally and in some cases more proficient at removing tyrosine, lysine, aspartic acid, glycine and lactate.

Stephens (1963) reported on the uptake of radioactive amino acids by the maldanid worm *Clymenella torquata*. He established that the uptake of glycine-C^{14} need not involve the digestive tract as a site of uptake. Utilizing manometric techniques he concluded that the oxidation of glycine, phenylalanine, lysine and valine could account for a significant amount of the oxygen consumed by *C. torquata*.

In a study of euryhaline polychates, *Nercis limnicola* and *Mereis suceinea*, Stephens (1964) found significant uptakes of glycine oc-

[1]Present address: Cooperative Fisheries Unit, Department of Wildlife Management, Ohio State University, Columbus Ohio 43201.

cured only in solutions containing 200 meq Cl-per liter (about 13,000 mg/l total dissolved solids). The ability to accumulate glycine increased with salt concentration, in both polychaetes. Using 12 fresh-water genera he was unable to demonstrate the removal of glucose-C^{14} and glycine-C^{14}. Stephens' results with euryhaline nereids and negative results with strictly fresh-water species made it desirable to examine fresh water animals inhabiting inland bodies of brackish water.

EXPERIMENTAL ANIMALS AND COLLECTION SITES

Specimens of *Tubifex tubifex,* an aquatic oligochaete, were collected from Devils Lake, North Dkota, and the English Coulee in Grand Forks County, North Dakota. Total dissolved solids in Devils Lake fluctuate about 11,000 mg/l (Swenson and Colby, 1955), and the English Coulee water contained 1,650 mg/l dissolved solids on the day the worms were collected. The soluble component of the Kjeldahl-nitrogen from the mud water interface was determined to be 7.56 mg/l at the Devils Lake collection site and 4.63 mg/l in the English Coulee.

No attempt was made to analyze for specific small molecules. Peterson, Fred and Domogalla (1925) demonstrated that 50 to 60 per cent of the soluble organic nitrogen is present in the form of free amino acids.

There is a difference in the response of the two populations to changes in salinity. *T. tubifex* from Devils Lake were allowed adjust to English Coulee water for 23 days. About twice as many worms from the Coulee were set aside for the same length of time. All worms were in Coulee water, free of mud. They were fed a suspension of decaying grass every 2 or 3 days. On the 23rd day all worms were blotted dry and weighed.

Thirty of the Coulee worms were returned to the Coulee water. The other worms were placed in Devils Lake water. On reweighing 2 hours later worms collected at Devils Lake had lost 42 per cent of their weight compared to a weight loss of 24 per cent by the Coulee worms. The control group, in Coulee water, showed a 3 per cent loss.

We were unable to locate a population of *T. tubifex* growing in a habitat near Grand Forks. *Lumbriculus inconstans* Smith, another aquatic ohgochate, was collected from Lake Itasca, Minnesota (total dissolved solids 225 mg/l).

Both Devils Lake and Lake Itasca contain species of larval midge flies *Chironomus.* The Lake Itasca species is *Chironomus plumosus* L. The Devils Lake *Chironomus* was not identified to the species level. It is possible to acclimate larval *C. plumosus* to high salinities. One group showed no ill effects after 9 days in Devils Lake water. A second group withstood six hours exposure to Devils Lake water which had been increased in salinity to 22,000 mg/l by addition of NaCl.

METHODS

Uptake of phenylalanine by English Coulee worms was measured by using a modification of Stephens' (1963) technique. Rigler (1963) showed that *Daphina magna* Straus could be freed of bacteria by two 90-minute exposures to a solution containing streptomycin and penicillin. A sterilizing solution was prepared containing 600 mg/l NaCl, 105 units/l pennicillin and 250 mg/l streptomycin.

A large number of *T. tubifex* from the English Coulee were rinsed free of all mud and debris and stored in sterile saline (600 mg/l) for two days. After two days worms were exposed for 2 hours to the sterilizing solution for a second 2-hour period. Half the worms were killed in FAA immediately following the second sterilizing period. The other half of the worms were reurned to the sterile saline.

After fifteen minutes 1.0 ml of 40 micromolar DL phenylalanine-3-C^{14} (CEA(France) was added to each group. The addition provided each group of worms an amibient concentration of 1.6 micromoles per liter. Radioactivity of this medium measured 548 cpm/0.5 ml (corrected for background).

After 0.5, 1.0, 1.5 and 2.0 hours, portions of the worms were removed and place in 80 per cent ethanol for 24 hours. After extraction 0.5 ml aliquots of alcohol were evaporated (in triplicate) on aluminum planchets. Radioactivity was measured, using a thin window, gas flow, geiger tube. Planchets were stored frozen when radioactivity could not be read immediately.

Extracts from the phenylalanine were plated on nutrient agar. A heavy growth of bacteria resulted after 24 hours of incubation. The experiment was repeated with two changes in procedure. The experimental medium was prepared by diluting stock phenylalanine solution with sterilizing solution to find if uptake would occur in the presence of streptomycin and penicillin. L ive animals in antibiotics and in a sterile medium were compared with non-sterile live animals in a medium prepared with raw English Coulee water. The ambient concentration and radioactivity were the same as in the previous experiment. The experiment lasted 6 hours.

The Devils Lake population of *T. tubifex* was uesd to estimate the effect of salinity on uptake rates of glycine-I-C^{14}. Three cultures of different salinities were prepared (11,000 mg/l, 2,200 mg/l and 550 mg/l) by diluting raw Devils Lake water. All cultures were placed in mud from the collection site and fet a suspension of decaying grass every 2 to 3 days. Worms were acclimated for 27 days befre using them in experiments.

A stock glycine solution was prepared by dissolving 0.9 mg of glycine-I-C^{14} (CEA France) in 100 ml of distilled water. This stock solution was diluted 1:50 providing 2.4 micromolar glycine medium with a radioactivity of 1000 cpm/ml.

A salt solution was prepared to match the composition of Devils Lake water but to be lacking in organic matter, nitrates, and phos-

phates, so that microorganisms would not grow in it. Composition of artificial Devils Lake water (A.W.) is shown in Table I.

TABLE I

COMPOSITION OF ARTIFICIAL DEVILS LAKE WATER

	mg/l
$NaHCO_3$	1,050
K_2SO_4	450
$MgSO_4$	2,500
Na_2SO_4	5,000
$CaCl_2$	180
NaCl	1,800
	10,980

In the following experiments each culture was divided into four equal parts consisting of 20 to 30 worms. Animals were rinsed four times with sterile inorganic salt solution of the appropriate concentration. One group of each culture was killed with FAA and incubated in sterile A.W. of the proper concentration. A second group was incubated in Devils Lake water or dilutions filtered through a 0.45 micron filter (H.A. millipore). A third group was incubated in raw Devils Lake water (or dilutions thereof). The fourth group was incubated in autoclaved A.W. of the appropriate concentrations. All experiments lasted 2 hours.

After a 2 hours exposure to glycine-I-C^{14} worms were extracted with 80 per cent ethanol for 24 hours. The radioactivity of 0.5 aliquots of the extract was measured in triplicate.

Specimens of *T. tubifex* from the English Coulee could not be acclimated to Devils Lake water, and uptake of glycine was measured in media containing 1,650 mg/l and 550 mg/l total dissolved solids. The technique was nearly the same as the previous set of experiments. However, the worms were not allowed a long period of adjustment when uptake was measured in A.W. at 550 mg/l.

The ability of larval *Chironomus plumosus* to remove glycine was examined at different salinities. Uptake was measured in sterile and non-sterile media prepared from Lake Itasca water, (225 mg/l) Devils Lake water (11,000 mg/l) and concentrated Devils Lake water (22,000 mg/l). Ambient glycine and radioactivity were the same as in previous experiments. Animals were acclimated for 4 hours before radioactive glycine was added. Exposure was for 2 hours. An additional group of *C. plumosus* was tested in sterile Devils Lake water after a 9-day acclimation in raw Devils Lake water.

Uptake of glycine by *Lumbriculus inconstans* was measured over a 6-hour period. Animals were kept for 3 hours in sterile A.W. (550 mg/l) to clear their alimentary tracts. Uptake was measured from media millipore filtered and from raw Lake Itasca water.

RESULTS AND DISCUSSION

The uptake of phenplalanine by *T. tubifex* showed the radioactivity of the extract was linear with time for at least 60 minutes in both groups. Uptake by live animals was significantly greater than uptake by dead ones.

Confidence limits were established in phenylalanine experiments and all following experiments were established by use of the "t" test. Radioactivity count data are not normally distributed. However, the measurements from which the standard error of the means are calculated are themselves mean values of counts per minute based on 3000 counts. Therefore, the "t" test can be used to establish confidence limits.

The difference in uptake rates of phenylalanine is evidence that uptake is an active process in living animals. The small amount could be caused by absorption of radioactive material, but passive diffusion is the most likely process involved since worms were carefully rinsed before placing in 80 per cent ethanol. Results obtained by plating the used media indicated that bacterial populations were effectively retarded but not entirely eliminated.

Figures given in Table II and in all subsequent tables represent one fourth of the radioactivity that would be extracted from 100 mg dry weight of the animals. The greatest value recorded in Table II-A is approximately 150 cpm/100 mg dry weight and indicates that in 1.5 to 2 hours 100 mg dry weight of living *T. tubifex* would be expected to remove an amount of phenylalanine-C^{14}, having an activity of 600 cpm. The activity of the original medium was approximately 1000 cpm/ml and the phenylalanine-C^{14} concentration was 1.6 X 10.3 micromoles/ml. The amount of phenylalanine-C^{14} taken up in 1.0 to 2 hours in calculated as being approximately 10^{-3} micromoles/100 mg dry weight. One mm^3 O_2 is roughly equivalent to the oxidation of one microgram of amino acid (Stephens, 1963). Consider the QO_2 of *T. tubifex* to be about 150 mm^3 O_2/100 mg dry tissue/hour (Brazda and Rice, 1940). Then the amount of amino acid required to satisfy this respiration must be set at 150 micrograms/100 mg dry tissue/hour. This is two orders of magnitude greater than the uptake measured. It does not appear that the amount of amino acids acquired by the worms would support a significant portion of their metabolism. A portion of the radioactivity may not have been removed by the 80 per cent ethanol. However, it is safe to assume that any correction would be too small to make the uptake of phenylalanine important as a means of acquiring energy.

The uptake of phenylalanine by *T. tubifex* in sterile and non-sterile media showed that uptake in 2 hours was the same in the presence or absence of antibiotics (Table II-B). English Coulee water in the medium apparently stimulated the uptake of phenylalanine. These results established that uptake of phenylalanine occurs in the absence of particular matter. Indicating that *T. tubifex*

TABLE II

UPTAKE OF PHENYLALANINE* BY ENGLISH COULEE *T. TUBIFEX*

Experimental Conditions*		Uptake (cpm/100 mg dry weight)
A. Animals pretreated with antibiotics		
Dead animals, 1/2 hour		22 + 18
Live animals, 1/2 hour		56 + 7
Dead animals, 1 hour		55 + 14
Live animals, 1 hour		123 + 18
Dead animals, 1 1/2 hours		77 + 2
Live animals, 1 1/2 hours		154 + 40
Dead animals, 2 hours		84 + 13
Live animals, 2 hours		159 + 8
B. Sterile and non-sterile media compared		
Sterile medium,	2 hours	144 + 16
Non-sterile medium,	2 hours	243 + 10
Sterile medium,	4 hours	144 + 6
Non-sterile medium,	4 hours	366 + 18
Sterile medium,	6 hours	234 + 18
Non-sterile medium,	6 hours	257 + 17

*ambient concentration = 1.6 micromolar

acquire the phenylalanine directly from solution than by ingestion of microorganisms or non-living particulate matter.

Uptake of glycine by Devils Lake and English Coulee *T. tubifex* at different salinities showed no dependence on salinity of the medium (Table III-A). However, in every case uptake in significantly different from background and the uptake by live animals is significantlp greater than the uptake by dead ones. At 550 mg/l English Coulee worms took up twice as much glycine as the Devils Lake worms, even though the Devils Lake worms had 4 weeks to acclimate to the reduced salinity.

The larvae of *Chironomus plumosus* showed increased uptake of glycine at higher salinities than in its natural habitat after a suitable acclimation time (Table IV). *C. plumosus* were the only animals showing a decrease in glycine uptake on changing from a sterile to a non-sterile meadia. This may be an artifact caused by the addition of a small amount of Lake Itasca mud to each of the containers for the non-sterile experiments. Glycine may have been absorbed on the mud or by microorganisms within the mud making it unavailable to the larvae. The evidence of glycine uptake by larval chironomids is of special interest in light of Stephens and Schinske's (1961) negative results with arthropods.

TABLE III

UPTAKE OF GLYCINE* BY *T. TUBIFEX* AT DIFFERENT SALINITIES

Experimental Conditions*	Uptake (cpm/100 mg dry weight)
A. Devils Lake animals	
11,000 mg/l, sterile Devils Lake water	175 + 112
" " raw Devils Lake water	253 + 12
" " sterile A. W.	130 + 50
" " dead animals	77 + 13
2,200 mg/l, sterile Devils Lake water	190 + 38
" " raw Devils Lake water	110 + 10
" " sterile A.W.	259 + 198
" " dead animals	55 + 32
500 mg/l, sterile Devils Lake water	170 + 17
" " raw Devils Lake water	253 + 20
" " sterile A.W.	126 + 14
" " dead animals	53 + 10
B. English Coulee animals	
1,650 mg/l, sterile Coulee water	174 + 40
" " raw Coulee water	258 + 26
" " dead animals	85 + 28
550 mg/l. sterile A.W.	393 + 110
" " dead animals	49 + 16

*ambient concentration = 2.4 micromolar

TABLE IV

UPTAKE OF GLYCINE* BY *CHIRONOMUS PLUMOSUS* AT DIFFERENT SALINITIES

Experimental Conditions	Uptake (cpm/100 mg dry weight)
225 mg/l, sterile Lake Itasca water	109 + 13
11,000 " sterile Devils Lake water	23 + 3
22,000 " sterile Devils Lake water**	19 + 8
225 mg/l, raw Lake Itasca water	49 + 6
11,000 " raw Devils Lake water	17 + 12
22,000 " raw Devils Lake water**	24 + 4
11,000 mg/l, sterile Devils Lake water after 9 days acclimation	224 + 57

*ambient concentration = 2.4 micromolar

**concentrated by addition of NaCl

Uptake of glycine from raw Lake Itasca water by *Lumbriculus inconstans* after 6 hours exposure, was 1,055 + 38 cpm/100 mg dry weight, while uptake from sterile filtered water was 354 + 37. Ambient glycine concentration and radioactivity were the same as in previous experiments.

CONCLUSIONS

The data obtained from this study do not conclusively demonstrate that any aquatic invertebrate uses dissolved organics as a natural enegry source. Such a demonstration depends on several types of evidence.

First, it must be shown that the aquatic animals in question have a mechanism through which they are able to remove some dissolved substances from solution. The work described here had this objective. Two species of aquatic oligochaetes and two species of chironomid larvae repeatedly removed glycine and phenylalanine from dilute solutions.

Second, it must be shown that organic compounds which the animals are capable of using are present in the habitat and in the requisite quantities. There is strong circumstantial evidence that this is the case for the common amino acids. The concentration of free amino acids has been conservatively estimated as 40 per cent of the nitrogenous organic matter.

Finally, it must be demonstrated that animals actually remove materials from solution at a rate that is capable of satisfying their metabolic requirements. Calculations based on the radioactivity of ethanol extracts indicate that the absolute uptake of glycine and phenylalanine is negligible when compared to the animals' energy reqiurements.

One goal of this study was to determine what effect salinity has on the uptake of dissolved organic substances. The data give no unequivocable answer to this question. Uptake is less when measured at a salinity that differs widely from the animals' natural habitat. However, larvae of *Chironomus plumosus* had a greater uptake at higher salinities when properly acclimated. There is no pattern followed by the animals studied.

This study was financed in part by an NDEA traineeship at the University of North Dakota and an N.S.F. summer fellowship for graduate assistants at the University of Minnesota. Thanks is extended to the Department of Biochemistry of the University of North Dakota for the use of equipment and space.

LITERATURE CITED

BRAZDA, F. G. and RICE, J. C. 1940. The respiratory metabolism of the freshwater oligochaete, *Tubifex*. Jour. Cell and Comp. Physiol., 16:97-102.

KROUGH, A. 1931. Dissolved substances as food for aquatic organisms. Biol. Rev., 6:412-442.

PETERSON, W. H., FRED, E. B., and DOMOGALLA, B. P. 1925. The occurence of amino acids and other organic nitrogen compounds in lake waters. J. Biol. Chem., 23:287-295.

PUTTER, A., 1901. Die Ernahrung der Wassertiere und der Stoffhaushalt der Gewasser. Fisher, Jena.

RIGLER, F. H. 1961. The uptake and release of inorganic phosphorus by *Daphnia magna* Straus. Limnol. Oceanogr., 6:165-174.

RUTTNER, F., 1963. Fundamentals of Limnology. 3d. ed. Toronto: University of Toronto Press.

STEPHENS, G. C. 1962. Uptake of glucose by the solitary coral, *Fungia schtaria.* Biol. Bull., 123:648-659.

———— 1963. Accumulation of amino acids by the bamboo worm, *Clymenella torquata.* Comp. Biochem. Physiol., 10:191-202.

———— 1964. Uptake of glycine by brackish water annelids. Biol. Bull., 126: 150-162.

————, and SCHINSKE, R. A. 1961. Uptake of amino acids by marine invertebrates. Limnol. Oceanogr., 6:175-181.

SWENSON, H. A., and COLBY, B. R. 1955. Chemical quality of surface waters in Devils Lake basin, North Dakota. Geological Survey Water Supply Paper 1295. United States Government Printing Office. Washington.

PHYTOCHEMICAL STUDIES OF POISONOUS PLANTS OF NORTH DAKOTA—PART I[1]

J. R. Sinha, K. S. Nadodwalla, D. McCracken, L. J. Schermeister

College of Pharmacy
North Dakota State University, Fargo, North Dakota

and W. H. Bhatti

College of Pharmacy
University of Pittsburgh, Pittsburgh, Pennsylvania

INTRODUCTION

A phytochemical survey is an initial step in the effective evaluation of poisoning by plants and in the investigation of new compounds of economic and medicinal importance. Within the last decade an increased number of phytochemical surveys have been published. The majority of these surveys, however, are of plants not indiginous to the United States. Among these surveys which include native plants are the random sampling approach of Wall et al. (1-10) and the recent report on the biological and phytochemical evaluation by Farnsworth and co-workers (11-12).

[1]This investigation was supported in part by the funds from North Dakota Heart Association and from N.S.F. grants number G20980 and G3097.

This survey was undertaken to provide more complete phytochemical information on North Dakota plants. The flora of North Dakota includes about 139 species which have been considered to be chemically poisonous to animals and man by Pammel (13), Kingsbury (14), and Muenscher (15-16). The recent works of McCracken (17) and Nadodwalla (18) have indicated that 13 plants should be considered for addition to the list of poisonous plants of North Dakota since these plants were found to be toxic to living cancer cells. The work reported is a preliminary evaluation of the type of chemical compound that may be responsible for the poisonous properties of the native plants.

MATERIAL AND METHODS

Wild plants growing throughout the state of North Dakota were collected during June and July. Each plant under investigation was taxonomically classified, air dried at room temperature, separated into the respective plant parts, ground to a fine powder, and stored in air-tight containers. These plants were then extracted and tested for the presence of alkaloids, steroidal saponins, flavanoids, and tennins. These chemical groups were identified by the methods outlined by Persinos and Quimby (19) as follows:

I. Test for Alkaloids—

All plant extracts were tested for alkaloids with each of these reagents, Mayer's, Valser's, Bertraud's and Kraut's reageants. The precipitates produced were semiquantitatively estimated using standard atropine sulphate and quinine sulphate solutions.

II. Test for Saponins—

A positive saponin reaction was indicated when the plant extracts haemolyzed red blood cells. Complete haemolysis within one minute was given a value of 4+, while 5 minutes was kiven a value of 1+. The red blood cells were standardized using 0.1 per cent digitonin solution in 80 per cent ethanol.

III. Test for Flavanoids—

The flavanoids gave a distinct positive color reaction when reduced in alcoholic solutions with hydrochloric acid in the presence of magnesium turnings. Standard quercetin solution was used as control.

IV. Tannins—

Tannins were reported to be present in the plants only when the extracts produced both a precipitate with the gelatin salt reagent and a blue-black or green-black color with $FeCl_3$ solution. A standard solution of tannic acid was used as a control.

RESULTS AND DISCUSSION

Eighty North Dakota plants belonging to 23 families were tested for the presence of alkaloids, saponins, flavanoids and tannins. The results of these studies are presented in Table I.

The extracts of 155 plant parts were positive for one or more of the chemical groups tested. However, no positive tests for alkaloids, saponins, or flavanoids or tannins were found for 47 plant parts.

Alkaloids were found to be more widely distributed than saponins among the plants tested. Their presence was recorded in appreciable amounts in 53 parts of 27 plants, out of which 17 plants contained considerable amounts. These alkaloids were present in one, two, three or in all parts of a plant in the same or varying amounts. The data indicates that a higher percentage of flowers, leaves, and stems contain alkaloids than any other part. However, the plants found to possess alkaloids were generally the member of Asteracae, Fabaceae, Liliceae, Memispermaceae, Ranunculaceae, and Solanaceae families.

Saponins were present in 137 plants with 69 parts but only 24 plants contained considerable amounts. A small amount of saponins was present in many members of Fabaceae family, while almost all members of the plant families Asclepiadaceae, Caryophylaceae, Ranunculaceae, and Scrophulariaceae tested contained large amounts of Saponins. These Saponins were found to be present in one, two, three, or in all parts of a plant.

The distribution of flavanoids was found to be of a wider range than the alkaloids. Their presence was recorded in 68 parts of 41 plants. Out of these 68 parts, only parts of 5 plants contined considerable flavanoid content.

The tannins were found to be the most widely distributed of these four chemical groups. 44 plants with 93 parts contined considerable amounts of tannins.

This phytochemical survey may prove to be valuable not only in the complete evaluation of toxicity of local poisonous plants, but also in the determination of new biologically active plant chemicals.

The authors are continuing this work on the flora in order to provide complete phytochemical data for the interested investigators in this field.

Abbreviations and symbols used in Table I: Bd—buds, F—fruits, Fl—flowers, L—leaves, R—roots, Rh—rhizomes, S—stems, Sd—seeds, W—whole plant.

TABLE I

PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
1.	63-43	Acerates viridiflora Raf.* (Asclepiadaceae)	R	0	0	1+	1+
			L	0	1+	0	1+
2.	63-42	Achillea lanulosa Nutt. (Asteraceae)	R	0	0	0	0
			S	0	0	0	0
			L	0	0	0	0
			Fl	0	0	0	0

TABLE I (Continued)
PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
3.	64--46	Achillea sibirica Ledeb. (Asteraceae)	L	1+	3+	0	1+
4.	63-68	Actaea rubra Ait. (Ranunculaceae)	R	1+	0	0	0
			S	0	4+	0	0
			L	0	0	0	0
			F	0	0	0	0
5.	61-22	Amorpha canescens* Pursh. (Fabaceae)	R	0	1+	1+	1+
			S	0	1+	0	2+
6	64-113	Apocynum androsaemifolium L. (Gentianaceae)	S	0	0	0	0
			L	0	0	0	3+
7.	63-44	Apocynum sibiricum	R	0	1+	0	3+
	108	Jacq.	S	0	0	0	1+
		(Gentianaceae)	L	0	0	0	3+
8.	63-32	Asclepias ovalifolia	R-S	1+	0	0	0
		Dec.	L	1+	0	0	0
		(Asclepiadaceae)	Fl	1+	1+	0	0
9.	63-54	Asclepias pumila	R	2+	2+	1+	1+
		A. Gray	S	2+	2+	0+	1+
		(Asclepiadaceae)	L	1+	1+	0	1+
			Fl	0	0	1+	2+
10.	63-51	Asclepias speciosa	S	2+	3+	0	0
		Torr.	L	1+	0	0	0
		(Asclepiadaceae)	Fl	2+	0	0	0
11.	64-106	Asclepias syriaca	R	2+	2+	0	1+
		L.	S	2+	2+	0	1+
		(Asclepiadaceae)	L	3+	3+	1+	1+
12.	63-45	Astragalus bisulcatus	R	0	1+	1+	1+
		Hook.	S	0	0	0	1+
		(Fabaceae)	L	2+	1+	0	1+
13.	63-31	Astragalus caryocarpus	S	3+	1+	1+	1+
		Ker.	L	2+	3+	1+	1+
		(Fabaceae)					
14.	63-53	Astrgalus missouriensis	R	1+	0	0	0
		Nutt.	S-L	1+	0	0	0
		(Fabaceae)					
15.	-58	Astragalus racemosus	L	0	4+	1+	0
		Pursh.	S	0	2+	1+	0
		(Fabaceae)	R	1+	2+	0	0

TABLE I (Continued)
PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
16.	63-40	Astragalus striatus Nutt.* (Fabaceae)	R	1+	3+	0	2+
			S	3+	3+	0	2+
			L	2+	3+	0	2+
17.	63-34	Astragalus tenellus Pursh. (Fabaceae)	R	1+	0	0	0
			S	1+	0	0	0
			L-Fl	1+	1+	0	1+
			F	1+	4+	0	1+
18.	63-56	Astragalus lotiflorus* Hook. (Fabaceae)	R	0	2+	0	2+
			L	0	2+	0	2+
19.	63-89	Brauneria angustifolia* DC. (Asteraceae)	S	0	1+	1+	1+
			L	0	1+	1+	2+
20.	-167	Caltha palustris L. (Ranunculaceae)	L	1+	1+	1+	2+
21.	176	Camelina microcarpa Andrz. (Brassicaceas)	S	0	2+	0	0
			R	0	1+	0	0
			Sd.	1+	2+	0	2+
22.	64-116	Celastrus scandens L. (Celastraceae)	S	2+	0	0	0
			L	1+	0	0	0
23.	64-122	Cicuta maculata L. (Ammiaceae or Umbelliferae)	S	0	0	0	0
			L	2+	0	0	0
24.	1-127	Convolvulus arvensis L. (Convolvulaceae)	L	0	3+	0	1+
			S	1+	3+	0	3+
			R	1+	1+	1+	2+
			Fl	1+	2+	1+	2+
25.	63-61	Corydalis auroea Willd. (Brassicaceae)	R	3+	0	0	0
			R-S	3+	0	0	0
			S	3+	0	0	0
			L-Fl	4+	0	0	0
26.	63-73	Delphinium virescens Nutt. (Ranunculaceae)	R	2+	0	0	0
			S	1+	3+	3+	0
			L	2+	2+	0	0
			Fl	2+	4+	3+	0
27.	126	Eupatorium maculatum L. (Asteraceae)	S	0	0	0	0
			L	0	0	0	0

TABLE I (Continued)

PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
28.	63-65	Eupatorium perfoliatum L. (Asteraceae)	S	0	0	0	0
			L	0	0	0	0
29.	63-93	Euphorbia esula L. (Euphorbiaceae)	S	1+	0	1+	1+
			L-Fl	1+	2+	2+	4+
30.	63-80	Fumaria officinalis L. (Brassicaceae)	W	3+	2+	2+	0
31.	64-128	Gaura coccinea Pursh. (Onagraceae)	S	0	1+	0	2+
			L	0	3+	0	3+
32.	61-20	Glycyrrhiza lepidota Nutt. (Fabaceae)	S	1+	0	0	0
			L	1+	0	0	0
33.	-5	Grindelia squarrosa Pursh. (Asteraceae)	L	2+	2+	0	1+
			S	1+	2+	0	0
			R	1+	0	0	0
34.	64-121	Gysophila paniculata L. (Caryophyllaceae)	R	0	4+	0	0
			S	1+	0	0	0
			L-F	1+	1+	2+	0
35.	63-90	Helenium autumnale L. (Asteraceae)	S	1+	0	2+	0
			L	1+	0	3+	0
36.	-165	Iva Axillaris Pursh. (Asteraceae)	L	2+	2+	1+	2+
			S	2+	2+	0	2+
			R	1+	1+	0	2+
37.	64-119	Lactuca pulchella Pursh. (Asteraceae)	S	2+	0	0	0
			L	2+	0	1+	0
38.	63-64	Lathyrus oxhroleucus Hook. (Fabaceae)	S	1+	1+	0	0
			L	1+	1+	2+	0
39.	63-72	Lobelia spicata Lam. (Campanlaceae)	S-L	3+	0	2+	0
			L	2+	0	1+	0
40.	64-143	Lupinus argenteus Pursh. (Fabaceae)	S	4+	1+	1+	0
			L	4+	2+	3+	0
			Fl-F	4+	1+	3+	0
			W	4+	2+	2+	0

TABLE I (Continued)

PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
41.	64-120	Lychnis alba*	S	4+	1+	0	1+
		Mill.	L	2+	0	0	1+
		(Caryophyllaceae)					
42.	61-2	Lygodesmia Juncea	S	4+	4+	0	1+
		Pursh.					
		(Asteraceae)					
43.	63-59	Mamillaria vivipara	R	1+	0	0	0
		Nutt.	S-L	1+	0	0	0
		(Violaceae)					
44.	63-75	Menispermum canadense	R-Rh	4+	0	0	1+
		L.	S	4+	0	0	0
		(Menispermaceae)	L	1+	0	0	0
45.	64-100	Nepeta cataria	S	1+	3+	0	0
		L.	L	1+	4+	0	1+
		(Lamiaceae or Labiatae)					
46.	63-85	Nepeta hederacea	R	1+	2+	1+	1+
		L.	S-L	0	4+	2+	1+
		(Lamiaceae or Labiatae)					
47.	63-60	Opuntia polycantha	R	0	0	0	0
		Haw.	S-L	0	0	0	0
		(Elaeagnaceae)					
48.	61-18	Oxytropis lambertii	R	0	0	1+	0
		Pursh.	S	0	1+	1+	0
		(Fabaceae)	L	0	1+	1+	0
			F	0	0	1+	0
49.	63-47	Penstemon albidus*	R	0	2+	0	1+
		Nutt.	S	1+	1+	1+	1+
		(Scrophulariaceae)	F	1+	3+	0	1+
50.	63-49	Penstemon cristatus	R	1+	3+	1+	1+
		Nutt.*	S	0	0	1+	1+
		(Scrophulariaceae)	L	1+	4+	1+	1+
			F	1+	4+	0	1+
51.	61-1	Penstemon eriantherus*	S	1+	4+	1+	1+
		Pursh.	L	1+	4+	0	1+
		(Scrophulariaceae)	R	1+	0	1+	1+
52.	63-38	Penstemon gracilis*	R	1+	3+	0	1+
		Nutt.	S	0	2+	0	1+
		(Scrophulariaceae)	L	0	4+	0	1+
			Fl	1+	4+	3+	1+

TABLE I (Continued)
PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
53.	63-67	Penstemon grandiflorus Nutt. (Scrophulariaceae)	R	0	0	0	0
			S	0	0	0	0
			L	1+	0	0	0
			F	0	4+	0	0
54.	63-50	Penstemon nitidus Dougl. (Scrophulariaceae)	R	0	0	0	0
			S	0	0	0	0
			L	1+	0	0	0
			F	0	0	0	0
55.	63-33	Polygonatum commutatum Schult. (Liliaceae)	S	2+	0	0	0
			L	3+	0	0	0
56.	-8	Psoralea argophylla Pursh. (Fabaceae)	R	0	0	0	0
			S	0	1+	0	0
			L	0	1+	0	0
57.	64-103	Rhus radicans L. (Celastraceae)	R	0	1+	0	3+
58.	64-144	Rudbeckia hirta L. (Asteraceae)	S	0	1+	2+	0
			L-Bd	0	1+	1+	0
69.	64-161	Rumex mexicanus* Meissn. (Polygonaceae)	S	0	0	1+	4+
			Sd	0	0	3+	4+
			L	0	0	4+	4+
60.	63-76	Rumex persicarioides* L. (Polygonaceae)	S	1+	3+	1+	1+
61.	-144	Rumex crispus (Polygonaceae)	L	0	0	1+	2+
			S	0	1+	0	0
62.	64-162	Rumex stenophalus* Ledeb. (Polygonaceae)	S	0	0	1+	2+
			L	0	0	1+	1+
			Fl	0	1+	1+	1+
63.	64-109	Rumex venosus Pursh. (Polygonaceae)	R	0	2+	0	3+
			S	0	0	0	1+
			L	0	0	0	1+
64.	63-66	Saguinaria canadensis L. (Manispermaceae)	S	1+	0	0	0
			L	2+	0	0	0
65.	63-77	Senecio canus Hook. (Asteraceae)	R	1+	1+	0	1+

TABLE I (Continued)
PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
66.	61-17	Shepherdia argentea Nutt. (Elaeagnaceae)	S	2+	2+	1+	1+
			L	4+	2+	1+	1+
67.	64-115	Silene cserei Baum. (Caryophyllaceae)	R	0	3+	0	0
			S	0	2+	0	0
			L	0	4+	2+	0
68.	63-102	Silene noctiflora L. (Caryophyllaceae)	S	1+	3+	1+	1+
			L	0	4+	0	1+
69.	63-84	Sium cicutaefolium Gmel. (Ammiaceae or Umbelliferae)	R	0	0	0	1+
			S-L	0	0	0	1+
70.	63-78	Solanum carolinense (Solanaceae)	W	3+	0	0	0
71.	-150	Solanum rostratum Dunal. (Solanaceae)	L	2+	2+	0	1+
			S	2+	2+	0	1+
			R	2+	0	0	0
			F	2+	2+	0	1+
			Fl	1+	1+	0	1+
72.	63-88 or 145	Solanum triflorum Nutt. (Solanaceae)	S	0	3+	1+	1+
			L	0	4+	1+	1+
73.	64-140	Stachys palustris L. (Lamiaceae or Labiatae)	S	1+	0	0	0
			L	1+	0	0	0
74.	64-141	Stipa comata Trin. & Rupr. (Graminae)	S-L	0	0	0	0
			F	0	0	0	0
75.	64-142	Thermopsis rhombifolia Nutt. (Fabaceae)	R	3+	0	1+	0
			S	4+	0	2+	1+
76.	64-101	Triglochin maritima L. (Juncaginaceae)	R	1+	0	0	1+
			S	0	0	0	1+
77.	63-83	Veronica americana Schwein. (Scrophulariaceae)	W	0	0	0	0
78.	63-39	Vicia americana Muhl. (Fabaceae)	R	0	0	0	2+
			S	0	1+	0	1+
			L	1+	0	1+	1+

TABLE I (Continued)
PHYTOCHEMICAL SCREENING DATA

Ser. No.	Code No.	Scientific Name	Plant Part	Chemical Groups Tested: Alkaloids	Saponins	Flavanoids	Tannins
79.	63-76	Xanthium italicum Mor . (Asteraceae)	R	0	0	0	0
			L	3+	0	2+	0
80.	63.-37	Zigadenus elegans Pursh. (Liliaceae)	R	2+	0	0	0
			S	1+	0	1+	0
			L	1+	0	1+	0

*Plants in which cytotoxicity was observed by authors against living cancer cells.

REFERENCES

1. Wall, M. E., *et al.* 1954., "Steroidal sapogenins XV.", *U.S. Dept. Agric. ARS. Bull. AIC*-367.
2. Wall, M. E., *et al.* 1954., "Steroidal sapogenins XII.", *J. Am. Pharm. Assoc., Sci. Ed* .43:503-505.
3. Wall, M. E., *et al.* 1961., "Steroidal sapogenins LXI.", *Econ. Botany* 15:131-132.
4. Wall, M. E., *et al.* 1959. "Steroidal sapogenins LV.", *J. Am. Pharm. Assoc., Sci. Ed.*, 48:695-722.
5. Wall, M. E., *et al.* 1957. "Steroidal sapogenins XLIII.", *J. Am. Pharm. Assoc., Sci. Ed.*, 46:653-684.
6. Wall, M. E.,*et al.* 1955. "Steroidal sapogenins XXV.", *J. Amer. Pharm. Assoc., Sci. Ed.*, 44:438-440.
7 Wall, M. E., *et al.* 1955. "Steroidal sapogenins XXVI.", *U.S. Dept. Agric. ARS. Bull.*, ARS-73-4.
8 Wall, M. E., *et al.* 1961. "Steroidal sapogenins LX.", *J. Pharm. Cci.*, 50:1001-1034.
9. Wall, M. E., *et al.* 1954. "Steroidal sapogenins VII.", *J. Am. Pharm. Assoc., Sci. Ed.*, 43:1-7.
10. Wall, M. E., *et al.* 1954. "Steroidal sapogenins VII.", *U.S. Dept. of Agric., ARS. Bull.*, ARS-363.
11. Farnsworth, N. R., *et al.* 1966. "Biological and Phytochemical Evaluation of Plants. I. Biological Test Procedures and Results from Two Hundred Accessions.", *Lloydia* 29:101-122.
12. Farnsworth, N. R., 1966. "Biological and Phytochemical Screening of Plants.", *J. Pharm. Sci.*, 55:225-276.
13. Pammel, L. H., 1911. *A Manual of Poisonous Plants.* The Torch Press, Iowa.
14. Kingsbury, J. M., 1964. *Poisonous Plants of the United States and Canada.* Prentice-Hall, Inc., New Jersey.

15. Muenscher, W. C., 1962. *Poisonous Plants of the United States.* The Macmillan Company, New York.
16. Muenscher, W. C., 1940. *Poisonous Plants of the United States.* The Macmillan Company, New York.
17. McCracken, D., *et al.* "Phytochemical and cytotoxicity evaluation of Astragalus species of North Dakota" to be presented at American Society of Pharmacognosy in June 1967.
18. Nadodwalla, K. S., *et al.* "Cytotoxicity studies of North Dakota plants and preliminary isolation of cytotoxic component of Rumex Species," to be presented at American Society of Pharmacognosy in June 1967.
19. Persinos, A. J., Quimby, M. W. and Schermerhorn, John W., "A Preliminary Pharmacognostical study of Ten Nigerian Plants," *Econ. Bot.* 18:329-41. (1964.)

A METHOD FOR SPORIDIAL INNOCULATIONS FOR COVERED SMUT OF BARLEY

Dennis H. Lade and R. L. Kiesling

Department of Plant Pathology

North Dakota State University, Fargo, North Dakota

INTRODUCTION

Covered smut of barley caused by *Ustilago hordei* (Pers.) Lagerh. is a seedling infecting fungus. The organism invades only during the seedling stage and produces smut sori in the barley inflorescence and upper leaves. The environmental conditions immediately following inoculation are very important in determining whether or not the seedling will be infected and smutted heads produced.

A problem in inoculating barley is placing the inoculum underneath the seed coat which constitutes a barrier for fungal penetration. Seed dehulling has alleviated most of this problem along with different techniques of inoculation. Several different methods for inoculating are described (1). Among these are: the partial vacuum method in which the sporidia are forced underneath loosened hulls; the spore suspension method whereby the seedlings are allowed to grow up through a layer of infection hyphae; and a high speed electric homogenizer to force suspensions underneath seed coats.

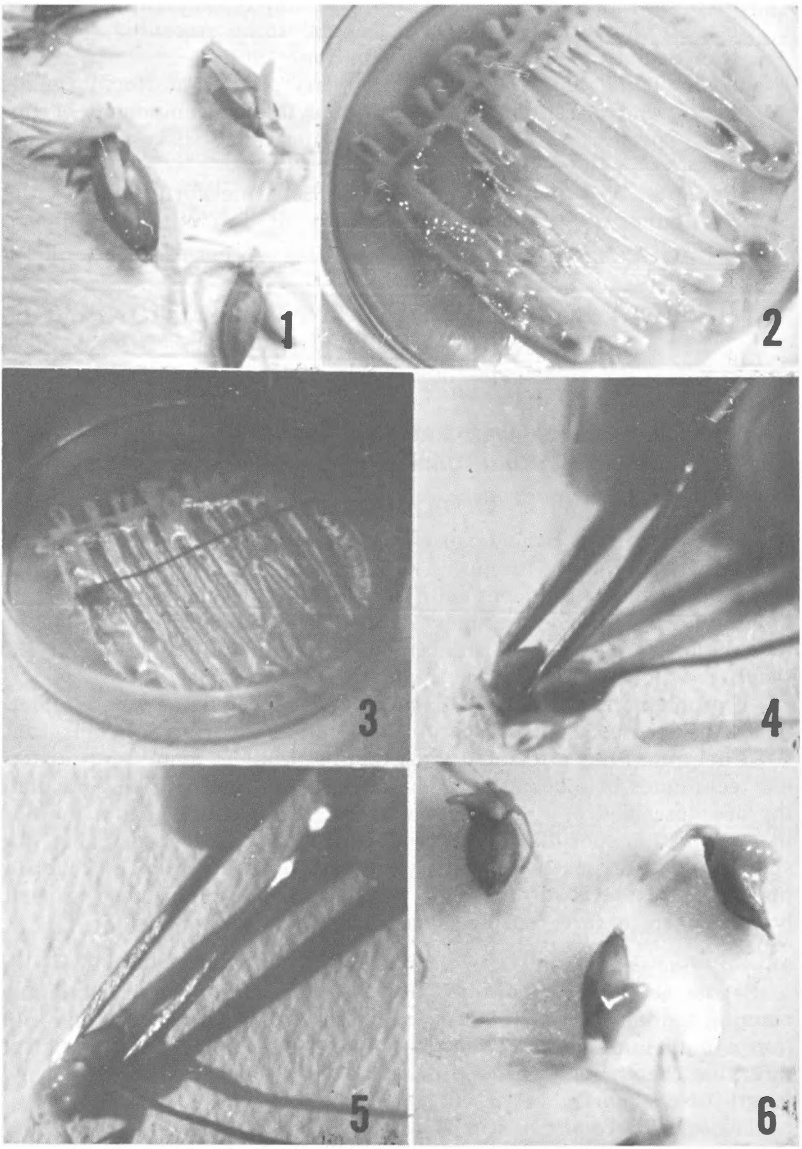
METHODS AND MATERIALS

Seeds were hand dehulled and germination was stimulated by running tap water over them for two hours. The seeds were then put into a high humidity incubator at 24 C for 24 hours. At the end of this time the emerging coleoptiles reached a length of 1/2 to 3/4 the length of the barley kernel (Figure 1).

The sporidial culture was streaked out on 2 per cent potato dextrose agar (PDA) to increase the amount of inoculum (Figure 2).

After incubating these cultures five to seven days they were used for inoculations .

Sporidial inoculum was gathered on a flattened inoculating needle (Figure 3) and transferred to the barley coleoptile (Figures 4 and 5).



FIGURES 1 through 6—See text.

Care was taken to get as much inoculum near the base as possible. The inoculated seeds were put into a petri dish containing moistened filter paper (Figure 6), and wrapped in aluminum foil to retain the humidity. The dishes were then placed in a 24 C incubator for 24 hours. The inoculated seedlings were then planted, 2 seeds per pot, in autoclaved soil in six-inch pots. The plants were grown to maturity under 16-hour days length at 22 ± 2C.

RESULTS AND DISCUSSION

A consistent level of at least 70 per cent smutted plants has been obtained using Odessa (C. I. 934) and Hannchen (C. I. 531) barleys. In many samples, 100 per cent smutted plants resulted. Other methods used may give infection percentages this high; however, their results have not been consistent. This may partly be explained by the fact that the environmental conditions necessary at the critical infection time are easily controlled while using the inoculation method described above. The growth chamber-incubator easily regulates the temperature and humidity needed for maximum infection. Another factor in producing high percentage of infected plans may be the accurate placement of inoculum at the base of the coleoptile (2).

Another advantage with this method was that the seed is pre-germinated and only the healthy growing seed is selected for inoculations. Selecting in this manner eliminates the seed which has been injured either by harvesting or dehulling. In methods whereby the seed is not pre-germinated, many seeds fail to emerge or die shortly after emerging. This often has been attributed to an infection reaction and has produced skewness in infection curves.

A possible disadvantage of this method would be a time factor in that this method is slower than some of the others described.

SUMMARY

A procedure for sporidial inoculation for covered smut of barley is described. This procedure provides good control of environmental conditions, insures the correct placement of inoculum, and eliminates poor seed that would otherwise not germinate or produce a mature plant. The percentage of infections obtained is as great if not greater than other methods used. High infections are consistently obtained.

REFERENCES

1. Fischer, G. W. and C. S. Holton. 1957. Biology and control of the smut fungi. The Ronald Press Co. New York. 622 p.
2. Kiesling, R. L. 1962. Effect of temperature and point of inoculation on the symptomology of barley covered smut. (Abstr.) Phytopathology 52:15.

ADSORPTION OF H_2O AND NH_3 VAPORS UPON LYOPHILIZED HUMIC ACID FROM LIGNITE[1]

Wasyl S. Hnojewyj

College of Chemistry and Physics
North Dakota State University, Fargo, North Dakota

ABSTRACT

a) Adsorption isotherms of water vapor at 25° and 35° upon dialytically purified lyophilized and vacuum-dried humic acid from lignite are presented. Five definite slopes in the isotherm at 25° are established in the region of total mono-layer coverage, which is ~ 19.15mmoles of H_2O per gram of dry humic acid.

The established higher adsorptivity, if compared to H-humate(s), is suggested to result from the further recovering of molecular structure by humic acid upon lyophilization.

Differential heats of water vapor adsorption as calculated from the isothermic data show several peaks, the highest of which is around 25 kcal/mole. From an amount equal to 5.5 mmoles/gm of adsorption, the differential heats continuously approach the heat of H_2O condensation with progressing sorption. The results of investigations are discussed in respect to the presence of functional groups in the humic acid molecule.

b) Desorption isotherms of NH_3 at 25° and 35° in the range 700-0 mm of equilibrium pressure are presented.

Each isotherm demonstrates only one slope at higher pressure, starting with an amount around 8 and 7 mmoles/gm adsorbed respectively, which, however, with slight fluctuation, depends on the method of preparation of the sample.

Saturation of humic acid up to the multilayer content of H_2O + NH_3 and equilibration with the atmosphere showed the capability of H-Ac(II) (holding them) at 25° and 35° equal to 263.5 and 184.0 mg of H_2O + NH_3 per gram of humic acid respectively.

INTRODUCTION

Investigations on lignites and related brown coal have shown the presence of different functional groups in their organic parts (1).

Studies done in our laboratory on H-humate(s) obtained from lignite (which seems to be the only organic constituent of lignite) showed its high stability under high vacuum as well as the high sorptivity to water vapor, which, however, demonstrated some isothermal slopes (2).

These led to conclusions that H-humate(s) from lignite is a compound of relatively high polymeric structure (because of stability in high vacuum) and containing functional groups of different activity

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

which cause the appearance of slopes in sorption isotherms analogously to natural proteins (3) and poly-amino acids (4) under similar experimental conditions.

The conductometric titrations of H-humat(s) partly supported the above mentioned suggestions (5); however, it appeared obvious that further investigations should be done on the chemically pure substances with highly developed surface which preserves the natural structural features if structural clarification is desired. It was established that humic acid is the most stable form of H-humate(s).

For preparation of humic acid from H-humate(s) satisfying the conditions of purity and possessing the maximum reopened structure methods were developed (6) which utilized an alkaline or alkaline-acidic pretreatment of H-humate(s) that was followed by dialysis against distilled water and, finally, lyophilization. The species of humic acid obtained by these methods were designed as H-Ac($_{Na}$) and H-Ac($_{II}$), respectively, and used for further investigations: conductometric titration (7), sorption of carbon dioxide (7,8), isotopic H→D exchangeability (7,9).

The presented work is the further study of the sorptivity of H_2O and NH_3 (or H_2O + NH_3) vapors upon the species of humic acids H-Ac($_{Na}$) and H-Ac($_{II}$), which were obtained and satisfy the conditions mentioned above.

EXPERIMENTAL

Materials:

Water was many times redistilled previously, degassed in vacuum system, and again redistilled by freezing and thawing. Its chemical purity was controlled by measuring of vapor pressures at different temperatures and finally spectroscopically.

Ammonia-anhyrous had a purity of 99.99 per cent from Matheson Co.

Humic Acids were prepared by methods described elsewhere in detail (6) using a commercial H-humate(s) from lignite, provided by Baroid Division, National Lead Co., Houston, Texas.

The methods of preparation of humic acids utilized the following steps in order: pretreatment of H-humate(s) with NaOH, filtration, dialysis and lyophilization. The resulting humic acid is desginated H-Ac($_{Na}$).

The humic acid designated H-Ac($_{II}$) was prepared by a slight variation in the above method, in which the solution was treated with HCl for complete regeneration of humic acid before dialysis.

Apparatus and Procedure:

The same high vacuum system in conjunction with a sealed-in McBain quartz spiral balance of sensitivity 1.498 ± 0.002 mg/mm was used as described previously (2).

The procedure of original desorption-drying of humic acid specimens as well as the determination of adsorption isotherms were followed in principle in accordance with previous work (2,3).

RESULTS AND DISCUSSION

Several samples of lyophilized humic acids H-Ac($_{Na}$) and some of H-Ac($_{H}$) were used repeatedly for investigations. They were first desorbed-dried in high vacuum at various temperatures in the range of 25° to 60°, as well as at the constant temperatures of 25° and 35° during the whole course of drying, i.e., until dry weights were established.

The plot of weight losses as a function of desorption-drying time demonstrated five definite regions (slopes) with the final one equal to 0.002-3 per cent per hour at 10^{-7} mm of pressure.

Variation of temperatures sems to have only slight influence on the total time of drying desorption. Comparing these data with the data obtained on commercial H-humate(s) (2), there is some similarity; however, the present data demonstrate slopes more sharply and their number increases. Furthermore, the total time required for drying on the present species of H-Ac($_{Na}$) and H-Ac($_{H}$) is three times shorter. That the final slope of desorption-drying equals 0.002-3 per cent per hour as compared to 0.005 per cent in the case of H-humate(s), together with the above mentioned, indicates higher reopening of the structure due to lyophilization.

A. *Adsorption of H_2O vapor on lyophilized humic acids*—The adsorption isotherms were obtained at 25° and 35° in the usual way by addition of increments of H_2O vapor to the completely desorbed dried sample of humic acid and equilibration to constant pressure.

It should be mentioned that reproducibility of the isothermic data is observed only if the sample is first saturated with H_2O vapor up to a multilayer content and then is completely desorbed-dried at the temperature of the desired isotherm. This phenomenon is suggested to be due to particular structural orientations of functionalities, and was first established on H-humate(s) where it was also extensively discussed (2), and on natural proteins (3).

Typical data of adsorption isotherms on lyophilized humic acids are plotted as the amounts of H_2O adsorbed in millimoles per gram of completely dried sample *vs.* equilibrium pressure of water vapor upon speciment and are shown in Figure 1.

The most complete adsorption isotherm is on a speciment of H-Ac($_{Na}$) at 25°, curve 1, which extends to a multilayer adsorption (coverage). Its shape, including the upermost portion, which is approximated up to the saturation pressure (2), demonstrates five definite slopes, the summation of the ordinates of which gives the total monolayer adsorption of 19.15 mmoles/gm.

Curve 2 represents the adsorption isotherm on the same sample determined at 35°, the shape of which shows only three initial slopes in the same range of equilibrium pressures. Measurements at higher equilibrium pressures, however, were limited by room temperature (since the whole system is not thermostated).

Two isotherms at different temperatures show similarity if the

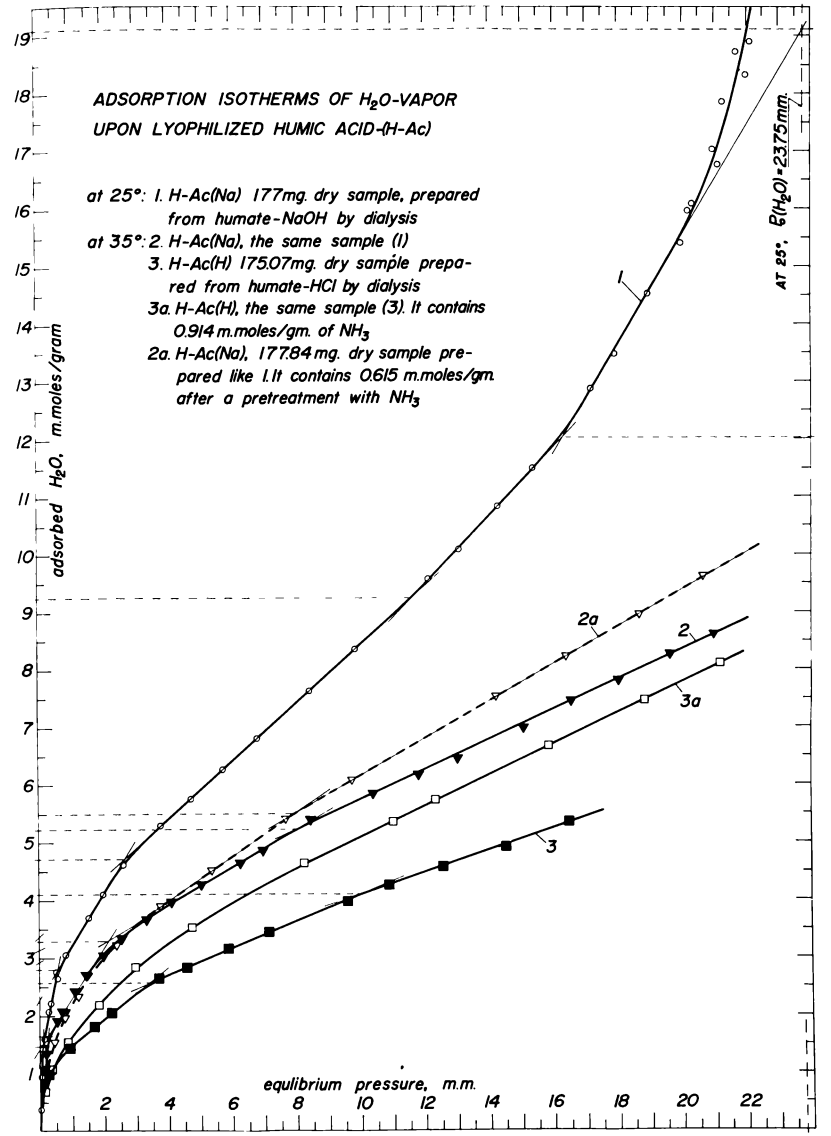


FIGURE 1—See text.

amounts of sorbed H_2O are referred to relative pressures in the range of measurements; however, the equilibration time at 35° is remarkably shorter. Therefore it may be concluded that no structural changes of H-Ac$_{(Na)}$ molecules occured in the region of investigation. Again, the appearance of slopes in isotherms seems, as previous-

ly discussed (2), to be due to the activity of different functional groups, and this should be re-emphasized here.

After the determination of isotherms was accomplished the sample was treated with NH_3 (additionally adsorbed), which formed ammonium salts, then desorbed during two days at 10^{-6} mm of pressure.

At the H-Ac(ₛ) containing only 0.615 mmoles/gm of NH_3 it was repeated an adsorption of H_2O vapor at 35°.

From the isotherm, curve 2a, it is evident that a noticeable increase in sorptivity of H_2O occurred, especially in the region of higher equilibrium pressure (physical adsorption), while the initial sorptivity slightly diminished.

On the other humic acid, H-Ac(ᵤ), determinations of adsorption isotherms were done which demonstrate similar shapes except the sorbed amounts of H_2O are lower at 35° as is shown in Figure 1, curve 3.

A treatment with NH_3 + H_2O which followed desorption and re-adsorption of H_2O showed a similar increase in adsorptivity of H-Ac(ᵤ) (see curve 3a). The increase in adsorptivity in both H-Ac(ₛ) and H-Ac(ᵤ) is evidently due to the presence of NH_4^+ ions; however, the mechanism of it is not known yet. There is some "confusion" in that H-Ac(ᵤ) with an even lover content of ash, and therefore considered to be of higher purity, still showed lower adsorptivity than the H-Ac(ₛ).

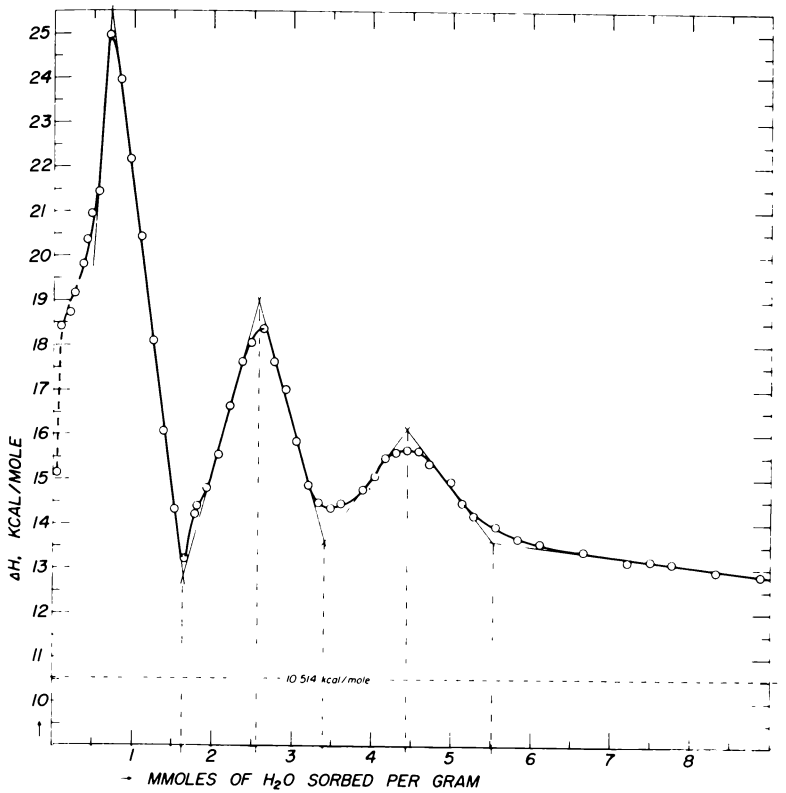
This may be caused by the method of preparation, where H-Ac(ᵤ) was regenerated by HCl before dialysis. It is known that HCl reacts with amino groups, if they are present, to form salts which block a number of adsorption centers for H_2O. Besides that, chemical regeneration is a more rapid process than the process of dialysis which caused some further disaggregation of the substance; i.e., it increases reopening of the structures.

Isothermic data on H-Ac(ₛ) were used for calculation of differential heats of adsorption by means of the Clausius-Clapeyron formula. The results are shown in Figure 2. From this figure it can be seen that the bond strengths diminish with progressing adsorption. These demonstrate several peaks which are the limitation of saturation of different functional groups participating in adsorption.

Now, comparing these data of H-Ac(ₛ) and H-Ac(ᵤ) with those earlier obtained on commercially produced H-humate(s) (2), it can be seen that purification (even a relative one) and especially lyophilization, increased the accessibility of functional groups in humic acid compounds. This accounts for the appearance of two additional slopes in the adsorption isotherms at 25°.

From the shape of the isotherm at 25° it may be concluded that at least five functional groups or their combinations take part in adsorption of H_2O vapor.

Although one is skeptical of attempting to determine the total surface area of a humic acid sample, it is felt that the data obtained



DIFFERENTIAL HEATS OF WATER VAPOR ADSORPTION UPON THE HUMIC ACID ($H\text{-}Ac_{(Na)}$) AT 25-35°

FIGURE 2—See text.

can be utilized for internal comparison to demonstrate the advantage of lyophilization.

Assuming average diameter of H_2O—molecule = 2.88 x 10^{-8} cm (10) and using the amount of adsorption in the total monolayer = 19.15mmoles/gm for H-Ac (۷,۱) its surface is estimated to be ≈ 747.5 m^2 gm, while for H-humate(s) with the respective amount of sorption 11.55 mmoles/gm (2), it is only 450.74 m^2 gm.

B. *Sorption of NH_3 or H_2O + NH_3 on lyophilized humic acids*—Knowledge of an interaction mechanism between humic acids from lignite and NH_3 or H_2 + $NH_3 \leftrightarrows NH_4OH$ is important not only for molecular structural clarification, but because it may be immediately utilized in the technology of fertilizers for agriculture.

This part of the study was concerned basically with obtaining preliminary data to the subject. First of all a high adsorptivity of NH_3 gas was not expected on a dry humic acid, because the chemical re-

action of neutralization does not occur in the solid state of an acid as in the case of solution. This was proved with Polyglutamic Acid (11), and it was concluded that the hydrogen bonds in the solid state of the acid could not be broken by NH_3 gas to start a normal ionic reaction.

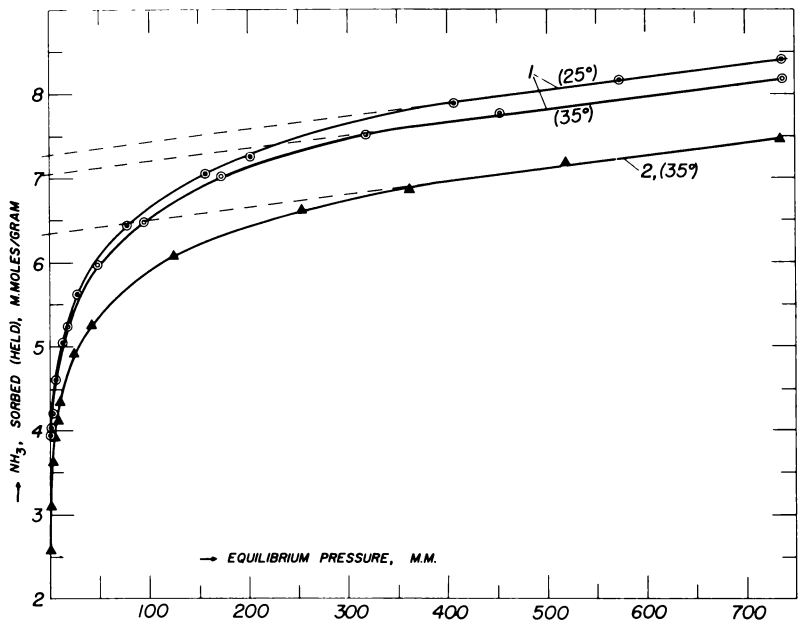
The preliminary attempts to determine the adsorption isotherms show the necessity of a very long time for equilibrations (up to 5 days for every new point); however, at higher pressures the sorption seems somewhat accelerated.

Therefore, it was decided to saturate the samples of H-Ac$_{(Na)}$ and H-Ac$_{(H)}$ with NH_3 gas at a high presure (up to 730 mm) exposing them for 5 days.

This resulted in the following amounts of NH_3 gas being adsorbed:

1) at 25° for H-Ac$_{(Na)}$ equal to 8.8 mmoles/gm,
2) at 35° for H-Ac$_{(Na)}$ equal to 8.35 mmoles/gm, and for H-Ac$_{(H)}$ equal to 7.00 mmoles/gm.

Starting from these amounts of NH_3 adsorbed, there were determined desorption isotherms at 25° an 35°, the data of which are shown in Figure 3.



DESORPTION ISOTHERMS OF NH_3 UPON HUMIC ACID AT 25 & 35°

1. H-Ac(H), SAMPLE PREPARED BY DIALYSIS FROM HUMATE-HCl
2. H-Ac(Na), SAMPLE PREPARED BY DIALYSIS FROM HUMATE-NaOH

FIGURE 3—See text.

The shapes of desorption isotherms are demonstrating straight lines which may be considered as the accomplishment of saturation. It is interesting that the extension of the linear portions of the isotherms to zero pressure meet approximately the value which is in region of the total acidity as determined by straight and backwards titrations of the same samples of humic acids (8).

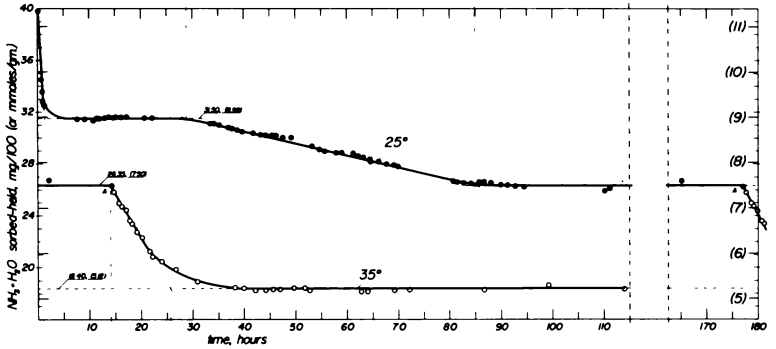
The titration curves (8), however, showed a number of approximated slopes (up to 9) for the isothermic areas which are represented here as curvatures, due probably to an overlapping effect. According to (11), it was suggested that hydrogen bonds can be broken by H_2O if it undergoes adsorption.

In an attempt to investigate the capability of H-Ac(H) (which is considered to be of higher purity) to hold NH_3 or H_2O + NH_3 at the condition of its equilibration with the atmosphere at 25° and 35°, its samples were exposed first to H_2O vapor until its adsorption reached 10 per cent, then to NH_3 gas at a pressure of ~ 720mm.

This resulted in amounts of adsorbed H_2O and nNH_3 (n>1) equal to ~60 per cent, which were reached during a short time (1 hour).

Now, holding the sample of H-Ac(H) + adsorbate at 25°, the system was opened and equilibrated with the atmosphere (of average barometric pressure at 725 mm, and room temperature of 26°). A rapid loss of weight was observed, which stops at a content of adsorbant equal to 31.50 mg/100, ~ 8.98 mmoles/gm, which during 26 hours remained constant. Then the loss of weight continued linearly during an additional ~ 59 hours, until a final constant value of content equal to 25.35 mg/100, ~ 7.50 mmoles/gm, was reached as is shown in Figure 4, for 180 hours of the total period of equilibration.

The change of temperature of H-Ac(H) + adsorbate (H_2O + NH_3) sample to 35° caused a further desorptive loss of weight. The finally equilibration during ~ 90 hours of time demonstrated a content of H_2O + NH_3 at 35° equal to 18.40 mg/100 ~ 5.12 mmoles/gm of dry H-Ac(H); see Figure 4.



Equilibration curves at 25 & 35° of the previously sorbed NH_3·H_2O by H-Ac(H), after it was exposed into atmospheric air

FIGURE 4—See text.

Complete desorptions of the samples of H-Ac(II) + adsorbate in vacuum at 25° and 35° showed a rapid further loss of weight; however, at a pressure of 10^{-4} to 10^{-5} a content of adsorbed H_2O + NH_3 was recorded in the range of 3.5 mg/100, which seems to be relatively strongly chemically bound to H-Ac(II).

A plot of content of H_2O + NH_3 as a function of desorption time indicated three (four) stages of desorption in the region of 10^{-6} to 10^{-7} mm of pressure during 58 hours. The final content reached amounted to about 1.5 mg/100.

SUMMARY

A. The results show that purification and especially lyophilization develops the surface of the complex aggregated molecular structures of humic acid, and that this improves further studies by means of sorptions. It may extend to an application of IR analysis in conjunction with sorption and titration for identification of functional groups.

B. Adsorptivity of NH_3 and H_2O + NH_3 have certified the suggestions concerning the behavior of acidic functionalities in the solid state. The presence of H_2O at least in small amounts causes extensive adsorptivity of NH_3 gas, which seems to be interacting with humic acid physically (by attractive forces) and chemically, although with a variety of bond strength.

ACKNOWLEDGEMENT

Thanks are due to Dr. C. D. Slater for his remarks and discussion during the preparation of this paper.

REFERENCES

1. Wood, J. C., Moschopedis, S. E., and den Hertog, W.; Fuel, *40* 491 (1961); Moshopedis, S. E.; Fuel, *41* 425 (1962).
2. Hnojewyj, W. S.; Proc. N. Dak. Acad. Sci. *19*, 91 (1965).
3. Hnojewyj, W. S., and Reyerson, L. H.; J. Phys. Chem *65*, 1694 (1961).
4. Hnojewyj, W. S., and Reyerson, L. H.; J. Phys. Chem. *67*, 711 (1963).
5. Bakken, G. S., and Hnojewyj, W. S.; Proc. N. Dak. Acad. Sci. *20*, 1 (1966).
6. Johnson, S. O., and Hnojewyj, W. S.; Proc. N. Dak. Acad. Sci. *20*, 180 (1966).
7. Ferche, D. S.; PhD Thesis—Surface Studies of H-humate(s) and Humic Acid(s) derived from Lignite, North Dakota State University, Fargo, N. Dak. 1967.
8. Ferche, D. S., and Hnojewyj, W. S.; Proc. N. Dak. Acad. Sci. *20*, 15 (1966).
9. Hnojewyj, W. S. Proc. N. D. Acad. Sci. *21*, 211 (1967).
10. Moore, W. J.; Physical Chemistry p. 179, 2nd Edition, Englewood Cliffs, N.J., Prentice-Hall, Inc.
11. Reyerson, L. H., and Hnojewyj, W. S.; J. Phys. Chem. *67*, 1945 (1963).

DIFFERENTIAL SPECTROPHOTOMETRIC DETERMINATION OF HAFNIUM IN THE PRESENCE OF ZIRCONIUM WITH MANDELIC ACID

Arnold R. Johnson, Jr.

Department of Chemistry
Minot State College, Minot, North Dakota

Harry Freund

Department of Chemistry
Oregon State University, Corvallis, Oregon

INTRODUCTION

The application of Mandelic acid (phenylglycolic acid) to the gravimetric determination of zirconium and hafnium mixtures, and the subsequent differential spectrophotometric determination of the hafnium, was investigated.

Mandelic acid is probably the most useful and convenient reagent for the separation and gravimetric determination of zirconium and hafnium. The use of mandelic acid as a specific reagent for zirconium and hafnium was first described by Kumins (10) in 1947. Hahn (4) later found that hafnium also precipitates quantitatively with this reagent.

Klingenberg, and Papucci (9) reported, that in the determination of zirconium using *p*-bromo or *p*-chloromandelic acid, the precipitates can be weighed directly without conversion to the oxide. However, Belcher, Sykes and Tatlow (1) found that it was necessary to ignite mandelic acid and *p*-bromomandelic acid to the oxides for the best accuracy. With the aid of Chevenard Thermobalance, Statchtchenko and Duval (11) carried out thermogravimetric analysis of zirconium mandelate and zirconium *p*-bromomandelate, and as a result, observed that the formula of the first compound closely approximates $Zr(C_6H_5CHOHCOO)_4$ while the second one had no definite formula. Hahn and Baginski (5) postulated that compounds of varying amounts of basic salts such as $ZrO(C_8H_7O_3)_2$ and $Zr(OH)(C_8H_7O_3)_3$ along with the pure zirconium tetramandelate were formed. This suggested that best results, for direct weighing, should be expected in strongly acid solutions. They found that the concentration of hydrochloric acid present at the time of precipitation greatly influenced the composition of the precipitate. The normal zirconium tetramandelate was obtained only in strong acid solution (5M or greater). However, it was found that quantitative precipitation of zirconium occurred at all acidities. The proper conditions for precipitation to be followed by direct weighing were also outlined.

Kumins (10) observed that zirconium tetramandelate dissolves in dilute sodium hydroxide followed by immediate precipitation of the

hydrous oxide. He found, however, that with ammonium hydroxide no precipitate was formed, thus indicating the probable formation of a soluble complex. Hahn and Weber (6) further studied this reaction and subsequently (7) developed an ultraviolet spectrophotometric method for zirconium. They found that an alcohol and ether wash was satisfactory for removing the excess mandelic acid from the zirconium tetramandelate precipitate. They also found that the amount of ammonia used to dissolve the zirconium tetramandelate was not critical but that after two days, the solutions started to hydrolyze with precipitation of the hydrous oxide. Absorbance measeurements were made at a wavelength of 258 mμ. It was postulated that the absorbance was caused by the phenyl groups of zirconium tetramandelate, as ammonium mandelate solutions gave an identical absorbance spectrum. These authors also concluded from their studies on zirconium tetramandelate that it must be a chelate type compound as postulated by Feigl (2).

Bricker and Waterbury (10) attempted to use *p*-bromomandelic acid instead of mandelic acid but found that each bottle of reagent was sufficiently different so that consistent and reproducible results could not always be obtained.

The papers of Freund and Holbrook (3) and Johnson and Freund (8) should be consulted for a discussion of the theoretical basis of the differential spectrophotometric method developed in this paper.

In the method described, standard solutions prepared from known weights of ZrO_2 and HfO_2 were precipitated with mandelic acid. The precipitates were dried and exactly the same weight (optimum concentration) of each tetramandelate standard was dissolved in 1.0M ammonium hydroxide and diluted to a given volume. The absorbance of each solution was then measured differentially by the "transmittance-ratio" method, the standard contining the largest amount of hafnium being used as the reference solution. A calibration curve of relative absorbance *vs.* per cent halfnium tetramandelate was constructed from these data.

In developing this method it was necessary to: (1) establish conditions for the mandelic acid precipitation of hafnium and zirconium mixtures for direct weighing of the mixed mandelates, (2) determine the absorption curves of mandelic acid, zirconium tetramandelate and hafnium tetramandelate in 1M ammonia, (3) find the optimum concentration of zirconium and hafnium tetramandelate for differential spectrophotometric measurement, in 1M ammonia solution, and (4) construct a calibration curve for the per cent of hafnium tetramandelate in mixtures.

EXPERIMENTAL

Apparatus—All absorbance measurements were made with the Beckman Model DU Spectrophotometer equipped with photomultiplier and ultraviolet light source. Matched silica cells were used for all absorbance measurements.

Reagents—Zirconium oxide. High purity ZrO_2 obtained from the United State Bureau of Mines, Albany, Oregon was used as the source of zirconium for all zirconium solutions. These solutions were standardized by the cupferron method.

Hafnium oxide. High purity HfO_2, $HfCl_4$ and $HfOCl_2.8H_2O$ obtained from the Bureau of Mines were used as the sources of all hafnium solutions. The solutions were also stadardized by the cupferron method.

Mandelic acid. Reagent grade mandelic acid was used for preparation of mandelic acid solutions.

Potassium pyrosulfate. Reagent grade, used for fusion of ZrO_2 and HfO_2.

Precipitation of zirconium and hafnium with mandelic acid—Standard solutions of ZrO_2 and HfO_2 were prepared from $ZrOCl_2.8H_2O$ and $HfOCl_2.8H_2O$ respectively. These solutions were 2M in HCl.

Twenty-five milliliter aliquots of these standards were pipeted into beakers, 25 ml of 2M HCl and the 35 ml of 15 per cent mandelic acid were added. The solutions were slowly heated to 85°C and allowed to digest at this tempertaure for thirty minutes. The resulting precipitates were filtered through previously weighed medium porosity sintered glass filtering crucibles. The precipitates were washed with a hot 2 per cent HCl + 5 per cent mandelic acid wash solution. They were then washed three times with acetone and twice with ether. Air was drawn through the precipitates for a few minutes and they were finally dried in an oven at 110°C for an hour. The results obtained are shown in Table I.

TABLE I

MANDELIC ACID PRECIPITATION OF ZIRCONIUM AND HAFNIUM

ZrO_2 Added	$Zr(M)_4$ Theory	$Zr(M)_4$ Found	Difference	Relative Error
(g)	(g)	(g)	(g)	(%)
0.0812	0.4585	0.4577	-0.0008	0.17
		0.4581	-0.0004	0.09
		0.4565	-0.0020	0.44
		0.4542	-0.0043	0.94
		0.4540	-0.0045	0.98
HfO_2 Added	$Hf(M)_4$ Theory	$Hf(M)_4$ Found	Difference	Relative Error
(g)	(g)	(g)	(g)	(%)
0.1173	0.4364	0.4384	0.0020	0.46
		0.4374	0.0010	0.23
		0.4397	0.0033	0.76
		0.4389	0.0025	0.57
		0.4382	0.0018	0.41
		0.4383	0.0019	0.44

Although the above conditions seemed to give satisfactory precipitations, the paper of Hahn and Baginski (5) suggested that the optimum conditions for the direct weighing of zirconium tetramandelate are: (1) the solution should be 5M or greater in hydrocloric acid, (2) the mandelic acid should be added dropwise with constant stirring to the hot (85° to 90°C) acid solution, (3) the solution should be cooled before filtration and (4) the precipitate should be washed with a saturated solution of zirconium tetramandelate followed by alcohol and ether.

The above conditions were tested for the precipitation of both zirconium and hafnium and the effect of varying the ratio of zirconium and hafnium to mandelic acid was determined. The concentration of zirconium and hafnium was varied by using 5.00, 10.00, 25.00 and 50.00 ml of each standard solution. Fifty milliliters of concentrated hydrochloric acid was added to each solution and then 50 ml of 15 per cent mandelic acid was added dropwise at 85°C followed by a thirty-minute digestion. The precipitates were finally cooled, filtered, washed and dried. The results are summarized in Table II.

These data seem to indicate that a large excess of mandelic acid gives the best results with zirconium and that no significant difference is observed for hafnium. This would imply that a large excess

TABLE II

EFFECT OF VARIATION OF ZIRCOIUM AND HAFNIUM TO MANDELIC ACID

ZrO_2 Added	$Zr(M)_4$ Theory	$Zr(M)_4$ Found	Difference	Relative Error
(g)	(g)	(g)	(g)	(%)
0.0210	0.1184	0.1184	0.0000	0.00
		0.1189	0.0005	0.42
0.1049	0.5918	0.5889	-0.0029	0.49
		0.5892	-0.0026	0.44
0.04196	0.2367	0.2376	0.0009	0.38
		0.2365	-0.0002	0.09
0.2098	1.1836	1.1686	-0.0150	1.27
		1.1694	-0.0142	1.20
HfO_2 Added	$Hf(M)_4$ Theory	$Hf(M)_4$ Found	Difference	Relative Error
(g)	(g)	(g)	(g)	(%)
0.0401	0.1492	0.1513	0.0021	1.40
		0.1505	0.0013	0.87
0.0201	0.0746	0.0755	0.0009	1.20
		0.0743	-0.0003	0.42
0.1003	0.3731	0.3737	0.0006	0.16
		0.3731	-0.0016	0.43
0.2006	0.7462	0.7476	0.0014	0.19
		0.7460	-0.0002	0.03

of mandelic acid should be used for precipitation of mixed oxides of zirconium and hafnium where the zirconium content is relatively high.

The next step in this study was the precipitation of solutions containing both zirconium and hafnium in varying amounts. These data are shown in Table III.

TABLE III

MANDELIC ACID PRECIPITATION OF MIXED OXIDES

ZrO_2 Added	HfO_2 Added	$Zr(M)_4+Hf(M)_4$ Theory	$Zr(M)_4+Hf(M)_4$ Found	Difference	Relative Error
(g)	(g)	(g)	(g)	(g)	(%)
0.0497	0.000988	0.2841	0.2825	-0.0016	0.56
			0.2824	-0.0017	0.60
0.0477	0.00198	0.2765	0.2733	-0.0032	1.15
			0.2727	-0.0038	1.37
0.0456	0.00296	0.2683	0.2675	-0.0008	0.30
			0.2667	-0.0016	0.60
0.0435	0.00395	0.2601	0.2576	-0.0025	0.96
			0.2586	-0.0015	0.58
0.0414	0.00494	0.2520	0.2505	-0.0015	0.59
			0.2506	-0.0014	0.56
0.0332	0.00889	0.2204	0.2183	-0.0021	0.95
			0.2192	-0.0012	0.54
0.0249	0.0128	0.1881	0.1876	-0.0005	0.27
			0.1862	+0.0001	0.05
0.0207	0.0148	0.1719	0.1721	+0.0002	0.17
			0.1701	-0.0018	1.05

From the preceeding data it can be seen that both zirconium and hafnium and mixtures of the two can be quantitatively precipitated with mandelic acid and weighed directely as the mandelates.

Absorbance curves of mandelic acid, zirconium tetramandelate and hafnium tetramandelate in 1M ammonium hydroxide solution were determined next.

Absorption Curves—Ultraviolet absorption curves of mandelic acid, zirconium tetramandelate and hafnium tetramandelate were run in 1M ammonia solution. The spectra obtained were essentially identical to each other with the absorption maxima occuring at 258 mμ. These spectra were in agreement with the ammonium zirconium tetramandelate spectrum obtained by Hahn and Weber (7) and seem to support their postulate that the absorption is due to the mandelate ion. These curves are shown in Figure 1.

Optimum Concentration—The optimum concentration of both zirconium and hafnium tetramandelate was determined by preparing a series of solutions contining each tetramandelate in 1M ammonia solution at a constant weight increment of the tetramandelate. The

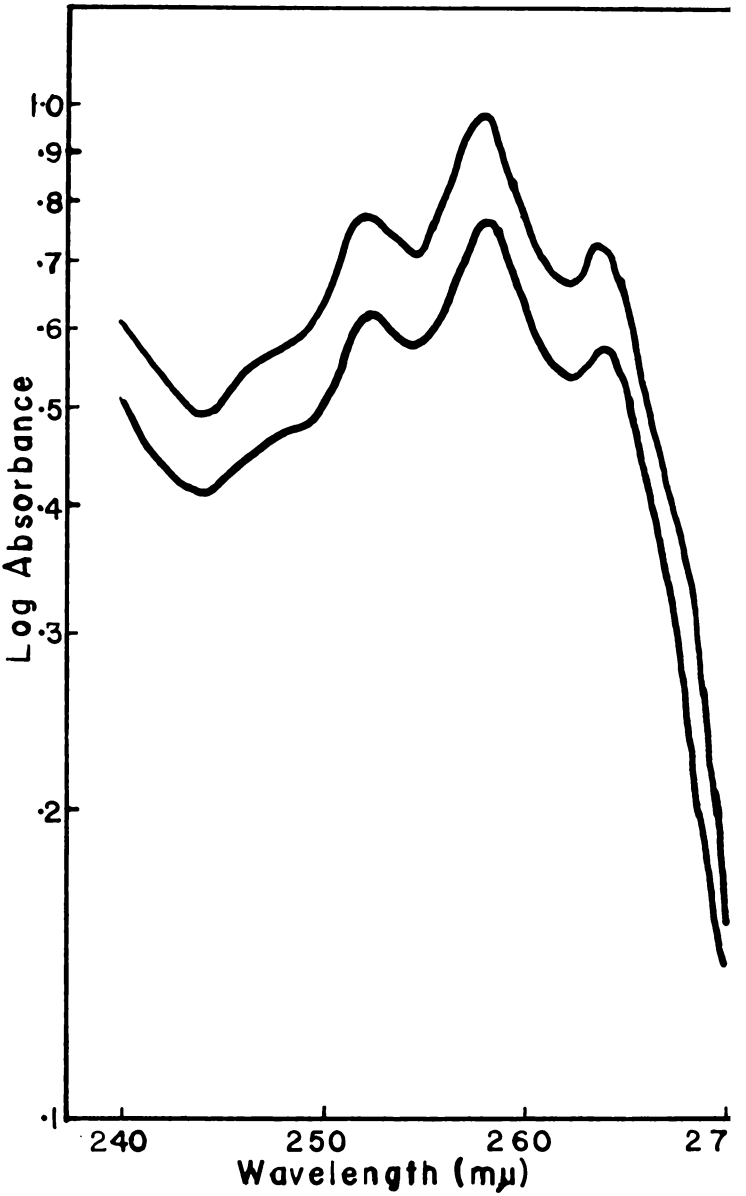


FIGURE 1—Absorption curves of zirconium tetramandelate, hai tetramandelate and mandelic cid in 1M ammonia. Upper curve 5.04 X 10^{-3} M mandelic acid and lower curve is for 9.9 X 10^{-4} M conium and hafnium tetramandelate.

absorbance of the most dilute solution was measured relative to the solvent. Then using this solution as reference, the absorbance of the solution of next higher concentration was determined, and so on until all solutions were measured. Using these absorbance values and knowing the concentration increment, the slope, $S = \Delta A/\Delta C$ was calculated. As previously mentioned (3,8) the optimum concentration is that concentration at which S x C is a maximum. From these measurements it was found that the optimum concentration was about 2.1 g/l for zirconium tetramandelate and about 2.8 g/l for hafnium tetramandelate. These data are shown in Tables IV and V.

TABLE IV

OPTIMUM CONCENTRATION OF ZIRCONIUM TETRAMANDELATE

To Set Zero	To Obtain Reading	Slit	Relative Absorbance	Slope	SxC
		(mm)	ΔA	(S)	
0.0000	0.4175	0.0225	0.461	1.104	——
0.4175	0.8350	0.04	0.502	1.202	0.502
0.8350	1.2525	0.07	0.460	1.102	0.920
1.2525	1.6700	0.13	0.495	1.186	1.485
1.6700	2.0875	0.24	0.438	1.149	1.752
2.0875	2.5050	0.40	0.434	1.140	2.171
2.5050	2.9225	0.64	0.318	0.620	1.909
2.9225	3.3400	0.88	0.269	0.644	1.882
3.3400	3.7575	1.15	0.130	0.311	1.039
3.7575	4.1750	1.30	0.112	0.268	1.007

Solution $Zr(M)_4$(g/l)

TABLE V

OPTIMUM CONCENTRATION OF HAFNIUM TETRAMANDELATE

To Set Zero	To Obtain Reading	Slit	Relative Absorbance	Slope	SxC
		(mm)	A	(S)	
0.0000	0.4698	0.0225	0.469	0.998	——
0.4698	0.9396	0.04	0.500	1.064	0.500
0.9396	1.4094	0.07	0.456	0.971	0.912
1.4094	1.8792	0.13	0.498	1.060	1.494
1.8792	2.3490	0.24	0.432	0.920	1.728
2.3490	2.8188	0.40	0.443	0.943	2.215
2.8188	3.2886	0.64	0.434	0.924	2.604
3.2886	3.7584	0.88	0.146	0.311	1.022
3.7584	4.2282	1.15	0.135	0.287	1.080
4.2282	4.6980	1.30	0.109	0.232	0.098

Solution $Hf(M)_4$(g/l)

The optimum concentration for mixtures of the tetramandelate: was taken to be about 2.4 g/l. Using this optimum concentration, a standard curve was next constructed.

Standard Curve for Determination of Hafnium in Presence o Zirconium—A series of standard solutions containing from 5.00 t 50.00 per cent hafnium tetramandelate in zirconium tetramandelat were prepared by weighing out the proper amounts of each com pound and dissolving and diluting to volume with 1M ammonia s that each solution contained exactly 2.400 g/l of mixed mandelates The absorbance of each of these solutions was then measuered rela tive to the 50 per cent hafnium tetramandelate as reference, since i was the least absorbing. A standard curve of ΔA *vs.* $Hf(M)_4$ wa then drawn. This is shown in Figure 2.

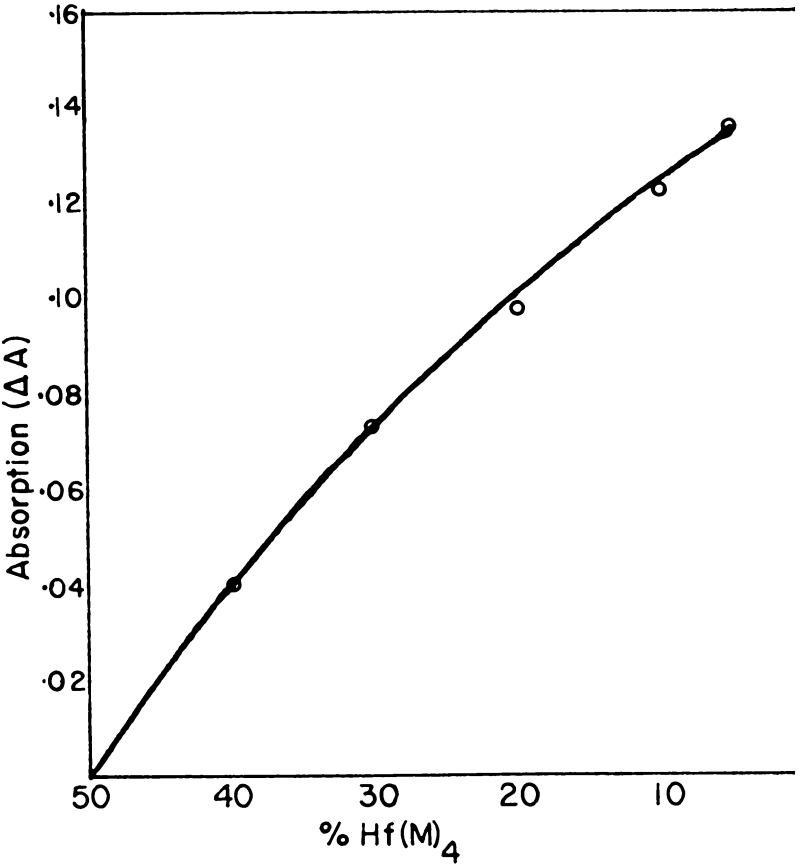


FIGURE 2—Standard curve for determination of per cent hafniu tetramandelate in zirconium tetramandelate.

DISCUSSION

The results of this investigation seem to indicate that relatively large ratios of HfO_2 to ZrO_2 can be determined by the procedure outlined.

The method developed has a certain advantage over other colorimetric methods, since the hafnium and zirconium are precipitated, weighed and measured spectrophotometrically as the mandelate. Unfortunately this method did not seem to be sensitive enough for the determination of very low percentages of hafnium in the presence of zirconium, with the instrument used in this investigation. However, further investigation of low ranges of hafnium using an expanded scale spectrophotometer might prove fruitful.

REFERENCES

1. Belcher, R., Sykes, A. and Tatlow, J. C., Anal. Chim. Acta., **1,** 34-37 (1954).
2. Feigl, F., CHEMISTRY OF SPECIFIC SENSITIVE AND SELECTIVE REACTIONS, P. 74. Acdemic Press, Inc., New York., 1949.
3. Freund, H. and Holbrook, W. T., Anal. Chem., **30,** 462-465 (1958).
4. Hahn, R. B., Anal. Chem., **21,** 1579-1580 (1949).
5. Hahn, R. B. and Baginski, E. S., Anal. Chim. Acta., **14,** 45-47 (1956).
6. Hahn, R. B. and Weber, L., J. Am. Chem. Soc., **77,** 4777-4779 (1955).
7. Hahn, R. B. and Weber, L., Anal. Chem. **28,** 414-415 (1956).
8. Johnson, A. R., Jr. and Freund, H., N. Dak. Acad. Sci. **20,** 72-78 (1966).
9. Klingenberg, J. J. and Papucci, R. A., Anal. Shem. **24,** 1861-1862 (1952).
10. Kumins, C. A., Anal. Chem. **19,** 376-377 (1947).
11. Stachtchenko, J. and Duval, C., Anal. Chim. Acta, **5,** 410-421, (1951).

ADAPTING URANIUM EXPLORATION TECHNIQUES TO HOST-ROCK ENVIRONMENTS

E. A. Noble

Department of Geology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In the present revival of uranium exploration, few surface bonanzas are likely to be found. Even companies with large exploration budgets should precede drilling with careful geologic thinking. Correct interpretation, mapping, and projection of a favorable host rock can greatly improve chances of intersection of ore by drilling.

Examples of exploration approaches are suggested for three recently discovered Argentine uranium deposits illustrating widely

different host-rock environments. Preliminary mapping of the favorable environment is necessary for efficient exploration in all cases, but, in detail, each deposit requires different exploration techniques for delineation of associated favorable zones. One deposit is in continental Cretaceous rocks in the marginal part of an oil-producing basin in Patagonia. Preliminary exploration here depends chiefly on reconstruction of paleo-stream patterns spatially related to a major unconformity.

A second deposit, in the pre-Cordillera of northwestern Argentina, is found in near-vertical faults within a thrust breccia where Carboniferous continental deposits have overridden Ordovician limestones. The possible function of certain Carboniferous sandstones as aquifers, and their position relative to the thrust plane, may have been important in the introduction of uranium, lead, and zinc.

The third and probably most unique deposit is in a clean, well sorted sandstone apparently formed in a transitional environment between continental arkosic sandstones and marine carbonates and argillaceous rocks along the margin of a Cretaceous basin in northern Argentina. Meaningful exploration must start with detailed mapping of the transitional continental environment to determine the distribution of the favorable lithology.

INTRODUCTION

One of the characteristics of a uranium exploration boom, such as that of the early 1950's and the one developing today, is the rush to acquire properties and drill out ore. Individuals, groups, and even established mining companies make hurried decisions for expenditures that would not be considered justifiable in ordinary times. Careful geologic work could accomplish remarkable economies, yet a close observer can point to costly examples of failure to include adequate geologic investigation.

The purpose of this paper is to emphasize that preliminary geologic investigation can simplify and reduce the scale and costs of exploration without reducing the chances for success to any great extent. A prime goal in mineral exploration is to limit the size of the target by differentiating favorable from unfavorable ground (Weir, 1952). The type of investigation described here involves interpretation of paleo-environment and reconstruction of geologic history. Paleohydrology is all-important; mapping of the "plumbing system" associated with a uranium occurrence is more practical at this early exploratory stage than is undue emphasis on genetic aspects such as pinpointing the primary source of the ore element. If genetic studies are made at this stage, they should concentrate on the problem of causes of ore deposition, and direction of movement of the ore-forming fluid.

Depending on the time, money, and facilities available, any number of extensions and refinements can be made to this preliminary type of geologic investigation. For example, equilibrium studies can

provide clues useful in tracing movements of radioactive compounds, particularly in the oxidizing environment generally encountered in exploration of surface and near-surface uranium manifestations. The important thing is to use these techniques in conjunction with geologic common sense.

The examples given here are of three distinctly different types of environment. Argentine deposits are chosen because they portray uranium exploration in its early stages. Two of the three uranium occurrences are unique, although one may have a rough analogue in the extraordinarily rich deposit at Marshall Pass in Colorado (present author, unpublished manuscript).

STREAM-CHANNEL ENVIRONMENT

Uranium has been discovered in a conglomeratic sandstone in the Rio Chubut drainage basin of north-central Chubut province, Argentina (Figure 1). The area of interest lies in the northern part of the San Jorge basin, a subnegative area in extra-Andean Patagonia (Jenks, 1956).

Exposed at the surface are poorly consolidated mudstone, siltstone, sandstone and tuffaceous rocks of Cretaceous age. Erosion has locally excavated the Cretaceous rocks to expose knobs that are a

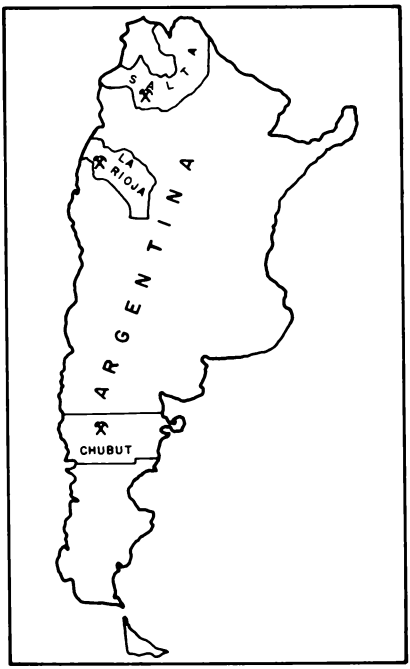


FIGURE 1—Index map of Argentina, showing locations of uranium occurrences discussed in text.

part of a buried topography developed on a Jurassic "basement" composed of rocks folded and indurated to a greater degree than the Cretaceous and younger rocks. To the southeast, in the deeper portions of the San Jorge basin, the Jurassic "basement" is more deeply buried by Cretaceous sediments, which in turn are covered by sediments of Tertiary age (Dr. P. N. Stipanicic, oral communication, 1963).

The uranium occurrence consists of secondary uranium minerals in sandstone and conglomeratic sandstone believed to represent a Cretaceous stream-channel deposit. Present exposures have obviously been oxidized, so an unknown proportion of the total uranium has probably moved out, in the direction of ground-water movement. Search for local concentrations should thus be conducted in this direction.

Economical exploration of the fluviatile environment involves narrowing the target area by mapping the channel systems that appear to have functioned as aquifers. Strong mineral alteration is one criterion by which these aquifers can be distinguished. Marginal zones of these channel deposits, or zones along the outer margins of strongly altered portions of these deposits (Shawe, 1956; Harshman, 1962; Noble, 1963) are especially favorable. By delineating these selected environments before and during the early stages of drilling, progressive mining companies have shown that the costs of exploration can be greatly reduced.

THRUST-BRECCIA ENVIRONMENT

An area in the pre-Cordillera of northwestern Argentina, near the Guandacol River in La Rioja province (Figure 1), is host to two types of uranium deposits, in addition to deposits of lead and zinc. The rugged topography exposes a thick section of continental Carboniferous rocks thrust over older rocks, chiefly marine limestone of Ordovician age.

Prior to 1963, the only known uranium occurrences were small, but locally high-grade, deposits associated with carbonaceous debris in small paleochannel deposits within the continental Carboniferous sequence. These occurrences, not discussed further here, are a common type in which the uranium was apparently extracted from through-going ground water by organic material.

In 1963, uranium minerals were discovered in a limestone thrust breccia within a few kilometers of the Carboniferous paleochannel deposits. The uranium-bearing breccia, consisting largely of Ordovician limestone of irregular thickness, is presently situated in front of the forward edge of the partially eroded overthrust plate of Carboniferous continental deposits (Figure 2). Uranium mineralization, presently evidenced only by secondary minerals, appears to be concentrated along small, high-angle fractures in the breccia. The vertical extent of the mineralization was not determined, as the exposure was limited to a depth of about two meters.

Two features of this breccia occurrence of uranium may be sig-

nificant in explaining its presence. One feature is the apparent localization of uranium in the breccia at or near the intersection, or projected intersection, of coarse Carboniferous sandstone units with the brecciated Ordovician limestone present on the thrust plane (Figure 2). The second feature is the apparent concentration of uranium long minor fractures in the relatively thick accumulations of breccia.

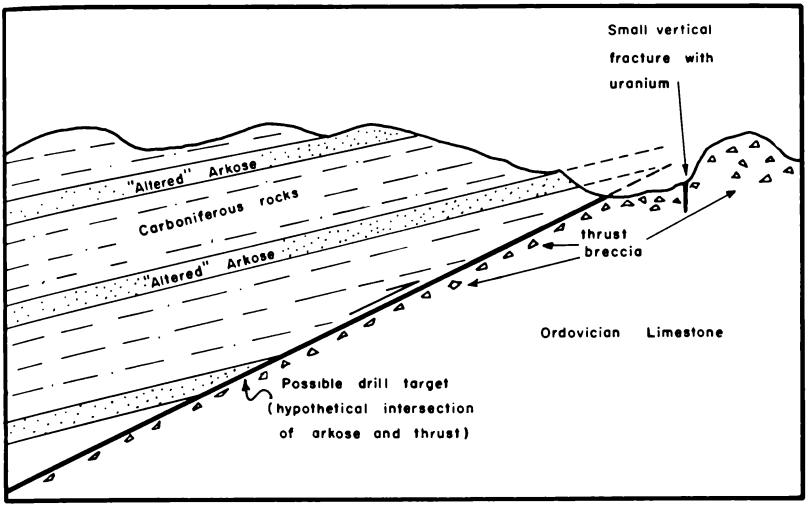


FIGURE 2—Diagrammatic interpretation of uranium occurence in thrust breccia, La Rioja Province, Argentina.

The explanation of the first feature may lie in the relatively high transmissivity of the sandstone units, which could have permitted them to function as aquifers carrying uranium-bearing formation water (Noble, 1963) being expelled from the thick pile of Carboniferous sediments shoved forward along the thrust. The bleached or altered appearance of the sandstone suggests the passage of solutions. Macroscopic examination and comparison with samples from relatively less altered zones indicate that these sandstones were highly arkosic. This probability further encourages prospecting, because arkoses are generally believed to indicate a terrane favorable for yielding uranium for concentration into deposits. The environment encountered by the uranium-bearing water as it passed from the sandstone into the fault zone would likely have been favorable for precipitation.

The importance of finding an explanation for the second feature, the concentration of uranium along minor fractures in the breccia, is believed to be less critical than in the case of the first. The fractures apparently provided local permeability for ore solutions brought to the breccia zone by the sandstone aquifers.

Selection of target areas for further exploration in the thrust-fault environment can be accomplished by projecting the strongly altered Carboniferous arkosic units to their intersections with the sole of the thrust. Particularly favorable areas may be those in which the thrust breccia is relatively thick, locally silicified, fractured, and in places piled up in the manner of moraine near the toe of a thrust block. Attention should be paid to pre-fault topography on the Ordovician limestone, because the surface configuration and orientation of topographic features seems to have affected the distribution and thickness of the breccia. In addition, the possibility should be considered that concealed Carboniferous aquifers may intersect fault breccia in the subsurface (Figure 2); here could lie the real potential of the area, for oxidation and erosion would have taken less toll.

SHORELINE ENVIRONMENT

Uranium has been found in Cretaceous sedimentary rocks that, dipping steeply, and in places overturned, crop out on both sides of the Tonco Valley syncline in south-central Salta province, Argentina (Figure 1). The Tonco Valley syncline, presently flanked on both sides by Precambrian granitic rocks, is a down-folded and down-faulted remnant of rocks deposited along the margin of a Cretaceous basin. Remarkable exposures representing shoreline and near-shore depositional environments are present along both flanks of the synclinal valley, while at the center of the valley, or trough of the syncline, is filled with flat-lying continental sediments of Tertiary age. Lithologies exposed include conglomerate, arkose, uncemented sandstone, carbonate-cemented sandstone, siltstone, mudstone, and carbonates.

Uranium is found in sandstone units, suggesting that relatively high transmissivity is a factor in its accumulation. Moreover, it is found only in light gray sandstone associated with greenish-gray shales; this may indicate that the uranium was initially concentrated in a reducing environment, although it is now seen in the form of yellow oxidized minerals. The most distinctive host unit is a clean, friable, fine-grained quartz sandstone containing black tourmaline grains and thin beds of finely comminuted muscovite. Presumption that these sediments are locally derived from a terrane of granite and granite pegmatites is reinforced by noting that some of the red arkosic conglomerates contain abundant pebbles of quartz and tourmaline of typical pegmatitic texture. Presumption is also made that the uranium, too, is locally derived as a product of the weathering of uranium-bearing granite and granite pegmatite.

Exploration in this environment should be based on careful mapping and projection of the Cretaceous shoreline, with care taken not to overlook the possibility of oscillatory repetition of deposits. The favorable environment would probably be readily identified during drilling. It should appear as a mudstone-sandstone sequence of

greenish gray to light gray color, distinctly different in appearance from overlying reddish arkosic clastics and underlying carbonates and carbonate-cemented clastics.

Here, as in other near-surface occurrences of uranium, the investigator must consider not only the geologic and hydrologic conditions that brought about early concentration of uranium, but also the subsequent history. Oxidation and ground-water movement may have dispersed the uranium, or dispersed and reconcentrated it, many times (Gruner, 1956).

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Argentina's Comision Nacional de Energia Atomica and to the International Atomic Energy Agency for making possible the writer's acquaintance with the uranium deposits described in this paper.

REFERENCES

Gruner, J. W., 1956, Concentration of uranium in sediments by multiple migration-accretion: Econ. Geology, v. 51, p. 495-520.

Harshman, E. N., 1962, Alteration as a guide to uranium ore, Shirley Basin, Wyoming, *in* Geological Survey Research 1967: U.S. Geol. Survey Prof. Paper 450-D, p. D8-D10.

Jenks, William F., Ed., 1956, Handbook of South American Geology: Geol. Soc. America Memoir 65, 378 p.

Kelley, V. C., 1955, Regional tectonics of the Colorado Plateau and relationship to origin and distribution of uranium: N. Mex. Univ. Pub. in Geol. no. 5, 120 p.

Noble, E. A., 1963, Formation of ore deposits by water of compaction: Econ. Geology, v. 58, p. 1145-1156.

Shawe, Daniel R., 1956, Significance of roll ore bodies in genesis of uranium-vanadium deposits on the Colorado Plateau, *in* Page, L. R., Ed., Contributions to the geology of uranium and thorium: U.S. Geol. Survey Prof. Paper 300, p. 239-241.

Weir, D. B., 1952, Geologic guides to prospecting for carnotite deposits on Colorado Plateau: U.S. Geol. Survey Bull. 988-B, p. 15-27.

PETROGRAPHY OF CORE AND WELL SAMPLES FROM LAKE AGASSIZ AND ASSOCIATED SEDIMENTS, GRAND FORKS, NORTH DAKOTA

James D. Ross and Frank R. Karner

Department of Geology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Lake Agassiz and associated sediments have been divided into nine lithologic units in the Grand Forks area after preliminary petrographic study of 273 feet of drill core and well samples from two

sites on the University of North Dakota campus. The units are listed below in stratigraphic sequence with the oldest at the bottom:

9. Brown and gray brown silty loam with clay laminae, 20 feet
8. Gray clay with lenses of silty loam, 26 feet
7. Dark gray clay, 37 feet
6. Gray clay with some gravel, 44 feet
5. Dark gray clay, 14 feet
4. Grayish brown sand, 15 feet
3. Dark gray clay, 51 feet
2. Gray clay with some gravel, 34 feet
1. Gravelly clay loam, 32 feet (base not determined).

The units are distinguished and characterized by lithologic properties observable in hand specimens, engineering properties, sand-silt-clay ratios, and qualitative and quantitative mineralogic composition as determined by X-ray diffraction.

The units have been grouped into five sediment types which have been interpreted as follows:

Type A.—unit 1—gravelly clay loam—till;

Type B.—units 2 and 6—gray clay with some gravel—ice-marginal lacustrine deposits;

Type C.—unit 3 and lower and middle parts of 5, 7 and 8—dark gray clay—relatively deep-water lacustrine deposits;

Type D.—unit 9 and upper parts of 7 and 8—brown and gray silty loam—relatively shallow water lacustrine deposits;

Type E.—unit 4—grayish brown sand—fluvial deposits.

Gradational relationships between types B and C, and C and D suggest a complex history with at least two periods of glacial advance, and lacustrine deposition under varying conditions of water depth and distance from ice margins.

INTRODUCTION

Beginning essentially with the work of Upham (1896) Lake Agassiz and related sediments of North Dakota and Minnesota have been studied by many workers. Laird (1964) has recently outlined these studies and has attempted to provide a synthesis of the lake's history in these states. As a result of previous work the topographic forms of the lake deposits, the surface distribution of the lake sediments and their general structural and textural features are quite well known. Subsurface samples have been less thoroughly studied. Much general subsurface information is recorded in the well logs and cross sections of the geologic and ground water studies of the North Dakota State Water Conservation Commission and the North Dakota Geological Survey. Rominger and Rutledge (1952) utilized soil mechanics data in a study of cores of Lake Agassiz sediments in Grand Forks and Fargo. Our preliminary study summarizes textural and mineralogic variation observed in 273 feet of drill core and well samples from two sites on the University of North Dakota campus in Grand Forks. Nine major lithologic units and five sediment types are recog-

nized and can be related to two or more periods of glacial advance, and lacustrine deposition under varying conditions.

METHODS

Sampling.—Core and thin-wall samples were taken from the top 150 feet of sediment at the site of the new Mathematics and Physics building on the University of North Dakota campus in Grand Forks (U.N.D. sample 4201, NE ¼, SE ¼, SE ¼, SE ¼, Sec. 5, T. 151 N., R. 50 W.) by the Soil Exploration Company of St. Paul, Minnesota. In coring, a motorized drilling rig which converts to a small pile driver for the core barrel is used. The auger is drilled the length of auger section (5 feet), it is then pulled out of the hole, a core barrel is connected to the auger shaft and driven downward until a one-foot core sample is obtained. After trimming the samples are approximately 5 inches long and 1.5 inches in diameter. They are stored in wax-sealed jars to retain their natural moisture. In the upper 10 feet, the normal sample interval was 2.5 feet, below this, 5 feet. At 20-foot intervals thin wall samples were taken. These are large diameter cores (2.5 to 3 inches) which are taken by slowly pressing a thin-edged tube into the sediment. This almost completely eliminates the shearing distortion present in the regular core samples.

The samples from 150 feet to 270 feet are grab samples which represent about five feet of section each. They were taken from a water well drilled by the U.S. Geological Survey on the U.N.D. campus (U.N.D. sample 4702, SE ¼, SE ¼, Sec. 5, T. 151 N., R. 50 W.,). The samples were taken from the circulation mud pits of a water-well drilling rig. The time that it takes for the mud to circulate from the pit to the bottom of the hole and back to the pit determines the location of the sample. The sample is caught in a screen at the surface before it enters the mud pit. The samples are dried and stored in paper envelopes.

The cores were split lengthwise and a 40-gm sample the shape of a triangular prism, was cut from the inside. The sample was thoroughly mixed then split by the cone and quarter method. One half was wrapped in aluminum foil and returned to the sample bottle along with the remaining core. From the remaining twenty grams, a five gram split was dried for 24 hours at 60° to 80°C to determine water content and was then used for X-ray mineralogical analysis. The remaining 15 gm were used for particle-size analysis. For grab samples only one twenty-gram portion for X-ray and size analysis was used.

Study of Texture and Sedimentary Structures—Sand-silt-clay ratios were determined by a simplified method developed to obtain samples of these fractions for mineralogical determination by X-ray analysis. The sample of approximately 15 gm, corrected for water content, was disaggregated for 24 hours in 500 ml of Calgon solution by repeated stirring. Total clay (less than 4 microns) was determined using a 25 ml pipette sample taken at a depth of 4 cm after 75 min-

utes. A 25 ml sample of clay (less than 2 microns) was also taken for X-ray analysis. Clay was removed by decantation and silt and sand were separated by decantation followed by sieving after drying. This yielded a pure sand fraction (some gravel included) and a relatively pure silt fraction for X-ray analysis. The percentage of silt was determined by weight difference of the original sample and the amount of sand plus clay as determined above. Repeated tests of this procedure on previously analyzed sediments give results which have average deviations from values assumed to be correct of less than 4 per cent (Harlan K. Friestad, Dept. of Geology, Univ. of N. Dak., unpublished study). Sand-silt-clay determinations are given in Table IV.

Complete size analyses were made for selected samples using the general technique of Krumbein and Pettijohn (1938). Particle-size statistics were calculated using a University of Missouri Fortran program (Kane and Hubert, 1964) modified for the University of North Dakota I.B.M 360 computer by C. F. Royse of the U.N.D. Department of Geology and by R. Fisher of the U.N.D. Computer Center. Results were extrapolated from 10 phi to 14 phi using the method of Folk (1957). Results are given in Table V. Sand-silt-clay ratios for units 1 to 3 in Table IV were taken from Table V.

Nomenclature of sediments in this study follows the U.S. Bureau of Soils Classification system as given in Table I.

TABLE I

U.S. BUREAU OF SOILS CLASSIFICATION SYSTEM

Sediment Name	Composition (in weight per cent)		
	Sand	Silt	Clay
Sand	90-100	0-10	0-10
Loamy Sand	80-90	0-20	0-20
Sandy Loam	50-80	0-50	0-20
Loam	30-50	30-50	0-20
Silty loam	0-50	50-80	0-20
Silt	0-20	80-100	0-20
Sandy clay loam	50-80	0-30	20-30
Clay loam	20-50	20-50	20-30
Silty clay loam	0-30	50-80	20-30
Clay	0-40	0-50	30-100
Sandy clay	50-70	0-20	30-50
Silty clay	0-20	50-70	30-50

Sedimentary structures and fabric were studied principally by megascopic and microscopic examination of cut cores. Bedding in clays of massive appearance was identified by thin-section study, X-ray radiography and X-ray diffraction. Radiography was done by Pamela G. Miller of the X-ray Department of the U.N.D. Student Health Service. X-ray diffraction was used to detect preferred ori-

entation of clay mineral particles in sediment slabs cut perpendicular and parallel to probable bedding directions in the cores. Preferred orientation, *i.e.*, bedding, is demonstrated when X-ray reflections of basal planes are enhanced relative to reflections from other planes of clay minerals.

X-ray Analysis—Mineralogy was determined primarily by X-ray diffraction. Volume percentages of minerals present in the samples were determined using relationships developed by Alexander and Klug (1948) and modified by Leroux *et al.* (1953) to:

$$X_1 = \frac{I_1}{(I_1)_o} \frac{\mu_s^*}{\mu^*_s}$$

where

X_1 = weight fraction of component 1 (or volume fraction)
μ_1^* = mass absorption coefficient of component 1
μ_s^* = mass absorption coefficient of powder sample
I_1 = intensity diffracted at a definite Bragg angle 2θ by a crystalline component 1
$(I_1)o$ = intensity diffracted at a definite Bragg angle 2θ by pure crystalline component 1

This formula shows that the weight per cent of component 1 is equal to the ratio of the intensities of the diffraction peaks for component 1 in the sample and pure component 1 after correction for absorption by multiplying by a factor *i.e.* the ratio of absorption coefficients for the sample and pure component 1. Leroux, *et al.* (1953) further established the following relationship in order to experimentally determine the absorption correction.

$$\frac{\mu_s^*}{\mu_1^*} = \frac{\rho_1 \log T_s/T'_o}{\rho_s \log T_1/T'_o}$$

where

ρ_1 = apparent density of a pure sample of component 1
ρs = apparent density of a sample contining weight fraction X_1 of component 1
T_o = intensity of incident X-ray beam
T_1 = intensity transmitted by pure sample of component 1
T_s = intensity transmitted by sample containing weight fraction X_1 of component 1

The following machine conditions were used for the X-ray analyses:

X-ray Generator (Philips constant potential 50 KV-50ma)
X-ray Tube (Machlett Cu tube-short anode)
45 KV, 18 ma.
Diffractometer (Philips high angle)
1°/minute scan speed,
1° divergence and anti-scatter slits,
0.006" receiving slit,
Ni filter,
Rotating sample holder-back loaded.

Detector (Philips scintillation-transistorized 52572)
1.0 KV.
Circuit Panel (Philips 12206/53)
PHA, width 9V, level 7V,
linear scale,
1 sec time constant
1 x 10^3 counts/sec (or greater) full scale.
Recorder (Bristol)
30"/hr. chart speed.

Peak intensities were measured from diffractometer charts in counts per second (c.p.s.) by subtracting background from peak height. Intensities for pure mineral components (100 per cent values) were determined empirically. A standard sample (U.N.D. 1901) was used for quartz. An average of two typical optically analyzed perthites from granite (F. R. Karner 238,272) were used for plagioclase and K-feldspar since the feldspars in our unknown samples have been derived from a variety of sources, dominantly granitic. Calcite and dolomite vary widely in crystallinity and grain size both of which strongly affect diffracted intensities. One hundred per cent intensities for calcite and dolomite were selected on the basis of peak width measured at one half peak intensity according to results obtained by Karner (paper in preparation). Results for calcite and dolomite obtained in this way were checked against values of total carbonate obtained by the titration method of Herrin, Hicks and Robertson (1958) assuming approximate calcite-dolomite ratios given by X-ray analysis. Results are given in Table II.

TABLE II
COMPARISON OF CARBONATE DETERMINATION
BY X-RAY AND CHEMICAL METHODS

U.N.D. Sample Number	Chemical Determination (in weight per cent)	X-ray Determination (in volume per cent)		
	Total	Calcite	Dolomite	Total
4213-45'	11	3	6	9
4201-55'	11	5	5	10
4224-75'	14	2	13	15
4201-135'	19	7	11	18
4217-110'	16	13	9	22
4201-100'	28	7	18	25
4206-14'	29	4	23	27
4206-99'	29	13	23	36

X-ray determinations of carbonate have average deviations of less than 3 per cent from chemically determined values. One hundred per cent intensities for total clay minerals were derived from purified clays separated from samples of unit 9 and unit 7. The peaks used for total clay determination are those suggested by Schultz

(1964). The peaks which were averaged to determine mineral percentages and the 100 per cent values are given in Table III.

TABLE III

PEAK POSITIONS AND INTENSITIES USED FOR 100 PER CENT VALUES IN X-RAY ANALYSIS

Mineral	Peak Position (in degrees 2θ)	Intensity Factor (in counts per second)
Quartz	20.9	2400
	26.7	11500
	36.6	810
	39.5	740
	50.2	1420
	60.0	950
K-Feldspar	27.5	2200
Plagioclase	28.0	5000
Calcite	29.4	3200
	Avg. 47.5-48.5	540
Dolomite	31.0	5000
Total Clay	19.9	285
	34.6	180
	61.9	115

Transmitted intensities used to determine absorption values are measured using standard Philips rectangular holders partly filled to known relative density. These were placed in front of the Ni-filter on the diffractometer and held by a specially designed bracket similar to that mentioned by Niskanen (1964). Following Lennox (1957) and Niskanen (1964) the X-ray beam is monochromatized with a sample of ground quartz in the normal sample position using the 26.7° 2θ peak with Cu radiation. X-ray transmission was measured for standard pure mineral samples and used with transmission values obtained for unknown samples to obtain the ratio

$$\frac{\mu_s^*}{\mu_1^*}$$

which is used as the correction for absorption.

Clay mineralogy was estimated from relative intensities of basal peaks of chlorite, kaolinite, illite, montmorillonite and mixed-layer clays in oriented slides X-rayed after each of three types of treatment; humidification by heating to 72°C in a water-saturated atmosphere, saturation with ethylene glycol, and heating to 550°C for one-half hour.

Results of mineralogical analyses are given in Table IV. Reliability of mineralogical compositions determined by these methods has been stated to be about 10 per cent of the amount present by most workers after repeated analyses of artificial mixtures (*e.g.* Shaw and

Weaver, 1965; Schultz, 1964). Our work suggests that this is realistic for minerals of relatively perfect and constant crystallinity (*e.g.* quartz). Comparable reliability for minerals of variable crystallinity can probably only be obtained by using standards which are derived from the material to be studied (*e.g.* separation of clay) or by conducting an independent evaluation (*e.g.* chemical testing for carbonates). Extreme variability of crystallinity of clays allows only semi-quantitative evaluation of individual clay minerals.

RESULTS

Nine lithologic units can be recognized in the 273 feet of drill core and well samples examined in this study. The units are listed below in stratigraphic sequence with the oldest at the bottom:

9. Brown and gray brown silty loam with clay laminae, 20 feet
8. Gray clay with lenses of silty loam, 26 feet
7. Dark gray clay, 37 feet
6. Gray clay with some gravel, 44 feet
5. Dark gray clay, 14 feet
4. Grayish brown sand, 15 feet
3. Dark gray clay, 51 feet
2. Gray clay with some gravel, 34 feet
1. Gravelly clay loam, 32 feet (base not determined).

Mineralogy as determined by X-ray analysis and sand-silt-clay contents are given in Table IV and illustrated in Figure 1. Particle size distributions of the units are given in Table V and illustrated in Figure 2.

Only well cuttings were available for study of units 1, 2 and 3. Unit 1 is a poorly sorted gravelly clay loam with a bimodal particle-size distribution. It contains subangular to subrounded granules and pebbles of a variety of rock types including gray shale (probably Pierre Shale of Cretaceous age), limestone, dolomite, granitic and basaltic rocks. Unit 2 is a gray clay with some gravel similar to that in unit 1. The composition of unit 2 is intermediate to that of unit 1 and unit 3 which is a dark gray clay (Figures 1, 2). Unit 4 was encountered in the test hole for which core samples are available but very little was recovered. It is a grayish brown sand with pebble-size limonitic concretions.

Cores of units 5 through 9 were studied. Unit 5 is a dark gray clay with scattered weathered carbonate pebbles. The lower part is silty. Unit 5 appears to grade upward to unit 6, gray clay with some gravel, by decrease of clay and silt and increase of sand (Figures 1, 2). No bedding was found in unit 6. Subangular to subrounded pebbles and granules similar to those in units 1 and 2 are common.

The top of unit 6 is characterized by an increase in clay and a decrease in sand grading to unit 7 which is a dark gray clay with weathered carbonate pebbles. Faintly visible bedding has been observed only in the upper part of unit 7. The upper part also contains fragments of ostracod valves. It is marked by an increase in soil

TABLE IV

MINERALOGICAL COMPOSITION AND SAND-SILT-CLAY RATIOS OF LAKE AGASSIZ AND ASSOCIATED SEDIMENTS

		Particle Size			Mineral Composition (volume per cent)							
Unit	Sample Depth (feet)	Sand (with some gravel)	Silt	Clay	Quartz	K-feldspar	Plagioclase	Calcite	Dolomite	Total Clay* (with some mica)	Total	Mass Absorption Coefficient ($CuK\alpha$)
9	7.5	3	72	25	27	6	6	1	18	37	95	45
	13.5	3	29	68	14	4	6	3	11	63	101	44
8	25	1	25	74	14	3	4	3	7	75	106	44
	35	1	20	79	18	5	5	1	7	71	108	49
	45	1	9	90	11	2	4	1	4	84	106	48
7	50	1	31	68	8	3	5	2	3	84	105	47**
	55	1	26	73	8	1	3	3	3	89	107	49
	60	1	25	74	8	3	4	4	3	90	111	47**
	65	3	21	76	9	3	3	8	8	72	103	47**
	75	12	28	60	18	5	5	8	15	48	99	48
6	85	25	28	47	26	4	9	8	14	47	104	48
	95	26	29	45	22	4	5	10	27	31	99	47
	100	21	46	33	27	5	7	7	17	43	106	49
	105	28	29	43	29	5	8	10	20	41	113	48
	115	27	28	45	26	5	6	7	14	35	93	43
	125	28	29	43	26	4	8	9	15	39	101	47
5	130	1	38	61	—	—	—	—	—	—	—	—
	135	1	43	56	18	4	6	7	10	61	106	48
4	145	90	5	5	—	—	—	—	—	—	—	—
3	160	6	17	77	8	4	8	6	5	58	89	45
	180	4	24	71	11	5	8	7	6	50	87	47
	190	5	16	79	13	5	7	6	7	63	101	46
2	210	21	17	63	18	4	6	7	4	48	87	47**
1	245	50	14	36	30	7	9	8	11	33	98	48
	270	48	13	39	28	5	10	9	8	44	104	48

*Total clay is approximately 0.5 montmorillonite, 0.4 illite and 0.1 kaolinite.

**estimated

strength which was determined from Soil Exploration Company's logs. The combination of these two factors suggests a drying surface according to Rominger and Rutledge (1952) who have identified and interpreted this surface at Grand Forks, Fargo, and Crookston, Minnesota.

TABLE V

PARTICLE-SIZE DISTRIBUTION (WEIGHT PER CENT) OF LITHOLOGIC UNITS

	Phi-Size	Unit 9		Unit 8		Unit 7		Unit 6		Unit 5		Unit 3			Unit 2	Unit 1	
		7.5 ft.	12.5 ft.	25 ft.	35 ft.	65 ft.	95 ft.	115 ft.	125 ft.	130 ft.	135 ft.	160 ft.	178 ft.	190 ft.	208 ft.	243 ft.	270 ft.
Gravel	-1	0.1	0.3	—	—	—	4.3	2.1	0.1	—	2.3	1.4	—	0.6	3.9	1.1	6.0
	0	0.8	0.9	—	—	0.1	1.3	2.3	2.1	0.1	0.4	1.2	0.6	0.7	4.8	10.1	15.4
	1	0.6	0.6	—	—	0.0	2.5	2.6	2.9	0.3	0.3	1.0	0.5	1.0	3.7	12.3	9.6
Sand	2	0.6	0.4	—	—	0.3	4.0	4.3	4.8	0.4	0.5	0.9	0.7	1.1	3.9	13.4	7.6
	3	0.3	0.3	—	—	0.7	5.1	6.2	6.3	0.5	0.5	0.7	0.5	0.9	2.7	9.2	6.5
	4	0.6	0.4	0.1	0.1	1.0	4.9	6.5	6.3	0.0	4.8	0.5	0.3	0.6	1.6	4.0	3.0
	5	17.5	9.2	0.0	0.7	2.1	7.9	4.6	8.4	3.6	3.6	2.0	1.7	2.2	1.5	3.3	3.4
	6	49.5	32.3	2.9	1.0	2.1	9.3	12.5	8.7	10.7	4.9	1.4	0.6	6.3	2.7	2.2	2.3
Silt	7	10.5	20.6	12.0	12.3	3.4	10.9	7.7	12.1	10.9	8.3	4.8	4.2	0.7	6.1	3.1	3.1
	8	4.3	9.5	15.3	19.9	9.5	12.0	12.6	11.8	12.6	13.9	8.7	7.1	6.4	7.5	4.8	3.7
	9	3.4	9.2	12.9	10.6	15.8	9.2	8.8	7.8	13.3	12.2	10.1	12.6	12.9	7.1	4.6	6.8
	10	2.8	1.6	12.2	12.7	13.6	5.4	6.1	5.6	12.9	10.3	17.7	19.1	20.5	16.7	8.0	8.0
Clay	11**	4.1	7.2	21.9	20.9	24.9	11.3	12.5	11.4	17.6	19.1	23.0	25.1	23.0	20.0	12.3	12.5
	12**	2.6	4.1	14.4	13.9	16.0	6.8	7.0	6.8	10.5	11.8	16.3	17.2	13.4	11.2	6.9	7.3
	13**	1.3	2.2	6.4	5.4	8.0	3.6	3.1	3.5	5.0	5.2	7.6	7.6	6.8	5.6	3.4	3.5
	14**	0.8	1.0	1.9	1.9	2.6	1.5	1.2	1.5	1.5	1.8	2.8	2.3	2.0	1.7	1.3	1.3
Median (phi-size)		6.1	6.8	8.9	9.0	10.1	6.6	6.7	6.6	8.3	8.3	9.2	10.1	9.1	7.1	4.8	4.8

*The phi-size is the negative logarithm to the base 2 of the grain diameter in millimeters.

**Values for these sizes were determined by extrapolating from 10 phi the last point determined by pipette to 100 percent at 14 phi using a straight line plot on arithmetic paper (Folk and Ward, 1957).

Unit 8 is a dark gray clay with thin lenses of gray-brown silty loam. The lenses occur at 1- to 2-foot intervals near the bottom of the unit increasing to 1/2- to 1-inch intervals near the top.

Unit 9 is a gray and brown mottled clay with cross-bedded lenses and laminae of silt and sand. Silt increases and clay decreases toward the top where limonite has stained the sediment and is concentrated in friable elongate concretions up to about 10 cm long. These generally contain tubular cavities up to 1 to 2 mm in diameter. Gypsum occurs in unit 9 as small crystals about 1 mm long disseminated through the sediment and also as partial fillings of cavities up to about 1/2 cm in diameter. Ostracod valves of the genus *Candona* (identified by L. D. Delorme, personal communication) are found throughout the unit.

DISCUSSION

Sediment Types—The nine recognized units can be divided into the following five sediment types:

Type A—unit 1—gravelly clay loam (poorly sorted, bimodal size distribution, angular rock fragments);

Type B—units 2 and 6—gray clay with some gravel (similar to type A but contains less sand and gravel and more clay, poorly sorted, bimodal?);

Type C—Unit 3 and the lower and middle parts of unit 5, 7 and 8—dark gray clay (comparatively well-sorted, containing weathered carbonate pebbles, bedded (in part?));

Type D—unit 9 and the upper parts of units 7 and 8—brown and gray silty loam (comparatively well-sorted to bimodal, interbedded silty and clay layers, contains ostracods and limonitic concretions);

Type E—unit 4—grayish brown sand (contains limonitic concretions).

Types A and B are similar in mineralogical and textural properties and may be gradational. Types B and C are gradational in properties and units of B and C type have gradational contacts in the samples studied *i.e.* unit 2 to 3?, unit 5 to 6, unit 6 to 7 (Figures 1, 2). Type C and D are also gradational in properties and often occur within a single unit which grades upward from type C to D *i.e.* units 7 and 8 (Figures 1, 2).

Origin of Sediments—The sediment types are believed to have originated as follows:

A. Ice deposits (till)
B. Ice-marginal lacustrine deposits (water-laid till?)
C. Relatively deep-water lacustrine deposits (lake clay)
D. Relatively shallow-water lacustrine deposits (interbedded lake silt and clay)
E. Stream deposits (fluvial sand).

Gradations of B and C suggest lateral movement of glacial ice in a lake basin. For example, units 5, 6 and lower part of unit 7 may

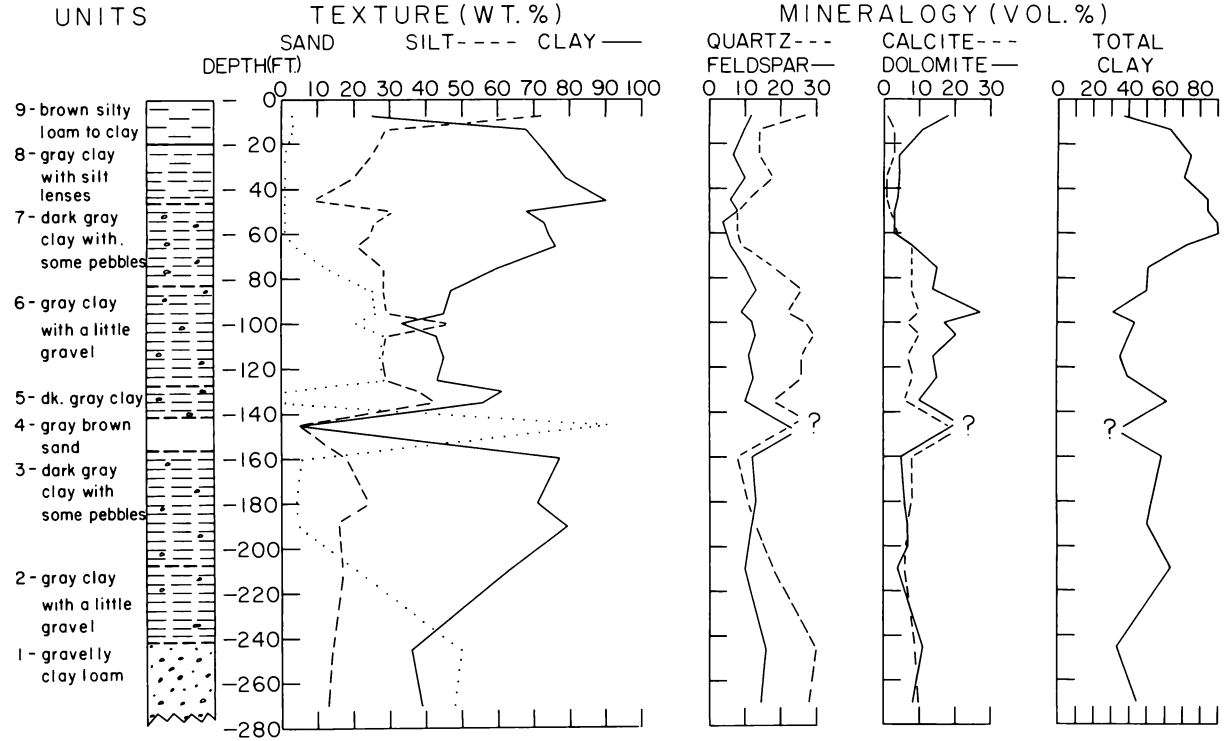


FIGURE 1—Mineralogical composition and sand-silt-clay variation in Lake Akassiz and associated sediments, Grand Forks, North Dakota.

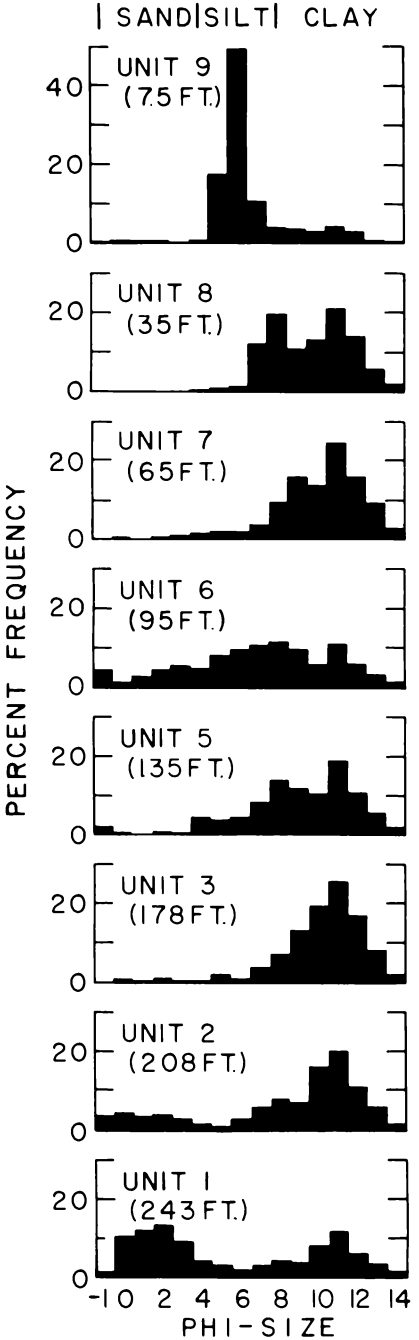


FIGURE 2—Particle-size distribution of Lake Agassiz and associated sediments, Grand Forks, North Dakota.

indicate ice advance into an area of lacustrine deposition and subsequent retreat. Gradations of types C and D may indicate shallowing of a lake. In Lake Agassiz this may indicate a shift of drainage from southern outlets to lower eastern or northern outlets. The bedding characteristics of type D may indicate strong currents in the lake along shorelines or possibly relatively rapid fluctuations in lake level because of fluctuation at the lake's outlet(s).

The well-sorted silt of unit 9 may indicate wind transportation. In this unit the limonite concretions containing tubular holes may have formed around plant stems or roots.

Sediment sources are indicated by the bulk mineralogy of the samples. By fractionation of various particle sizes, till similar to type A could be a source for all of the other sediment types. Type A is approximately equivalent to a mixture of about one-half Pierre Shale (Schultz, 1964) one-fourth granitic Precambrian rocks and one-fourth Paleozoic and Mesozoic limestones and dolomites.

Suggested Future Studies—At this time a complete sequence of events cannot be established for the lithologic units investigated but may be possible after similar studies have been made to determine the horizontal variation of the units and the details of the contacts between units. Part of our work has involved testing of possible methods of study of Lake Agassiz and associated deposits. Of these methods, sand-silt-clay ratios are the most useful for recognizing relationships between lithologic units. Mineralogical determinations support these data and allow considerations of source. Detailed size analyses of major units are also helpful as are hand specimen and thin-section studies. In further work these methods should be included.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the National Science Foundation through the Undergraduate Research Participation Program (Grant GY 375) in which the senior author was a participant during the second semester of the 1965-66 academic year, the summer of 1966 and the first semester of the 1966-67 academic year. Special thanks are due to Wilfred A. Wahl of the Soil Exploration Company, St. Paul, Minnesota, who supplied the core samples used in this study, and L. D. Delorme of the Department of Energy, Mines and Resources, Alberta for his identification of ostracod specimens. We have benefitted greatly from discussions and advice on methods and interpretation of our results with Edward Callender, Dr. Lee S. Clayton, Dr. Alan M. Cvancara, Dr. Wilson M. Laird, Dr. John R. Reid and Chester F. Royse of the Department of Geology at the University of North Dakota and Sidney B. Anderson of the North Dakota Geological Survey.

REFERENCES

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

Folk, R. L. and Ward, W. C., 1957, Brazos River Bar: A study in the significance of grain size parameters: Jour. Sedimentary Petrology, v. 27, p. 27-31.

Herrin, E., Hicks, H. S., and Robertson, H., 1958, A rapid volumetric analysis for carbonate in rocks: Field and Laboratory, v. 26, p. 139-144.

Kane, W. T. and Hubert, J. F., 1963, Fortran program for calculation of grain-size textural parameters on the I.B.M. 1620 computer: Sedimentology, v. 2, p. 87-90.

Krumbein, W. C., and Pettijohn, F. J., 1938, Manual of sedimentary petrography: New York, Appleton-Century Co. Inc., 549 p.

Laird, W. M., 1964, The problem of Lake Agassiz: N. Dak. Acad. Sci. Proc., v. 18, p. 114-134.

Lennox, D. H., Monochromatic diffraction-absorption technique for direct quantitative x-ray analysis: Anal. Chem. v. 29, p. 766-170.

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

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

Rominger, J. F., and Rutledge, P. C., 1952, Use of soil mechanics data in correlation and interpretation of Lake Agassiz sediments: Jour. Geology, v. 60, p. 160-180.

Shaw, D. B., and Weaver, C. E., 1965, The mineralogical composition of shales: Jour. Sedimentary Petrology, v. 35, p. 213-222.

Schultz, L. G., 1964, Quantitative interpretation of mineralogical composition from x-ray and chemical data for the Pierre Shale: U.S. Geol. Survey Professional Paper 391-C, 31 p.

Upham, W., 1896, The glacial Lake Agassiz: U. S. Geol. Survey Monograph 25, 658 p.

COMPARISON OF ERYTHROKINETICS IN MACROCYTIC AND MICROCYTIC ANEMIAS

Dorothy Shiesl Good, W. H. Bhatti, James H. Coffey and Leo J. Schermeister

College of Pharmacy

North Dakota State University, Fargo, North Dakota

ABSTRACT

Erythrokinetics, the body mechanism relating to the manufacture and hemolysis of erythocytes, is of vital interest to diagnostic clinicans in the medical field. A study of the patient's erythrokinetics should be all-inclusive, since there is no existing instrument by which the physiological needs may be measured directly.

Preliminary studies were done in order to determine the normal daily fluctuation possible in hemoglobin, due to hemoconcentration or hemodilution. Investigation of the anemias of patients included hemoglobins, erythrocyte counts, hematocrits, blood cell indices, reticulocyte counts and erythrocyte survival times.

In vitro culture studies were also conducted on the bone marrow speciments, in an attempt to obtain the erythroid:non-erythroid mitotic ratio.

INTRODUCTION

Erythrokinetics is the body mechanism relating to the manufacture and hemolysis of erythrocytes. Erythrohpoiesis is the formation of red blood cells. "Effective erythropoieses" has been described by Finch (6) as representing the number of viable and functional erythrocytes available for physiological needs, reflecting a balance between the number of cells produced and their life-span. "Total erythropoiesis" merely records the total number of erythrocytes, with no indication as to whether an adequate number of cells is being made available.

It is only reasonable that a study of the patient's erythrokinetics be all-inclusive, since there is no existing instrument by which "effective erythropoiesis" can be measured. The cells should be studied from their origin in the mitotic state, through all of the maturation stages, including their survival time.

In the past many causes of anemias have been suggested, (Baldini (1), Carnot (2), Crafts (3), Finch (7), Jacobson (11), Kracke (12), Matoth (13), Neumann (15), Wintrobe (22)) and many have been proven to be valid. However, the numerous factors known to produce anemia have often made it difficult to determine the exact cause. This study was therefore undertaken to compare the erythrokinetics of a marocytic and microcytic anemias.

MATERIALS AND METHODS

Patients at St. Luke's Hospital, Fargo, North Dakota, were selected

whose case histories and peripheral blood smears indicated that there was a problem of a hematological nature. In evaluating the patients' hematological state during the research period, the following tests were performed on all patients.

RETICULOCYTE COUNTS

Bone Marrow—A sternal bone marrow puncture was performed by the pathologist, who aspirated approximately 2.5 milliliters. An aliquot of the marrow was vital-stained for the study of reticulocytes, according to the method of Orten (16), three thousand erythrocytes and reticulocytes were counted, and the percentage of reticulocytes calculated.

Peripheral Blood—Peripheral blood reticulocyte counts were performed, using the same technic, each time venous blood was drawn for the red cell survival study. A comparison of the bone marrow reticulocytes to peripheral reticulocytes was also made in order to establish comparative variations.

RED BLOOD CELL SURVIVAL STUDIES

Sodium chromate Cr^{51} was used to tag the red blood cells, according to Strumia's (20) modification of the method of Gray and Sterling (8). In this study polythylene centrifuge bags[1] were used as containers for tagging the red blood cells. Fifty microcuries of sodium chromate Cr^{51} were administered to Patients 1, 2, 3, and 5. Patient 4 received 300 microcures of Cr^{51} to enable the physician to check the isotope level in the stool. The exact time of injection was noted, so that the blood sample representing 100 per cent of tagged red cells could be drawn exactly twenty-four hours later, since there should be no sigificant red cell-fatality within that period. Subsequent specimens of venous blood were withdrawn at intervals during the next ten to thirteen days, depending on the patient's availability for testing. Five milliliters of each sample of blood were scanned simultaneously by a scintillator[2] and the counts per second recorded for use in the calculations. The survival time was plotted on semi-log paper, and the erythrocyte half-life determined for each patient.

RED BLOOD CELL INDICES

It was deemed advisable to calculate the red blood cell indices to determine if a change in the cell size and/or hemoglobin content was occurring during the test period. The indices were calculated according to the method of Wintrobe (22). The hemoglobin determinations were performed in triplicate. Drabkin's (5) modification of the Sanford and Sheard (17) technic was used. The micro-hematocrit values were determined in triplicate, using the method of Hamre (9).

Erythrocyte counts were done in duplicate, using an electronic

[1]Unitag Centrifuge Bag, Abbott Laboratories, North Chicago, Ill.

[2]Scintillator Model AS-III, Curtis Nuclear Corporation, Los Angeles, Calif.

particle counter[3] and the Coulter technic (10). Each diultion of the cells in saline was counted at least twice to assure accuracy. Various threshould levels were checked during the counting, which insured the inclusion of the smaller cells. A check of the patients' leukocyte counts indicated that they were not present in sufficient number to affect the erythrocyte count significantly.

BONE MARROW DIRECT SMEARS FOR MITOSIS

Immediately after aspiration of the marrow, some of the fresh smear preparations were fixed with methanol. Trujillo and Ohno's (21) method of benzidine:hydrogen peroxide staining was applied to the slide, followed by Wright's staining. The smears were examined for mitosis, as described by Snyder (19), the identification of the mitotic figures of erythropoietic elements being based on the high sensitivity of benzidine for hemoglobin. A mitotic figure was classified as erythroid only if the cytoplasm showed sufficient hemoglobinization so that its characteristics were unequivocally those of a normoblast. All other cells in mitosis were classified as non-erythroid. The number of mitotic figures in three smears from each patient's bone marrow were recorded.

BONE MARROW CULTURE STUDIES IN VITRO

The method used for the bone marrow culture was essentially that of Trujillo and Ohno (21). After the cells were arrested with colchicine, they were subjected to a hyptonic treatment with warm distilled water, which enhanced the swelling. The cell mass was then exposed to methanol, acetic acid fixatve, to prevent distortion of the chromosomes. Following smear preparation and staining with Wright's stain, the slides were examined to determine the number of erythroid cells in mitosis as compared to myeloid cells in mitosis.

DETERMINATION OF HEMOCONCENTRATION OR HEMODILUTION

Preliminary studies were done by the author to determine the normal daily fluctuation possible in hemoconcentration or hemodilution. All factors were kept as constant as feasible. The donor received no medication for three days prior to and during the time of testing, and the diet was essentially normal. She had no symptoms of hypoxia or bleeding. Blood was collected for hemoglobin analysis by both venous and capillary technics twice daily. Three analysts performed the tests each time, to ascertain if any variation was due to personal error, or to difference within the donor *per se*. Drabkin's (5) modification of the Sanford and Sheard (17) technic was used.

RESULTS AND DISCUSSION

Erythrokinetic studies were performed on five patients. Patient 1 had no evidence of a hematological disorder. Patient 2 showed a macrocytic anemia with symptomology and diagnostic tests (hemo-

[3]Coulter Counter, Model A, Coulter Electronic Sales Corporation, Hialeah, Florida.

globin, leukocyte differential, red blood cell mophology) pointing to an untreated pernicious anemia. A severe microcytic anemia with rheumatoid arthritis complications was noted in Patient 3. The hematological pictures of Patients 4 and 5 appeared to be those of simple iron-deficiency anemias.

Table I lists the results of the preliminary studies of the normal daily variation of hemoglobin which are possible, due to hemoconcentration or hemodilution. During the three-day period of testing, there was a fluctuation of two grams per cent. It was shown that, all other factors being constant, a person's hemoglobin may vary at least two grams from day to day, without any known pathological condition being present. This is attributed to variation in fluid intake, electrolyte intake, loss of fluid through evaporation from the skin, the patient's activity, and other physiological factors.

The results of the erythrokinetic studies are discussed according to the individual tests.

RETICULOCYTE COUNTS

Patient 1 had a peripheral blood reticulocyte average of 3.43 per cent, compared to the bone marrow reticulocyte count of 3.4 per cent. According to Baldini and Pannacciulli (1), this slight elevation of the peripheral count may be due to a delayed maturation rate, increased numbers of younger reticulocytes with a longer life-span, or a delayed disappearance rate in the reticulum substance. Normal values for the bone marrow, as stated by Finch (6) are 1.5 reticulocytes to 1.0 peripheral reticulocytes. Miale (14) lists the normal adult peripheral reticulocyte range as 0.5 to 1.5 per cent of erythrocytes.

The values of Patient 2 were 1.2 per cent bone marrow reticulocytes, and an average of 1.4 per cent peripheral blood reticulocytes. Since the bone marrow exhibited erythroid hyperplasia, this would appear to be a prolonged maturation time, as described by Baldini and Pannacciulli (1) in pernicious anemia. No variation was noted during the ten-day interval in Patients 1 and 2.

Patients 3 and 5 appear to have a normal ratio of bone marrow reticulocytes to peripheral reticulocytes, with no fluctuation during the testing period. It is interesting to note the rise in the circulating reticulocytes of Patient 4. Two days after the baseline reticulocyte count was established, there was a significant rise of 0.7 per cent. This leveled off approximately to the original level by the fifth day. It was learned that the patient had received intravenous iron injection the day prior to the base-line count, and this was evidence of the bone marrow's response to therapy.

RED BLOOD CELL SURVIVAL STUDIES

The results of the radioactivity scanning of the blood samples are graphically depicted in Figure 1. The values for normal erythrocyte half-life times listed by the isotope laboratory where the testing was performed is 27 to 36 days. The erythrocyte half-life of Patient 1 was

33.5 days. Studies on Patients 2 and 3 revealed hyperhemolysis, with their red cells having a half-life of 19.6 days and 22.5 days, respectively. The results of the survival time of Patient 2, whose hematological picture is that of a macrocytic anemia, parallels the findings of Singer *et al.* (18), who revealed that the red cell survival studies of patients with pernicious anemia have shown an average destruction rate of three times the normal rate. Patient 4 had an erythrocyte survival time decreased to such a small degree that it is probably not pathological. The results of the Cr^{51} test in Patient 5 were found to be in the range of normal values.

TABLE I

QUANTITATION OF HEMOGLOBIN USING DIFFERENT PERSONNEL AND VARYING TIMES OF BLOOD COLLECTION

	Analyst "A" Grams per cent		Analyst "B" Grams per cent		Analyst "C" Grams per cent	
TIME	Finger-stick	Venous	Finger-stick	Venous	Finger-stick	Venous
10:30 a.m 3-29-65	13.0	13.1	13.0	12.8	12.8	13.1
4:30 p.m. 3-29-65	12.5	12.0	12.0	11.9	12.2	11.8 (low)
10:30 a.m. 3-30-65	12.6	12.7	12.7	12.7	12.6	12.9
4:30 p.m. 3-30-65	12.7	12.3	12.2	12.4	12.7	12.5
10:30 a.m. 3-31-65	13.7	13.6	13.6	13.7	13.5	13.6
4:30 p.m. 3-31-65	13.8 (high)	13.7	13.6	13.7	13.5	13.5

DETERMINATION OF HEMOCONCENTRATION OR HEMODILUTION

The results obtained by determinations of the hemoglobin, erythrocyte and hematocrit values of the blood samples collected at the same intervals at which the red blood cell survival specimens were drawn are relatively constant. The hemoglobins of all five patients appear to be within the normal daily variation as determined by the preliminary work. The erythrocyte counts parallel the hemoglobins and hematocrits fairly well.

RED BLOOD CELL INDICES

Using the normal values listed by Davidsohn and Wells (4), the red blood cell indices were studied according to their individual measurements. The normal range for the mean corpuscular volume (MCV) is 80 to 94 cubic micra. It is important to note that the aver-

age MCV of 145 cubic micra of Patient 2 is indicative of macrocytosis. A brisk deviation in the opposite direction was found in the microcytosis of Patients 4 and 5, with mean values of 66.5 and 69.5 cubic micra, respectively. Patient 3 had an average MCV of 88.3 cubic micra. Of the patients studied, only Patient 1 had a normal mean corpuscular hemoglobin (MCH), an average of 29.2 micromicrograms. The nomral MCH range is 27 to 32 micromicrograms. Patient 3 had a borderline MCH of 26.9. Patients 4 and 5, with MCH values of 17.3 and 18.7 micromicrograms, are in the iron-deficiency class as described by Miale (14), with a reduction in the hemoglobin concentration being proportionately greater than the reduction in the red blood cells. The normal range of the mean corpuscular hemoglobin concentration (MCHC) which takes into account the cell size is 32 to 38 per cent. Although all five patients displayed some hypochromia, the only clinically significant cases were those of Patient 4 (26.2 per cent) and Patient 5 (26.8 per cent).

DIRECT SMEAR MITOTIC STUDY

Most of the cells were found in metaphase or anaphase, excluding the interphase, of course. Table II depicts the total number of mitotic cells classified on examination of each patient's direct bone marrow smears, as well as the ratio of the erythroid cells to the non-erythroic mitotic cells.

TABLE II

RATIO OF ERYTHROID MITOTIC CELLS TO NON-ERYTHROID MITOTIC CELLS FOUND ON DIRECT SMEARS OF BONE MARROW

	Total Mitotic Cells	Erythroid:Non-Erythroid Mitosis	Mitosis
Patient 1	15	4	1
Patient 2	41	9	1
Patient 3	21	6	1
Patient 4	18	7	2
Patient 5	18	5	1

The was not the abundance of cells in the mitotic stage that had been anticipated. Of the five patients, Patient 2 had the greatest ratio of erythroid mitotic cells to non-erythroid cells (9:1). Since the total of less than 25 mitotic cells were found on examination of the smears from Patients 1, 3, 4, and 5, it is felt that no significant conclusion can be drawn concerning their relation to the patients' clinical status. Further work is suggested in this area.

BONE MARROW CULTURES

Of the five bone marrows which were cultured, cells in mitosis were found only in the culture of Patient 2. It is assumed that death of both the erythroid and myeloid series was due to the change in

environment, since the cells *in vivo* of the same patients, when studied by the direct smear technic, exhibited the various stages of mitosis.

The cytoplasm which differentiates the erythroid from the non-erythroid series was destroyed during the process, making identifica-

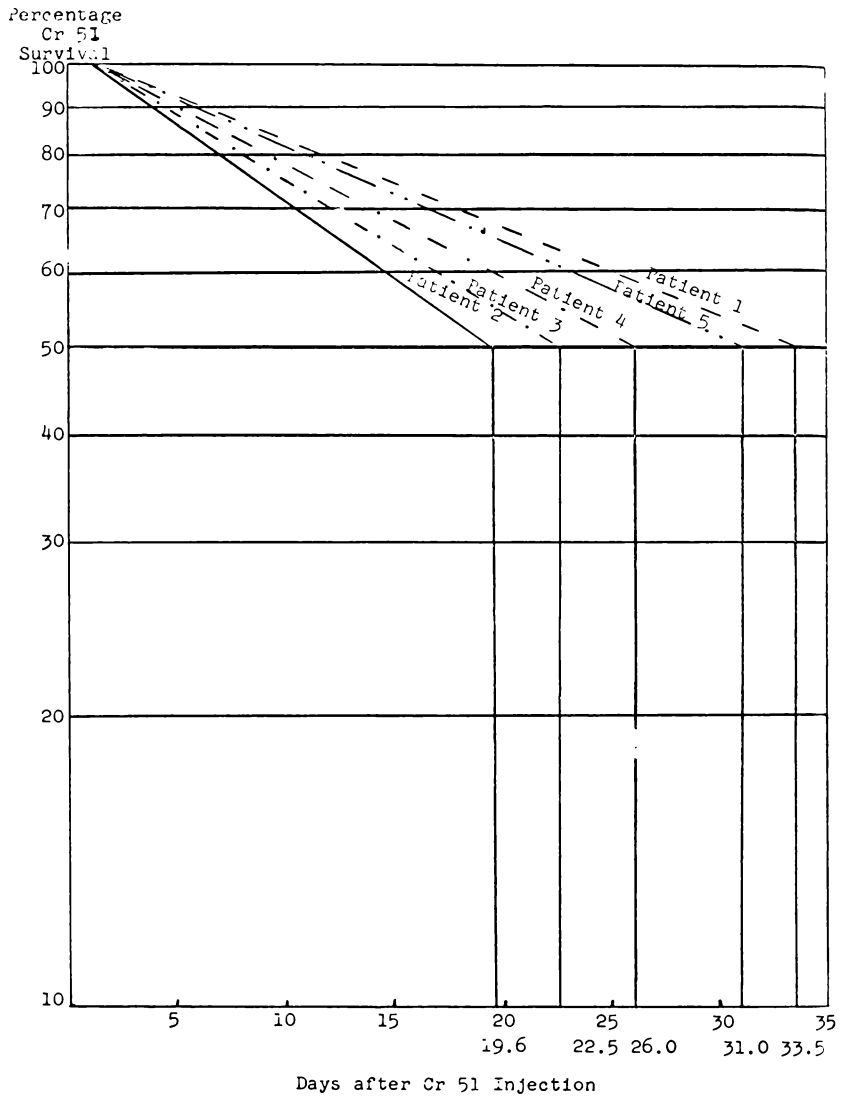


FIGURE 1—Erythrocyte Survival Half-Life of Patients Studied

tion of the series impossible. Many modifications of this procedure were tried, but all met with the same negative results concerning identification of the series of the cell from which the chromosome had come. The data obtained from this procedure are insufficient to be of consequence.

SUMMARY

The anemias of four patients have been investigated, and compared to a patient exhibiting no hematological disorder. On the basis of the findings in this study, in addition to symptomology, it is possible to confirm the diagnosis of pernicious anemia in Patient 2. This conclusion is justified by the following deviations from normal: severe macrocytosis; decreased erythrocyte survival; decreased hemoglobin; acutely decreased erythrocyte count; slight increase in bone marrow mitosis, indicating stimulation to stress; and fairly consistent hemoglobin, erythrocyte, and hematocrit levels.

Results of the investigation indicate that Patients 3, 4, and 5 have anemias based on iron-deficiency. This conclusion is supported by the findings of: hypochromia; microcytosis; slightly decreased red cell half-life; normal ratio of bone marrow reticulocytes to peripheral reticulocytes; consistently stable elevated erythrocyte counts; and fairly constant hemoglobin hematrocrit levels. Patient 4 also exhibited a brisk elevation of peripheral reticulocytes, followed by a slight rise in the erythrocytes and hematocrit, after intravenous iron therapy.

In vitro cultures were also prepared on all five bone marrows in an attempt to obtain the erythroid:non-erythroid mitotic ratio. Due to the loss of the cell cytoplasm, it was impossible to differentiate between the mitotic figures of the two series. In view of this difficulty, the present methods would have to be improved to obtain satisfactory results.

The preliminary studies done to determine the normal daily fluctuation possible in hemoglobin due to hemoconcentration or hemodilution revealed that, all other factors being relatively constant, a person's hemoglobin may vary at least two grams from day to day, without any evidence of a pathological condition being present.

The findings of normal erythrokinetics may be as important as those of pathological erythrokinetics, since they are of value in the elimination of diagnosis of specific blood dyscrasias. With a more inclusive picture of erythrokinetics, more effective therapy may be administered.

REFERENCES

1. Baldini, *et al.*, *Blood—The Journal of Hematology*, 15, 614 (1960).
2. Carnot, P., and Deflandre, C., *Comptus Rendus*, 143: 432 (1906).
3. Crafts, Roger C., and Meincke, Howard A., *Annuals New York Academy of Sciences*, 77, 501 (1959).

4. Davidsohn, Israel, and Wells, Benjamin B., *Todd-Sanford Clinical Diagnosis by Laboratory Methods,* Thirteenth Edition, Philadelphia: W. B. Saunders, 1962, p. 94.
5. Drabkins, D., *American Journal of Medical Sciences,* 217, 710 (1949).
6. Finch, Clement A., *Annals of New York Academy of Sciences,* 77, 501 (1959).
7. Finch, C. A., Coleman, D. H., *et al., Blood—The Journal of Hematology, II.*
8. Gray, S. J., and Sterling K., *Journal of Clinical Investigation,* 29, 1604-1614, (1950).
9. Hamre, C. J., *Journal of Laboratory and Clinical Medicine,* 25, 547-551 (1940).
10. Henderson, Floyd, Coulter Electronic Corporation, *Personal Communication* (1961).
11. Jacobson, Leon O., Goldwasser, Eugene, *et al., Annals New York Academy of Sciences,* 77, 553 (1959).
12. Kracke, R. R., and Garver, H. E., *Diseases of the Blood and Atlas of Hematology,* Second Edition, Philadelphia: J. B. Lippincott Co. (1941).
13. Matoth, U., and Ben-Porath, E., *Journal of Laboratory and Clinical Medicine,* 54, 722 (1959).
14. Miale, Jonn B., *Laboratory Medicine—Hematology,* Second Edition, St. Louis: C. V. Mosby Co. (1962), pp. 522, 490, 491, 425, 323, 492.
15. Neumann, E., *Zentralblatt für die Medizinschen Wissenschaften,* 6, 689 (1868).
16. Orten, J. M., *Yale Journal of Biology and Medicine,* 519 (1934).
17. Sanford, A. H., and Sheard, C., *Journal of Laboratory and Clinical Medicine,* 15, 483-489 (1930).
18. Singer, K., King, J.C., *et al., Journal of Laboratory and Clinical Medicine,* 33, 1068 (1948).
19. Snyder, Lawrence H., *The Principles of Heredity,* Boston: D. C. Heath Co., 4th Edition, 1951, pp. 26-31.
20. Strumia, M. M., *American Journal of Clinical Pathology,* 24, 260 (1954).
21. Trujillo, Jose M., and Ohno, Susumu, *Acta Haematologica,* 29, 311 (1963).
22. Wintrobe, Mawell M., *Clinical Hematology,* Fifth Edition, Philadelphia: Lea and Febiger (1946).

A THEORETICAL SCHEHMA OF PINEAL FUNCTION

R. E. Wiederanders, S. M. Hirsch and G. W. Evans

Harmon Park Research Laboratory, Williston, North Dakota

The basic problem we are attempting to explain is the fact that the pineal gland is involved in the rat's female sex cycle, but is not indispensable: pinealectomized (PND) animals can have babies and raise them like normal rodents, but they have prolongation of their estrus period, ovarian changes, and a decreased birth rate. In this laboratory we have noted about a fifth as many babies from PND animals as from their sham operated controls.

The review that follows is not complete, touching only on the data germain to the thought and if a more compendium is desired we suggest the recent one by Relkin (1). Nor are these data as cut and dried as they may seem for the work surrounding the pineal gland is still highly controversial and most of the ideas quite unsettled. The pineal gland is a cyclic organ in the female, connected to the lateral eyes by way of the sympathetic nerves and thus to the light-dark or circadian cycle (2). Light stimulates 5-hydroxytryptophan decarboxylase activity to produce serotonin (3) whose midday level averages about 90 nanograms per pineal. With the onset of darkness, serotonin is depleted at the rate of 25 nanograms per hour to a midnight low of ten nanograms per gland (4). This decrease is partly accounted for by conversion of serotonin to N-acetylserotonin and secondarily O-methylation of N-acetylserotonin to melatonin via an enzyme peculiar to the pineal gland (5): hydroxyindole-O-methyl transferase (HIOMT). Thus melatonin is the peculiar product of this gland (2). Serotonin production continues to build up and decline according to the circadian cycle in blinded rats and those kept in constant darkness, but this rhythm is abolished by constant light (6). Darkness stimulates the activity of HOIMT and thus the production of melatonin while light suppresses it (17). This is the situation in the rat, a nocturnal animal, but in the hen, a diurnal animal it is exactly reversed (8) ,serotonin being produced at night and melatonin during the day. Another cycle, not clearly as distinct, is hinted at in Quay's (4) report where a small increase in the pineal gland serotonin was found in the morning hours between the second and third day of the estrus cycle.

Not only the female rat's serotonin-melatonin rhythm is abolished but the weight of their pineal glands (9), irrespective of the state of the other endocrines (10), their body and adrenal weights (11), and their ovarian weights (12) are also decreased, if the animals are subjected to constant light for nine to ten weeks. They develop continuous estrus and have many well devoloped follicles in their ovaries but no corpora lutea. The change in adrenal weight is not due to increased catabolism of the corticoids as shown by finding the same

half-life of injected corticoids for animals under light-dark and continuous light conditions; but the continuous light group fail to show the three-fold afternoon increase shown by the light-dark animals, indicating an alteration in corticosteroid production. Constant light inhibits the uptake of tagged melatonin by the ovary which takes it from the blood stream and concentrates it (13). Melatonin (14) and cervical sympathectomy oppose the effect of four weeks of constant light (15), decreasing the incidence of estrus from 85 to about 38 per cent.

The effect of prolonged (9 to 10 weeks) constant light and PND on the estrus cycle have been compared, but in reality are different. Prolonged light leads to constant estrus and absent diestrus while PND only prolongs estrus. Their effects on the ovary are also at variance. A great deal of work has been gathered by Kitay and Altschule (16) showing statistically significant increase in ovarian size following PND and an increase in the numbers of corpora lutea in the examined ovaries. This is confirmed by more recent work showing enhanced ovarian growth in the PND rat (17) while melatonin injection is associated with inhibition of both ovarian and pituitary growth and increased concentration of pituitary leutinizing hormone in the young rat (18).

Let us turn now to a consideration of the ovary and some of the mechanisms which regulate it. It is directly under the control of the pituitary gonadotrophins which are three in number in the rat: follicle stimulating hormone (FSH), leutinizing hormone (LH) and prolactin. Their primary function has been worked out with purified preparations in the hypophysectomized rat. FSH acts directly on the egg to bring about formation and maturation of the follicle and increase in ovarian weight. LH sustains the ovarian interstitial tissue, acts with FSH to cause estrogen production, and in a ratio of FSH: LH-10:1, brings about ovulation. Prolactin is triggered by coitus and is required in the rat for the production of progesterone by the corpus lutem.

The progress of the estrus cycle is judged by the type of cells being desquamated from the vagina; the cycle divided into four phases on this basis. A very brief review may be advantageous here. At the start of proestrus, the pituitary elaborates FSH, the egg is brought to maturity, the follicle formed and estrogen is released, aided by LH. Under the influence of the increasing quantities of estrogen the vagina shows cornified epithelium characteristic of estrus and the pituitary increases the LH output to the point where ovulation occurs, ushering in metaestrus. The empty follicle fills with yellow fat cells forming the corpus lutem which secretes progesterone under stimulation of prolactin. If pregnancy occurs the corpus lutem and associated progesterone persist and FSH is inhibited. If not, the corpus lutem degenerates, progesterone decreases and diestrus ensues to release FSH and begin the cycle anew.

But this is not the whole story, for the primary control of this cycle lies not with the pituitary but with the hypothalamus, and apparently quite discreate areas within it. Electrolytic lesions in the pre-optic area (POA) in both rats and guinea pigs give rise to persistent estrus and polycystic ovaries without corpora lutea (19, 20, 21). Direct stimulation of this area will induce ovulation in the normal rat (22) as will injections of luteunizing hormone release factor (23), but not in an animal treated with small doses of testosterone at birth. It is sensitive to internal influences as well as to external light. The effect of prolonged light on the ovary, mentioned above, can be blocked by optic enucleation and by producing "small midline lesions at the level of the suprachiasmatic nuclei" which apparently conduct light stimulation to the POA; however, destruction of the lateral hypothalamus area does not block the effect of light on the estrus cycle (24). Other work (25) shows an anterior hypothalamus center that regulates FSH, for estrogen inhibits FSH production to a lesser degree in rats with lesions placed in the hypothalamus than in normal rats.

Giving 0.5 to 1.25 mg of testosterone in oil to female rats at birth (26) suppresses the POA and produces small, cystic ovaries without corpora lutea. Ovulation can be forced in these animals if stimulation of the arcuate-ventromedial nucleas (AVN) is done after, but not before, injection of progesterone. These authors feel this is a result of relative exhaustion of LH in the pituitary which repairs itself during the respite. They postulate the AVN tonically drives LH production but is stimulated to produce an "ovulatory surge" at the time of ovulation by the POA (25).

Since the POA inhibits FSH production, decycling it with testosterone at birth would release FSH inhibition and secondarily increase estrogen. Estrogen increases adrenal activity so hypertrophy and increase in blood corticosteroids would be expected, and these have been found in the rat 120 days after testosterone injection (27).

These data suggest a "hormonostat" (28), and while its authors have not assigned it to an exact location it could well be the same area as this POA. It is feminine at birth with cyclic activity and sensitive to both female and male hormones. In the normal state as long as only female hormones act on this area (female) the female cycle is established; but if by the "critical" tenth day, testosterone acts here (male), the acyclic pattern is established.

The final necessary concept has been borrowed from Yates and Urquhart (29). Their theory explains the regulation of the adrenal corticoids in the blood, incorporating a closed loop feed-back circuit with a set point against which the controlled variable may be compared and therby regulated by the contorl mechanism. This point is tentatively located in the hypothalamus. There are four types of controllers that may act to rectify the error between the set point and the controlled variable: only the simplest or "off-on" control need

be used here. When the difference between the variable and the set point becomes great enough, the controller is activated to repair the difference.

In combining these data into Figure 1 we have made several assumptions.

1. That a center exists in the hypothalamus that tonically drives the secretion of FSH, to explain the tonic output of FSH in those instances where the POA is deactivated.
2. That the FSH and LH secretion levels correspond to the estrus changes as displayed in the vagina.
3. That diestrus and proestrus appear distinct on vaginal histology but we believe they are two phases of proestrus. For proestrus is the time when FSH is stimulating the egg, and since FSH is tonically secreted there is not really a period of rest or diestrus, but rather a period of relatively low estrogen production at the beginning stimulation of the egg which begins immediately after metaestrus. Therefore, a prolonged proestrus in indistinguishable from prolonged diestrus. Diestrus has been retained but indicates the early phase of ovarian stimulation.

Observing one normal estrus cycle using these facts we begin with the animal in proestrus and the pituitary secreting FSH, driven by "X" (Figure 1). At the same time the AVN is tonically stimulating LH but always at a level that results in an FSH:LH ratio of greater than 10:1 (30), for at this ratio ovulation may occur. Thus there is continuous estrogen production which increases as the follicle is brought to maturity. When the ovary has established a sufficient, critical level of estrogen, the POA is triggered to stimulate the AVN and bring about the "ovulatory surge" (25) of LH, bringing the FSH: LH to 10:1, releasing the egg and stimulating corpus luteum formation. The POA also inhibits the "X" region at this time to suppress further FSH, while prolactin, triggered by coitus, excites progesterone production from the corpus luteum (30). What progesterone does is uncertain. It may suppress the AVN and/or the POA to bring on metaestrus. When progesterone declines sufficiently, the tonic action of FSH is again exerted and the cycle begins anew.

Attempting to fit the pineal gland to this schema several approaches were tried. It was first put directly into the cycle, saying for example that estrogen stimulates the pineal which in turn stimulates the POA to initiate the cycle. But if this were so pinealectomy would result in sterility. A second action was suggested by the concentration of melatonin by the ovary (2). If this were its only site of action (since it decreases the organ's weight it is considered a depressant) it would have to either block or inhibit the output of estrogen or progesterone or the action of the gonadotrophins. Consider first the effect of diminished estrogen output. This would increase the time necessary for the estrogen build-up to initiate estrus. This

would prolong diestrus but it would also prolong proestrus and subject the ovary to a longer action of FSH and LH with overproduction of follicles and subsequent enlargement while a decreased ovarian size is reported in this instance (18). Conversely, PND would now have as its only action that of releasing the restriction on estrogen production which would build up more rapidly, trigger the POA in a shorter time, etc. The ratio of estrus to diestrus would be preserved and estrus would not be prolonged.

Blocking or decreasing the gonadotrophin action on the ovary would result in a smaller ovary with melatonin administration and a larger one after PND as would be expected.2 Melatonin would diminish the output of estrogen, increasing both the diestrus and proestrus periods. Chu *et al.* (14) noted lengthening of the diestrus phase in relation to the estrus phase which would appear to coincide with our scheme. But they lumped the proestrus and estrus phases together, so, if the action of melatonin were only to block the gonadatrophin action, we would expect them to find both the estrus and diestrus phases prolonged. Therefore, we conclude that this is not the only site of melatonin action as this would not alter the time ratio of diestrus to estrus.

Another approach was sought: thus the addition of the pineal gland to Figure 1 and the lines connecting it to the ovary and POA Now we see the FSH being elaborated and the egg responding to form the follicle and estrogen being produced. When the estrogen level is high enough the POA will activate and as long as it is functioning at all an estrogen level will finally be reached that will cause it to act. But we postulate that the pineal can cause it to react to a lower level of estrogen and it may work in this way; when the follicle is ready to ovulate, it signals, perhaps by nervous stimuli, to the pineal which produces a spurt of melatonin, lowering the threshold of the POA to estrogen which then fires to activate the AVN and inhibit region "X". By assuming this POA to be sensitive to the amount of melatonin the pineal is placed in a position to regulate the lengths of the phases of the cycle. If it is active then melatonin is increased and the POA discharges at a lower level of estrogen. This does not affect diestrus, for the same time is required for the egg to begin to form a follicle, but it shortens estrus as the follicle would just barely ripen when the POA would respond. Conversely, if the pineal is absent and the level set high, this again does not alter diestrus but it does force the ovary to take time to generate a higher level of estrogen before the POA will respond and thus the estrus phase is increased in length and degree.

To digress for a moment, there appear to be two related but quite different phenomena: those centered about the POA itself and those affecting the POA. In the first group we place electrolytic lesions of the POA, neonatal tetsosterone administration and prolonged continuous light. Each of these leads to the same condition; failure of the

POA with secondary stimulation of FSH, increased estrogen, persistent estrus and small, polycystic ovaries. The ovarian changes resulting from the action of an increased FSH to LH ratio can bring the follicle to maturity but cannot release the egg or form corpora lutea. Since the male has already decycled his POA with neonatal testosterone, we would not expect him to react to continuous light in the same manner. Fiske (11) noted that continuous light was associated with an absolute decrease in adrenal gland weight in the female but not in the male rat. The second group includes such situations as intermediate light exposure, PND and melatonin administration.

Returning now to the discussion of the theory, let us see how well it explains the findings of intermediate light exposure. Since the effect of light on the POA is cumulative the pineal would be nearly inactive after four weeks of continuous light, explaining the decreased uptake of tagged melatonin. The gland is producing less melatonin and the POA is relatively insensitive to estrogen. Estrogen increases causing prolonged estrus, diminished diestrus and increase in ovarian size, both due to increase in gonadotrophins and the formation of rare but significant corpora lutea. Melatonin administration sensitizes the POA with ovulation and progression of the rest of the cycle and a return to the normal length of the next estrus phase. Animals in prolonged light may respond to melatonin in the same fashion as their POA would probably be intact.

If the pineal gland is excised the animal is in essentially the same condition as the animal in intermediate light exposure. The POA is again set at a high level but can still respond and when the estrogen reaches a sufficient level it will react, suppress the gonadotrophins and make pregnancy possible but less frequent. Thus without the intrusion of the pineal at the critical moment we have a desynchronization between the ovarian and the hypothalamic cycles.

During pregnancy the pineal apparently plays a very slight role as its weight decreases at this time (31).

We have assigned to melatonin the role of setting the POA as it is influenced most strongly by rhythmical light and is the unique product of the gland. It may not be this hormone that is responsible; other hormones could be implicated, e.g., serotonin. This produces no effect on the ovary (15) although it does increase the adrenal weight. Bovine pineal gland extracts inhibit the stimulatory effect of human chorionic gonadotrophin and human menopausal gonadotrophin (32), and both ubiquinone (33) and lysine vasotocin (34) have been isolated from the gland. This organ is complex and there may be yet unknown substances responsible for its action.

As pineal atrophy occurs under the same circumstances (prolonged light) that decycle the POA, this suggests the POA as the pineal driver, that which causes the perpetuation of the circadian serotonin-melatonin cycle under conditions of constant dark. When

exposed to the light-dark cycle the POA would implicate itself. The temperature cycle shows these same characteristics of persistence but gradual loss of phase in continuous dark, and disruption on total light (35).

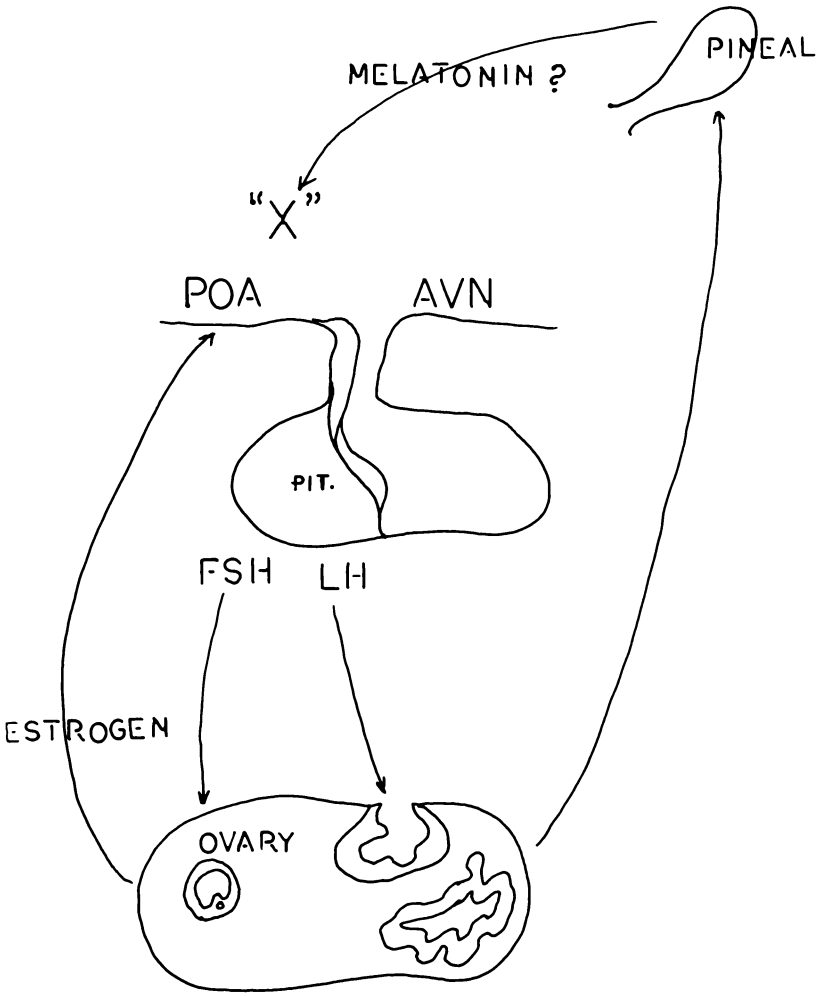


FIGURE 1—Presents a schematic of the presented theory. Both "X" and the AVN exert a tonic stimulatory effect on their respective gonadotrophins and are regulated by the POA. The "set point" directed by the pineal gland determines what level of control the POA will exert and when it will allow ovulation.

SUMMARY

The pineal is the exclusive site for melatonin production which is concentrated in the ovary and presumably affects it.

Continuous light of four to five weeks duration and pinealectomy (PND) produce similar effects on the estrus cycle which are opposed by melatonin.

Prolonged continuous light produces constant estrus and cystic ovaries without corporea lutea while PND does not show this effect.

FSH stimulates the ovary to form the follicle.

LH acts synergistically with FSH to stimulate estrogen output, cause ovulation and secondary progesterone elaboration.

These hormones are regulated in their output by discrete areas in the hypothalamus, currently assigned names of "X," pre-optic area (POA) and arcuate-ventromedial nucleus (AVN).

If melatonin acted only on the ovary it would produce the changes in ovarian weight but not the relative changes in lengths of the parts of the estrus cycle that have been reported.

To obviate this difficulty of timing the cycle, the pineal gland has been assigned the role of sensitizing the POA. This allows the gland to prolong the estrus phase without affecting the diestrus phase, by decreasing its activity, and to accomplish the opposite effect by increasing its activity.

ACKNOWLEDGEMENTS

We thank John Davidson, Jr., for preparing the illustration.

REFERENCES

1. Relkin, R., New England J. Med., 274, 944 (1966)
2. Barchas, J. D., A. B. Lerner, J. Neurochem., 11, 489 (1964)
3. Snyder, S. H., J. Axelrod, J. E. Fisher, R. J. Wurtman, Nature 203, 981 (1964)
4. Quay, W. B., Gen. Comp. Endocr. 3, 473 (1963)
5. Axelrod, J., P. D. MacLean, W. R. Albers, H. Weissbach, Regional Neuro-Chem. 161, 307 (1961)
6. Snyder, S. H., M. Zweig, J. Axelrod, J. E. Fisher, Proc. Nat. Acad. Sci. U.S.A., 53, 301 (1965)
7. Wurtman, R. J., J. Axelrod, L. S. Phillips, Science 142, 1071 (1963)
8. Axelrod, J., R. J. Wurtman, Nature 201, 1134 (1964)
9. Fiske, V. M., G. K. Bryant, J. Putnam, J. Endocrinology 66, 489 (1960)
10. Fiske, V. M., J. Pound, J. Putnam, J. Endocrinology 71, 130 (1962)
11. Fiske, V. M., H. H. Lambert, Endocrinology, 71, 667 (1962)
12. Fiske, V. M., S. E. Leeman, Ann. New York Acad. Sci. 117, 231 (1964)
13. Wurtman, R. J., J. Axelrod, L. F. Potter, J. Pharmacol. Exp. Ther. 143, 314 (1964)
14. Chu, E. W., R. J. Wurtman, J. Axelrod, Endocrinology 75, 238 (1964)

15. Wurtman, R. J., J. Axelrod, E. W. Chu, Ann. New York Acad. Sci. 117, 228 (1964)
16. Kitay, J. I., M. D. Altschule, The Pineal Gland, Harvard University Press, 1954
17. Wurtman, R. J., M. D. Altschule, U. Holmgren, Amer. J. Physiol. 199, 1109 (1959)
18. Adams, W. C., L. Wan, A. Sahler, J. Endocr. 31, 295 (1965)
19. Dey, L. F., Endocrinology 33, 75 (1943)
20. Hillarp, T., Acta. Endocrin. (Kobenhaben) 2, 11 (1965)
21. Greer, M. A., Endocrinology 53, 380 (1953)
22. Flerko, B., V. Bardos, Acta. Neuroveg. 20, 248 (1959)
23. Harris, G. W., M. Reed, C. P. Fawcett, Brit. Med. Bull. 22, 266 (1966)
24. Critchlow, V. J., J. DeGroot, Anat. Rec. 136, 179 (1960)
25. Barraclough, C. A., R. A. Gorski, Endocrinology 68, 68 (1961)
26. Barraclough, C. A., Endocrinology 68, 62 (1961)
27. Hacik, T., Arch. Int. Physiol. 74, 1 (1966)
28. Levine, S., R. F. Mullins Jr., Science 152, 1585 (1966)
29. Yates, F. E., J. Urquhart, Physiol. Rev. 42, 359 (1962)
30. Best, C. H., N. B. Taylor, Physiological Basis of Medical Practice, Williams and Wilkins Co. p 973 1961
31. Huang, C. Y., A. V. Everitt, J. Endocr. 32, 261 (1965)
32. Soffer, L. J., M. Fogel, A. Z. Riedausky, Acta. Endocr. 48, 561 (1965)
33. Fabre, L. F. Jr., R. C. Banks, W. M. McIsaac, G. Farrell, Amer. J. Physiol. 208, 1275 (1965)
34. Pavel, S., Endocrinology 77, 812 (1965)
35. Halberg, F., Circadian Clocks, North Holland Publishing Co. p 13 1965

THE COMPLETE BLOOD COUNT AS A TOOL IN THE DIAGNOSIS OF ANIMAL DISEASES

Rose M. Morgan Parsons

Department of Veterinary Science
North Dakota State University, Fargo, North Dakota

The value of the Complete Blood Count (CBC) has, at times, been underestimated, chiefly due to its apparent simplicity of performance. The CBC has shown itself to be a reliable, as well as versatile, tool in the diagnosis of various animal diseases.

In most laboratories, the CBC consists of the hemoglobin determination, total leukocyte count (WBC), hematocrit (packed cell volume or PCV), and differential leukocyte count (including red cell examination for morphology and blood parasites). At times, the total erythrocyte count (RBC) and erythrocyte sedimentation rate (ESR) may be included in the CBC (Table I).

TABLE I

COMPLETE BLOOD COUNT (CBC)

Test	Reported as:
Hemoglobin	Grams per cent
Total leukocyte count	#/cu. mm.
Hematocrit (VPC)	Volume per cent
*Total erythrocyte count	#/cu. mm.
Differential	Per cent
(Including erythrocyte morphology and search for blood parasites)	
*Erythrocyte sedimentation rate	mm. fall/60 min.

*These tests are optional.

In most cases, the individual tests comprising the CBC are non-specific for any one disease. However, this test in conjunction with other clinical and physical findings, proves to be of greatest value as a diagnostic procedure and is relied upon in animal, as well as human, disease diagnoses.

I. *Hemoglobin*

The hemoglobin molecule is a protein having a molecular weight of approximately 66,000. It is composed of protoporphyrin, native globin, and ferrous iron.

Several different methods for the determination of hemoglobin are available. The most commonly used is the cyanmethemoglobin, although the oxyhemoglobin method may be used. Other methods may be used.

Normal values depend on many factors, such as age and species of the animal (Table II).

TABLE II

NORMAL HEMOGLOBIN VALUES FOR DOMESTIC ANIMALS

Animal	Gm per cent
Dog	12-18
Cat	8-14
Cow	8-14
Sheep	8-16
Goat	8-14
Horse (cold)	8-14
Horse (hot)	10-18
Pig	10-16

II. *Total Leukocyte Count*

The total leukocyte count is calculated as the number of white blood cells per cubic millimeter of blood. Variations in the total count may be due to a number of factors, such as age, sex, species, etc., of the animal (Table III). In certain disease conditions, the count

rise *above* the normal. This is termed a *leukocytosis* (Table IV). In other disease conditions, the count may drop *below* normal and is termed a *leukopenia* (Table V). Quite often, the white cell count rises and falls much like a barometer to indicate the course of disease or the progress of infection.

The WBC may be performed by either the hemocytometer method, or the electronic particle counter method. In our laboratory, both methods are utilized.

TABLE III

NORMAL LEUKOCYTE VALUES FOR DOMESTIC ANIMALS AT BASAL ACTIVITY

Species	per cu. mm.
1. Canine	6,000-17,000
2. Feline	5,500-19,500
3. Bovine	4,000-12,000
4. Porcine	11,000-22,000
5. Equine	5,500-12,500

TABLE IV

LEUKOCYTOSIS (INCREASED WBC)

1. Epinephrine administration
2. ACTH administration
3. Kidney abscesses
4. Acute blood loss
5. Canine distemper
6. Cellulitis in canine
7. Hemolytic crisis
8. Infectious hepatitis
9. Leptospirosis
10. Others

TABLE V

LEUKOPENIA (DECREASED WBC)

1. Bacterial infection (overwhelming type)
2. Following excessive barbiturates, benzene, D.D.T., chloramphenicol, sulfonamides, etc., therapy
3. Canine distemper
4. Multiple myeloma (equine)
5 Feline panleukopenia
6. Vitamin B_{12} deficiency
7. Acute bovine mastitis
8. Radiation syndrome
9. Nicotinic acid deficiency (canine)
10. Other viral diseases

III. *Hematocrit*

This measures the total length of a column of blood centrifuged at a predetermined force and time. The hematocrit may vary in health and disease, with changes in body fluid. For example, in pregnancy, the body gains fluid and the cells become more dilute; consequently, the hematocrit falls below normal values. In severe burns, the body loses fluid; the red cells become more concentrated, and the hematocrit rises above the normal values. It drops below normal in anemia and leukemia and rises above normal in dehydration.

IV. *Leukocyte Differential*

The differential is employed as a ratio of the kinds of leukocytes present in the peripheral blood. Normally the 5 kinds found in the peripheral smear are the polyneutrophils, lymphocytes, monocytes, eosinophils, and basophils. In diseased conditions these cells may be found in abnormal numbers. For example, in a viral disease there may be an increase in the number of lymphocytes; in a severe infection ("shift to the left") the juvenile (stab) forms may be increased to great numbers, indicating that the reserve is being called upon.

The slide also should be observed for morphology of the red blood cells (Table VI). In addition, the blood smear must be screened for blood parasites. Therefore, good technique in preparing the slide is *essential.*

TABLE VI

MORPHOLOGICAL TERMS OF ERYTHROCYTES

1.	Anisocytosis	Variation in size of erythrocytes
2.	Poikilocytosis	Variation in shape of erythrocytes
3.	Hypochromasia	Decreased amount of hemoglobin in erythrocytes
4.	Polychromasia	Staining both with acid and basic dyes
5.	Crenation	The shrunken condition of erythrocytes

The blood film should be thin and rapidly air-dried. For best results, the blood should be taken from the animal and the smear made on a pre-cleaned slide immediately, without the use of an anticoagulant. A Romanovsky stain, such as that of *Giemsa's* stain, may be used Table VII).

Both parasitic and non-parasitic "bodies" may be found in the blood smear. These must be differentiated from artifacts (Tables VIII and IX).

V. *Non-Parasitic Bodies*

These include: Howell-Jolly bodies, basophilic stippling, Heinz bodies, and erythrocyte refractile (ER) bodies.

1. *Howell-Jolly Bodies*: Nuclear remnants found in young erythrocytes; increased in anemias; few normally found in blood of cats and dogs.

TABLE VII

GIEMSA STAINING METHOD FOR BLOOD PARASITES (1)

1. Prepare a thin blood film.
2. Air-dry rapidly.
3. Fix by immersing or covering the slide with methyl alcohol for 3 to 5 minutes.
4. Place 40 drops of water on the slide and then add 4 drops of the Giemsa concentrate.
5. Allow the mixture to stand for 30 minutes.
6. Rinse, air-dry, and examine.

TABLE VIII

NON-PARASITIC BODIES

Inclusion	Increased in:
1. Howell-Jolly bodies	Anemias; increased bone marrow examination
2. Basophilic Stippling	Anemias; heavy metal toxicity; during periods of increased erythrogenesis.
3. Heinz bodies	Associated with phenothiazine toxicity in horses; commonly found in drug toxicities in man.
4. Erythrocyte Refractile (ER) bodies	Degenerative processes of the cat erythrocyte.

TABLE IX

BLOOD PARASITES

A. Bovine (Anaplasmosis, Babesiosis)
   1. *Anaplasma marginale*
   2. *Basesia bigemina*
B. Equine (Piroplasmosis)
   1. *Piroplasma equi*
   2. *Piroplasma caballi*
C. Canine (Hemobartonellosis, Babesiosis, Ehrlichiosis)
   1. *Hemobartonella canis*
   2. *Babesia canis*
   3. *Ehrlichia canis*
D. Feline (Hemobartonellosis)
   1. *Hemobartonella felis*
E. Porcine (Eperythrozoonosis)
   1. *Eperythrozoon suis*

TABLE X
FACTORS INFLUENCING ERYTHROCYTE SEDIMENTATION RATE (2)

Increased Rate	Decreased Rate
1. Increased rouleau formation	1. Increased albumin
2. Leukocytosis	2. Hemoconcentration
3. Increased fibrinogen	3. Low temperature
4. Decreased albumin	4. Delay before testing
5. Increased globulin	5. Excess anticoagulation
6. Increased cholesterol	6. Poikilocytosis, anisocytosis
7. Decreased erythrocytes (anemia)	7. Sulfonamides, glucocorticoids
8. X-ray irradiation	8. ACTH
9. Pregnancy (dogs)	9. Increased lecithins

2. *Basophilic Stippling*: Small blue granules found within the erythrocytes which are the result of precipitation of cytoplasmic substance; found in anemias, heavy metal toxicity, and during periods of increased erythrogenesis.

3. *Heinz Bodies*: Small, refractile irregular bodies within and on the exterior of the erythrocytes; result of an oxidative injury to the hemoglobin of the cell (4); found in drug toxicities; associated with phenothiozine toxicity in horses.

4. *Erythrocyte Refractile (ER) Bodies*: Structures appearing on one edge of the cat erythrocyte; result of a degenerative process within the erythrocyte.

VI. *Erythrocyte Sedimentation Rate (ERS)*

The ESR is a non-specific determination, influenced by many variables (Table X). The value of the results depend on the ability of the clinician to weigh these against other physical and laboratory findings. The ESR is of more value in canine species than any other species (2).

VII. *Preparation of Blood Smears*

All too often the entire blood study is sacrificed because of the inability to make a good smear. Two methods are commonly employed:

1. Glass slide.
2. Cover slip.

VIII. *Available Stains*

Numerous stains are avilable from most commercial supply houses (Table XI).

IX. *Collection and Preservation of Specimens*

Blood presents more problems in sampling than any other single tissue because of the wide variety of potential tests.

Blood is composed of a fluid (plasma) in which float cells, platelets, proteins and other dissolved substances. Methods of collection can significantly alter determinations of these constituents; however,

TABLE XI
AVAILABLE STAINS

1. Wright's Method (standard stain)
2. Giemsa's Stain
3. Camco Quik Stain (Cambridge Chemical Co., Dearborn, Mich.)
4. Hemal Stain (Hemal Stain Co., Danbury, Conn.)
5. La Mar Stain (La Mar Laboratories, Inc., Oceanside, N.Y.)

TABLE XII
ANTICOAGULANTS

	Testing
1. Heparin (sodium heparin)	Test performed within 10 hours
2. EDTA	Blood shipped or delayed testing
3. Ammonium-potassium oxalate	Test performed within 4 hours

use of an anticoagulant can preserve blood in a state similar to its circulating form. Most tests can be run on blood collected in this form but occasionally serum from a clotted sample is preferred.

Hemolysis, or destruction of the cells, can be caused by moisture, rough handling, osmotic imbalances, and hemolytic bacteria.

X. *Anticoaqulants*

1. When testing is to be done within 10 hours, the anticoagulant of choice is heparin (sodium heparin) (Table XII). The syringe is "lined" with heparin—this prevents a clotted or hemolyzed sample. Heparin also can be placed in collection tubes at the rate of 1 drop of 1 per cent solution for each 5 ml of blood collected.

2. When blood is to be shipped or testing is delayed, the anticoagulant of choice is ethylenediaminetetraacetate (EDTA). It is available in tablet, powder, or liquid form as both disodium and dipotassium salts. Both salts cause a slight increase in blood urea nitrogen (BUN) and can decrease the hematocrit if excessive amounts are used. If using powder, 2 mg/ml blood of EDTA is desired.

3. If storage is anticipated, solutions containing sodium citrate should be used. EDTA, oxalates, and fluorides should not be used for transfusions. Sodium citrate is a poor anticoagulant for use in hematology.

4. Blood smears should be made from fresh blood. It is best, if possible, to make the smear directly from the tip of the needle.

REFERENCES

1. Morgan, H. C.: Blood Parasites: Celular. Vet. Med. 61: 10, Oct. 1966.
2. Morgan, H. C.: The Value of the Erythrocyte Sedimentation Rate in Veterinary Medicine. Vet. Med. 61: 5, May, 1966.
3. Schalm, Oscar W.: Veterinary Hematology. 2nd ed., Lea and Febiger, Philadelphia, Pa., 1965.
4. Wintrobe, M. M.: Clinical Hematology. 5th ed. Lea and Febiger, Philadelphia, Pa., 1961.

COAT-COLOR POLYMORPHISM IN NORTH PLAINS RED FOX POPULATIONS

Donald R. Johnson

Department of Biology

Minot State College, Minot, North Dakota

ABSTRACT

Red, cross, and silver color phases occur in red foxes (*Vulpes vulpes*). It is assumed that two pairs of autosomal, non-linked genes determine coat color (Warwick-Hanson hypothesis). The genotype determining red pelage is designated as $A^RA^RC^RC^R$. The Alaskan (A^D) and Canadian (C^D) alleles interact to produce eight other phenotypes (smoky-red, two of the cross and five of the silver phase).

These dark-phase genes have been almost eliminated from North Plains fox populations. Based on the average annual fur returns of the early 1830's from Fort Union (junction of the Yellowstone and Missouri Rivers), the frequency of the Alaskan and Canadian genes were 0.059 and 0.087 respectively. Now, both range in frequency from 10^{-4} to 10^{-5}. Thus, the occurrence of dark phases in North Plains populations is probably the result of mutation and, perhaps, some immigration from higher latitudes.

In present environmental conditions, dark phases have a very low adaptiveness, most likely because the genes producing them have unfavorable physiological, as well as the obvious morphological, effects. The amelioration of the climate during the past century is the probable cause for the elimination of the Alaskan and Canadian genes from these populations since, historically, cross and silver phases occured in highest frequency in rigorous climates.

DEVELOPMENT OF LATICIFERS IN THE EMBRYO OF *EUPHORBIA ESULA*

M. Arif Hayat[1]

Department of Botany

North Dakota State University, Fargo, North Dakota

ABSTRACT

Approximately 2 mm long mature embryos were excised from the seeds of *Euphorbia esula*. Serial transverse and longitudinal sections 8μ in thickness were cut by a rotary microtome. The first laticifer initial develops at the level of the future cotyledonary node during an early stage in embryo development. This initial cuts off

[1]Present address, Department of Biology, University of Dayton, Dayton, Ohio 45409.

laticifers on four faces. From this stage onward, laticifers develop and branch rapidly in both longitudinal and horizontal directions of the embryo axis. The tip of the laticifer exhibits a special form of intrusive growth. Laticifers show both uni- and bidirectional growth.

In the radicle/root laticifers form two rings, an outer one located below the epidermis and an inner one present directly below the prospective endodermis. Those rings constitute only a single row of laticifers. The most extensive branching of laticifers is found in the coetyledonary node. The nuclei of these highly specialized and complex laticifers undergo repeated mitotic divisions without being followed by cytokinesis, which results in the coenocytic condition of these cells. The number of nuclei in each laticifer varies from 1 to 20 depending upon the length and location of these specialized cells. The differences in the pattern of distribution of laticifers of the embryo and the seedlings are more of a quantitative than a qualitative nature. Thus, fundamental aspects of laticifer development and distribution are exhibited in the embryo.

ABSCISSION IN *KOCHIA SCOPARIA* (L.) SCHRADER

Donald A. Becker

Department of Biology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Kochia scoparia L. is an introduced annual common to waste areas and cultivated fields in this region. Like many other species of the Chenopodiaceae, it becomes a tumble-weed when mature. In some areas of Grand Forks County abscission occurred from September 21 to November 1, although in other areas many plants remained intact until Spring, 1967. Abscission occurred only during periods when winds reached velocities of 25 m.p.h. or more.

When compared anatomically with stem areas above and below, the abscission zone at the base of the stem was found to be structurally weak. The sources of weakness in this area include: (1) knots, particularly large in bushy plants, (2) cross grain of the wood, (3) amount of parenchymatous tissue relative to fibrous tissue, (4) a reduction in width of the fiber walls and (5) reduced lignification.

During high winds the abscission zone is subject to great tension and compression forces as the bushy plant sways back and forth. Separation of the abscission zone tissues often occurs suddenly, produring a loud crack, w h i c h suggests mechanical rupture. The woody, abscised ends are ring-shaked, exhibit various degrees of smoothness or roughness, and many have been obviously discolored

[1]Currently at Department of Biology, Midland Lutheran College, Fremont, Nebraska.

and decayed by fungi. Microscopic examination of such ends indicated no previous cell division, and that separation had occurred across the cell walls, rather than between the cells through the middle lamella.

Microchemical tests for lignin, cellulose, and pectic material presented no evidence of a chemical dissolution of the middle lamella through active metabolism. Staining sections from over 80 plants with cotton blue revealed that fungus mycelia had grown extensively within the abscission zone, with less penetration of the denser wood of the root and stem. The invasion of the mycelium into the host was accompanied by degradation of the cell walls of the periderm, phloem, and immediately adjacent tissue rich in cellulose and pectic materials. Degradation was not detectable, with the exception of pit-like holes, in slightly lignified tissues such as xylem vessels and fibers. Examination of intact plants indicated that infection begins prior to abscission, possibly several weeks before. The fungus was isolated and identified as a species of *Rhizoctonia*, a common soil-inhabiting fungus.

The above data suggests that abscission in *K. scoparia* is primarily a mechanical process. At maturity the structurally weak zone at soil level is weakened further by the invasion of a fungus. Hence, the wood becomes brash or brittle, yielding readily under the tension and compressive forces created as the plant sways before the force of the wind.

VARIATION IN FREE AMINO ACIDS IN MALE-STERILE AND MALE-FERTILE SUDANGRASS

Sultanul Alam and P. C. Sandal

Department of Agronomy

North Dakota State University, Fargo, North Dakota

ABSTRACT

Anthers at the pre-meiotic and pre-pollen stages from male-fertile and male-sterile sudan-grass were analyzed for free amino acids by thin layer and two dimensional descending chromatography and a Technicon automatic amino acid analyzer.

Eight amino acids including alanine, aspartic acid, glycine, histidine, isoleucine, threonine, tryptophan and valine were separated fairly well by thin layer chromatography. In addition, proline was identified by its characteristic color and was abundant in the male-fertile line but very low in the male-sterile line.

Two dimensional descending chromatography detected 10 amino acids in anthers at the premeiotic stage and 15 at the pre-pollen stage. A very low amount of proline was present in either male-fertile or male-sterile lines at the pre-meiotic stage. However, at the

pre-pollen stage proline was high in male-fertile plants but largely absent in male-sterile plants. Conversely, asparagine content was high in male-sterile plants and very low in male-fertile plants at the pre-pollen stage.

Alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, lysine, phenylalanine, proline, threonine, tyrosine and valine were identified by automatic amino acid analysis in anthers at both the pre-meiotic and pre-pollen stages. In general, the free amino acid content of anther tissue was higher at the pre-pollen stage than at the pre-meiotic stage. No significant differences were noted in amino acid content between male-fertile and male-sterile lines at the pre-meiotic stage. At the prepollen stage, however, anthers from fertile lines had a significantly greater amount of alanine, glutamic acid, phenylalanine, proline and tyrosine than did comparable samples from male-sterile plants. Non-significant differences were found among the remaining amino acids between the male-fertile and male-sterile lines at the pre-pollen stage.

Based on the results of this study, the following association appeared to be consistent in anthers at the pre-pollen stage: proline was high and asparagine was low in male-fertile plants, whereas, asparagine was high and proline was low in male-sterile plants.

MICROBIOLOGICAL STUDIES ON LINATINE, A NEW CLASS OF ANTIBIOTIC COMPOUNDS

Teh-tseng Liu Wang, A. Diner,[1] *J. L. Parsons and H. J. Klosterman*

Departments of Bacteriology and Biochemistry
North Dakota State University, Fargo, North Dakota

ABSTRACT

Linatine, the name given to a vitamin B_6 antagonist isolated from flaxseed, has been identified as 1-[(N-γ-glutamyl)-amino]-D-proline. Its growth-retarding effect on young chicks, which was first observed after feeding oil-free linseed meal, was found to be reversed by addition of B_6 to the diet. Linatine and its free form, 1-amino-D-proline, are potent inhibitors of *Azotobacter vinelandii* O and other azotobacter species at sub-microgram quantities. The optical isomers of the aminoprolines vary considerably in their antibiotic effect. The *D*-forms are 50 times more active than the *L*-configuration. The glutamyl derivatives of the aminoprolines are 3.5 times more effective on a molar basis than the free aminoprolines. A variety of bacteria were found to be unaffected except by 100 μg quantities, and only by the *L*-configuration. The usual B_6 assay

[1]Department of Pathology and Bacteriology, Colorado State University, Fort Collins, Colorado.

microorganisms, *Leuconostoc mesenteroides, Saccharomyces carlsbergensis,* and *Neurospora crassa,* were not inhibited.

Growth inhibition of the non-symbiotic nitrogen fixing azotobacter occurs whether or not nitrogen is being fixed. Of all the water-soluble and fat-soluble vitamins, only B_6 reverses inhibition of *A. vinelandii,* and only for certain of the 1-aminoprolines. Linatine inhibition is not reversed by any of the B_6 forms; however, pyridoxal-phosphate reverses inhibition produced by 1-amino-D-proline, 1-amino-L-proline, and gammaglutamyl-1-amino-L-proline (L-isomer of linatine). Pyridoxal-hydrochloride only reverses inhibition produced by 1-amino-L-proline. Pyridoxal-phosphate has been shown to complex with the free 1-aminoprolines, which may explain reversal of inhibition of *A. vinelandii*; however, inhibition by linatine is not reversed by B_6. These results indicate two different mechanisms of inhibition.

Vitamin-free casein hydrolysate reverses inhibition produced by all of the amino-prolines. The neutral amino acids, especially isoleucine and alanine, are the most effective in reversal of inhibition; glutamic and aspartic acids have little effect; and lysine and ornithine increase the inhibition. Putrescine also increases the zones of inhibition.

Since the 1-aminoprolines are B_6 antagonists, all cells should be inhibited as B_6 is needed in metabolism. It would appear that since the *D*-configuration is the most effective for *A. vinelandii,* permeability may explain the insensitivity of the microorganisms tested. This lack of permeation, however, may be overcome by use of other types of hydrazino acids and their peptidyl derivatives. The specific requirement for the *D*-configuration to inhibit *A. vinelandii* and the relative insensitivity of other microorganisms, offers considerable promise for the development of other optically-active hydrazino acids that preferentially attack only certain cell types. This would make possible the control of a given pathogen without harm to the host.

ISO-ENZYMES OF TWO CHLORELLA DEHYDROGENASES

John L. Tjostem and Murray E. Duysen

Department of Botany

North Dakota State University, Fargo, North Dakota

ABSTRACT

The iso-enzymes of lactic acid dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) of *Chlorella pyrenoidosa,* strain 7-11-05, were investigated. A comparison was made between the dehydrogenases obtained from cells cultured in light inorganic medium and cells grown in dark on glucose-enriched medium. *Chlorella* was cultivated in chemostates at 39°C and enzyme extracts were

prepared from cultures in the logarithmic growth phase. The iso-enzymes were demonstrated on disc electro-phoresed acrylamide gel columns by coupling the enzymatic reaction to the reduction of nitro blue tetrazolium.

LDH and G-6-UDH were present in extracts of both heterotrophically and autotrophically cultured cells. Only the d-lactate, which is biologically less common than l-lactate, served as a substrate for LDH. Two iso-enzymes of LDH were detected in extracts of both heterotrophic and autotrophic cultures. The iso-enzyme nearest the origin, A band, was more dense than the B band in dark-grown cells; the B band was more concentrated than the A in light-grown cells.

A difference was noted in the iso-enzymes of G-6-PDH obtained from cells grown in light compared to cells grown in dark. Only one major iso-enzyme was detected in light-grown cells whereas extracts of dark-grown cells exhibited four visible isoenzymes.

THE EFFECTS OF COLD EXPOSURE AND FASTING ON GLYCONEOGENESIS FROM ALANINE IN RATS[1]

James D. Longpre[2] and S. J. Brumleve

Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The effects of cold exposure and fasting on glyconeogenesis from alanine were studied in 3 groups of male rats (150 to 250 g) that were maintained at either 30°C, 10°C, or 2°C for 10 days. Each group of rats was fasted 0 to 16 hours at the temperatures indicated. One hour before the end of the fast and sacrifice, the rats were injected intrathoracically with 0.059 M moles of uniformly labelled C^{14}-alanine. After sacrifice, the amount of liver glycogen was determined and the radioactive carbon present in the glycogen assayed. Statistical analysis of the data suggests that both the environmental temperature and the fasting liver glycogen levels present at the time of sacrifice contributed to the variations in the rates of glyconeogenesis from alanine. The data were significant at the 5% level. The relationships between glyconeogenesis, liver glycogen levels, and environmental temperature were shown mathematically. These relationships are expressed in the equation: Relative per cent of alanine dose incorporated into glycogen = (112) (per cent liver glycogen $^{-0.50}$) (environmental temperature $^{-0.76}$). Observed data were compared with that from the predicting equation. The Chi-square Test, Sign Test, Wilcoxan Signed Rank Test and Run Test indicate that the observed data and predicted data could have been from the same basic population.

[1]Supported in part by National Institutes of Health Grant.

[2]National Institutes of Health Fellow.

RAT KIDNEY GLYCEROPHOSPHORYLCHOLINE DIESTERASE

J. Baldwin and W. E. Cornatzer

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, Notrh Dakota

ABSTRACT

Glycerophosphorylcholine diesterase (E. C. 3. 1. 4. 2.) catalyzes the following reaction:

Glycerylphosphorylcholine (GPC) + H_2O → Choline + α-glycerolphosphate (αGPO_4)

Utilizing the method described by Bublitz and Kennedy (J. Biol. Chem., **211,** 951, 1954) for the estimation of αGPO_4, this enzyme has been studied in the rat. Of the tissues tested, muscle, intestine, brain, liver, heart, lung, spleen and kidney, the kidney preparation showed the highest specific activity. The selective inhibitors of the true and pseudo cholinesterases — prostigmine and physostigmine (10^{-4}M)— showed no inhibition of the activity. Subcellular fractionation of the kidney homogenates demonstrated the highest specific activity of this enzyme to be located in the microsomes. The diesterase was found to be most active at pH 9.45. $MgCl_2$(1.1×10^{-6}M) stimulated the enzyme when the microsomes were isolated in the presence of 0.005M EDTA. The Michaelis constant was calculated to be 7.1×10^{-3}M at pH 9.5 for GPC.

Deoxycholate from 0.125 to 0.5% conc. (w/v) added to microsomal preparations showed slight stimulation of the activity with maximal stimulation observed at 0.5% (w/v). Tween-20 showed maximal activation at 0.125% conc. (w/v). Treatment of the microsomes with 0.375% conc. (w/v) deoxycholate resulted in solubilization of the enzyme. The enzyme was precipitated between 30 to 60% saturation with ammonium sulfate. Freezing and thawing a solution of the ammonium sulfate precipitate brought about further purification. Gel filtration of the most purified preparation on Biogel P-100, P-200, and P-300 suggested a high molecular weight. (Supported in part by U. S. Atomic Energy Grant No. (AT 11-1-1513) and Nat'l. Inst. of Health Grant No. (1-Fl-GM-32, 651-01).

PHOSPHOLIPID COMPOSITION OF MITOCHONDRIA AND MICROSOMES OF LIVER AND KIDNEY IN ADRENALECTMIZED, HYPOPHYSECTOMIZED AND ALLOXAN DIABETIC RATS

James D. Johnson and W. E. Cornatzer

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

Male albino rats of Sprague-Dawley strain were divided into two groups. The animals in Group I were adrenalectomized for 8 days; adrenalectomized for 8 days - treated with aldosterone (three injections each at 28, 16, and 6 hours before sacrifice); alloxan diabetic animals for 21 days (blood sugar 354 to 781 mg.%); alloxan diabetic animals for 21 days-insulin treated (4 days before sacrifice, at 12-hour intervals, 8 units of protamine zinc insulin were injected). Control animals received injected saline. The animals in Group II were hypophysectomized for 2 days, and 4 weeks; hypophysectomized for weeks—treated with daily injections (0.2 mg) purified bovine growth hormone. Control animals received saline. The animals were sacrificed, mitochondria and microsomes were prepared by differential centrifugation from liver and kidney tissues. An aliquot of each subcellular fraction was taken for protein determination. The phospholipids were extracted from the remainder of the subcellular fractions, separated by thin layer chromatography into sphingomyelin, phosphatidyl choline (PC), phosphatidyl inostiol (PI), phosphatidyl serine, and phosphatidyl ethanolamine (PE).

There is a decrease in concentration of the total mitochondria and microsomal phospholipid P of liver and kidney following adrenalectomy. This decrease is more apparent when expressed as mg phospholipid P/mg protein N in liver microsomes. However, this does not occur in kidney mitochondria and microsomes. The per cent of total lipid P composition of PC is greater in liver microsomes than mitochondria. Mitochondrial fractions of liver and kidney contains a greater concentration of PE than microsomes. There is apparently, very little change in the per cent of total lipid P for the individual phospholipids of mitochondria and microsomes in the adrenalectomized or adrenalectomized-aldosterone treated animals. There is a decrease in concentration of total mg phospholipid P/mg of protein in liver mitochondria and microsomes in alloxan diabetes and following hypophysectomy. The distribution of individual phospholipids is unaltered in diabetes. A decrease in concentration of PI in liver mitochondria following hypophysectomy was observed.

GLUCOSE-6-PHOSPHATASE, PYROPHOSPHATE-GLUCOSE PHOSPHOTRANSFERASE: EVIDENCE FOR A METALLOENZYME

Philip T. Johns and Robert C. Nordlie

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

Metals have been shown to be involved in enzyme catalyzed reactions both as a tightly bound, nondialyzable, metal and as a cofactor. The possibility of a metal being associated with this enzyme was suggested by Nordlie and Lygre (1) when they found that citrate and oxalate, metal chelators, inhibited glucose-6-phosphatase, pyrophospate-glucose phosphotransferase. Further work to determine the actual presence of a metal was thus indicated. Preincubation procedures, as used by Plocke (2) *et al.*, were employed here as were the normal procedures for determining inhibitor constants. The concentrations of chelator, with respect to the preincubation mixture, necessary for 50% inhibition after one hour preincubation are, first for the phosphatase and second for the transferase: o-phenanthroline—3.0 x 10^{-3} M and 5 x 10^{-3} M; diethyl-dithiocarbamic acid — 7.0 x 10^{-2}M and 7.3 x 10^{-2}M and; sodium azide — 1.36M and 0.88 M. The inhibitor constant for various chelators were determined by instantaneous inhibition as follows with respect to glucose-6-phosphate: $K_{cyanide}$ = 2.35 x 10^{-1} M, $K_{oxalate}$ = 2.44 x 10^{-2} M, K_{azide} = 8.79 x 10^{-2} M, with respect to pyrophosphate: $K_{cyanide}$ = 0.114 M, $K_{oxalate}$ = 9.52 x 10^{-3} M, $K_{o\text{-}phenathroline}$ = 5.67 x 10^{-2} M, and K_{azide} = 7.75 x 10^{-2} M; with respect to glucose: $K_{cyanide}$ = 9.9 x 10^{-2}, $K_{oxalate}$ = 2.46 x 10^{-2}, $K_{o\text{-}phenanthroline}$ = 2.39 x 10^{-2}M, K_{azide} = 1.51 x 10^{-1} M. These inhibitors showed competitive inhibition with respect to phosphate substrates and noncompetitive inhibition with respect to glucose. Microsomes were dialyzed for 16 hours in 10^{-3} M Tri 0.25 M sucrose with no loss of activity. Inhibition by chelators giving competitive inhibition with respect to the phosphate substrates indicates a metal at the active site and dialysis indicates that the metal is tightly bound.

LITERATURE CITED

1. Nordlie, R. C. and Lygre, D. G., J. Biol. Chem., **241,** 3136 (1966).
2. Plocke, D. J., Levinthal, C., and Vallee, B. L., Biochemistry, **1,** 373 (1962).

CATALYTIC PROPERTIES OF RABBIT INTESTINAL GLUCOSE 6-PHOSPHATASE-PHOSPHOTRANSFERASE

David G. Lygre and Robert C. Nordlie

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

Glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3. 1. 3. 9) has been recently shown to catalyze the hydrolysis of inorganic pyrophosphate (PP_i) and the synthesis of glucose 6-phosphate through PP_i-glucose and CDP-glucose phosphotransferase reactions in both rat liver and kidney (1, 2). Consequently, the intestinal enzyme was studied. New Zealand White male rabbits weighing 11 kg. and fasted 24 hours were used as the enzyme is not normally demonstrable in rat intestine. The four activities, routinely assayed at pH 6.0 with 3.mM phosphate substrates and 180mM glucose (for phosphotransferase reactions), were distributed quite uniformly along the intestinal mucosa, with slightly higher levels in the duodenum and jejunum with constant ratios of activities in each segment. Other similarities of these activities include the following: 1) all are primarily microsomal; 2) all show similar responses to thermal inactivation; 3) E_a = 12 Kcal/mole; and 4) all show similar patterns with varying concentrations of sodium deoxycholate, lysolecithin, and urea, optimal concentrations for activation being 5 mM, 0.0B0mM, and 1.5 M, respectively. The pH optima were approximately 6.2 for glucose 6-phosphatase and 5.7 for the other three activities. The above properties closely resemble those of liver and kidney glucose 6-phosphatase-phosphotransferase. Michaelis constant values at pH 6.0 were as follows: $K_{gl\text{-}6\text{-}P}$ = 1.8mM, K_{PP_i} = 2.1mM for both hydrolysis and transferase reactions, $K_{glucose}$ = 70mM for both phosphotransferase reactions, and K_{CDP} = 5mM. Of the twenty amino acids considered as normal constituents of mammalian proteins, the only compounds with a noticeable effect were L-leucine and L-cysteine, which each inhibited glucose 6-phosphatase 20% at 26.7mM inhibitor levels. Studies of 10mM metals showed glucose 6-phosphatase inhibition of nearly 100% by Cu^{2+}, Fe^{3+}, Hg^{2+}, and Zn^{2+}. Inorganic pyrophosphatase and PP_i-glucose phosphotransferase activities were inhibited nearly 100% by Ba^{2+}, Co^{2+}, Fe^{2+}, Cd^{2+}, Mn^{2+}, and Pb^{2+} in addition to the metals above. This inhibition by additional metals can be attributed to chelation of PP_i. This response to metals is similar to that observed with the liver enzyme. It is concluded that rabbit intestinal glucose 6-phosphatase-phosphotransferase is very similar to the hepatic and renal enzymes with respect to catalytic properties. This gives supporting evidence for the genetic

identity of liver, kidney, and intestinal glucose 6-phosphatase-phophotransferase.

LITERATURE CITED

1. Nordlie, R. C., and Arion, W. J., J. Biol. Chem., **239**, 1680 (1964).
2. Nordlie, R. C., and Soodsma, J. F., J. Biol. Chem., **241**, 1719 (1966).

STUDIES ON MESENTERIC LYMPH OF THE RAT

E. E. Largis, J. C. Crandall and F. A. Jacobs

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

In a study aimed at an investigation of the relationship of amino acids upon the intestinal absorptive processes of fatty acids, it was necessary to examine the chemical nature of various components of the lymph draining the upper small intestine. The adult male rat was used in these experiments. These animals were maintained on Purina Laboratory Chow up to the time of the experiment. Prior to the surgical procedures in which the mesenteric lymph duct was cannulated the animals had been fed corn oil by stomach tube to dilate the lymphatic system. After recovery from the surgical procedures the animals were fed cream which contained mixtures of amino acids, on of which was uniformly labeled with radioactive carbon. The amino acids used in these studies were chromatographically pure L-threonine, L-leucine, and L-valine in various combinations. Lymph collected before and after the feeding of the radioactive material was analyzed for the following: 1) the rate of flow, 2) protein concentration, 3) free amino acids, 4) total radioactivity and its distribution into the protein and non-protein fractions (containing free amino acids), 5) electrophoretic distribution of protein, 6) electrophoretic distribution of lipid (lipoprotein), and 7) distribution of radioactivity in the latter two sets of components.

Results of these experiments have shown that fed amino acids appear in the lymph in the form of free amino acids and in the form of newly synthesized protein. It is also evident, during the active phase of absorption, that the synthesis of protein from the fed amino acid is most rapid. We attribute this newly formed protein found in the lymph to the synthetic reactions occurring within the intestinal mucosa. Supported in part by N.I.H. Grant AM 2023).

COMPARATIVE STUDIES OF *IN VITRO* EFFECTS OF SURFACE-ACTIVE AGENTS ON THE RESPONSE OF SYTHETIC AND HYDROLYTIC ACTIVITIES OF LIVER GLUCOSE 6-PHOSPHATASE TO HORMONAL ADMINISTRATION AND DEPRIVATION

Roy E. Snoke and Robert C. Nordlie

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

The *in vitro* effects of various surface--active agents on the levels of inorganic pyrophosphate-glucose phosphotransferase, glucose-6-P phosphohydrolase and inorganic pyrophosphatase activities of hepatic glucose 6-phosphatase (EC 3.1.3.9) have been examined. The detergents employed could be place in two groups: Group (a) activated the enzyme activities and consisted of anionic (deoxycholate and cholate), cationic (cetyltrimethyl ammonium bromide) and non-ionic (Triton X-100) detergents. Group (b) inhibited the three activities progressively with increased detergent concentration and was composed of anionic (sodium lauryl sulfate) and non-ionic (Tween 20 and Tween 80) detergents. The rather general nature of the detergent action was suggested by the non-specific detergent-induced responses. The *in vitro* detergent effects of deoxycholate and Triton X-100 were used to examine the synthetic and hydrolytic activities of this enzyme from adrenalectomized, cortisone-treated adrenalectomized, normal and alloxan-diabetic rats. In the absence of added detergent, all three enzyme activity levels were activated above normal levels in the diabetic animals and in the glucocorticoid administered animals. The activity levels were progressively increased in all animals with increased concentrations of both detergents, to an optimal concentration (0.2%). The ratio, activity in alloxan-diabetic animals/activity in normal animals, increased progressively with increased homogenate concentrations of either dexycholate or Triton X-100 for all enzyme activities. The ratio, activity in cortisone-treated adrenalectomized animals/activity in control animals, however, decreased with increased homogenate detergent concentrations. This different response to detergents by activity levels of enzyme from hormone administered or hormone deprived animals suggests that the mechanism of activation of the enzyme activity in the two conditions is not the same. The observed responses suggest that a detergent-like effect is involved in the *in vivo* glucocorticoid stimulation.

OBSERVATIONS OF MITOCHONDRIAL CATION LEVELS WITH RESPECT TO STRUCTURAL CHANGES

D. R. Myron and J. L. Connelly

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

Structural changes (swelling and contraction) of mitochondria have been attributed by some investigators to simple osmotic factors. However, ample evidence is available which demonstrates that control of mitochondrial structural states can be independent of mild osmotic differences. Nevertheless, in view of the knowledge that the varying of the structural states and the transport of ions across mem branes are both energy-requiring processes, it becomes important to elucidate any significant interrelationships which might exist. This work reports on the movement of mitochondrial inorganic cations under a variety of conditions employed to control the time and amount of structural change.

A relationship has been established between time of onset (TO) of mitochondrial swelling and loss of bound potassium. The rate of loss of potassium is maximal at TO under conditions employing various swelling agents, pH values, and respiratory states. Although the extent of swelling is directly related to pH from pH 6.0 to pH 8.0, within this pH range the quantity of potassium lost at TO remained constant. This indicates that potassium loss is independent of the extent of swelling. Protection from swelling by EDTA also prevented potassium loss. However, when a contraction of swollen mitochondria is induced by ATP + EDTA, the lost potassium was not reaccumulated. Therefore, it is apparent that the control of the structural state does not depend upon potassium movement.

In studies concerning other cations present at lower quantities in mitochondria, it was found that the sodium level remained relatively constant throughout structural variations. A large percentage of mitochondrial calcium and magnesium was relseased concurrently with TO in a manner similar to that seen with potassium. Hydrogen ion movement was significant only under phosphorylating conditions (i.e., in the presence of ADP, Pi and substrate) and could be inhibited by oligomycin.

It is proposed that the release of specific ions from the mitochondrion during swelling, relates to the mechanism controlling TO rather than to osmotic phenomena. The release of bound potassium can be correlated only with TO, thus providing further evidence for the distinction between the chemical machineries controlling TO, extent, and contraction.

INHIBITORY EFFECT OF ANTISERUM ON GERMINATION OF UREDINIOSPORES OF *MELAMPSORA LINI*

Marie A. Petermann and J. A. Doubly

Department of Bacteriology
North Dakota State University, Fargo, North Dakota

ABSTRACT

During the course of serological investigations on the specificity of interaction between certain races of flax rust (*Melampsora lini*) and rust differentiating varieties of flax it was noted that antiserum prepared against Race I suppressed the germination of urediniospores of Race I *in vitro*.

Antiserum was produced in rabbits by inoculation of a globulin antigen extracted from the urediniospores of Race I. Freund's adjuvant was used with the antigen. The resulting antiserum had a titer of 10,000.

Germination experiments were done with water-serum-spore suspensions on cover glasses over hollow ground microscope slides. After incubating at 4°C for twenty-four hours the preparations were examined microscopically. Quantitative estimates of germination were made on counts of at least one hundred spores for each test preparation. The optimal concentration of normal serum permitting germination was determined before the tests were started. The germination tests were then done on suspensions of: water and urediniospores; normal serum diluted 1:6 with water and urediniospores; unabsorbed Race I antiserum diluted with water and urediniospores; and absorbed Race I antiserum diluted 1:6 with water and urediniospores. The absorbed antiserum was treated with a saline solution of Race I antigen to remove as much antibody as possible from this antiserum.

With normal serum, 97% of the spores germinated; with water alone, 85%; with unabsorbed Race I antiserum 2%; and with absorbed Race I antiserum 18%.

Germination in normal serum was slightly better than in water. The almost complete inhibition of germination in unabsorbed Race I antiserum and the decreased inhibitory effect when antiserum is absorbed, suggests an antigen-antibody reaction.

PROPERTIES OF THE CELLULASES OF *STREPTOMYCES ANTIBIOTICUS*

B. P. Sleeper

Department of Bacteriology
North Dakota State University, Fargo, North Dakota

ABSTRACT

The filamentous bacterium, Streptomyces antibioticus, has been found to secrete at least five extracellular endocellulases when grown on cellulose (J. Bacteriol., **89**: 23-27, 1965). The enzymes appeared to be electrophoretically and immunologically homogeneous.

The cellulases have now been found to be contaminated with a multiple family of proteinases, and also contain some amylase and invertase activity. Density gradient column electrophoresis gave improved preparative scale separation of the cellulases, two of which were free of proteinase. Electrophoretic fraction II was purified up to 120-fold; it showed a single peak in the ultracentrifuge.

The physical properties of the cellulases and proteinases are similar. Attempts at removal of the proteinases on many different column supports designed to exploit size or charge differences have been fruitless. Most promising was passage of the mixture through a column of acrylamide gel equilibrated with 1% casein. The proteinases passed through at the void volume as casein-proteinase complexes. The cellulases were then separated from the casein by another pass through a column.

Estimations of the molecular weight of the cellulases have been made by determination of their elution volume from a column of Sephadex G-100 previously calibrated with proteins of known values. These experiments indicated a total of at least eight cellulases with weights of 16,000 to 37,000. There was a striking regularity in these values in that each component differed from its neighbors by 2500 to 3000. This suggests that the multiplicity of the enzymes may be due to polymerization of peptide subunits. Depolymerization of a "native" cellulase by the proteinase could also account for the data.

TECHNIQUE FOR THE STUDY OF STRICTLY ANAEROBIC BACTERIA AND SOME APPLICATIONS

Robert S. Fulghum

Department of Bacteriology
North Dakota State University, Fargo, North Dakota

ABSTRACT

Hungate (1) developed techniques for the isolation and culture of obligately anaerobic bacteria requiring pre-reduced media and protection from atmospheric oxygen during all phases of culture pro-

cedures. These methods have been modified to the culture of anaerobic organisms from a number of ecological niches. The modifications detailed by Moore (2) have been most successful in the culture and characterization of a large numbers of strains of anaerobic bacteria. This work not only confirms many of the findings of Prévot (3) but also will contribute greatly to our understanding of anaerobic bacteria, when complete.

Hitherto unknown genera and species of bacteria have been isolated and characterized from the bovine rumen using these techniques (4). Work is now in progress in several laboratories on obligately anaerobic, non-sporeforming bacteria from other ecological niches such as human and animal gastrointestinal tract, sewage sludges, anaerobic lesions, etc. Many workers are turning to the basic technique of Hungate.

Work in this laboratory centers on growth factors required by and produced by the bacterial species composing the flora of the bovine rumen (5). Additional work includes the isolation of anaerobic bacteria from sewage lagoons.

Pure cultures of ruminal bacterial requiring biotin will not utilize biotin solutions which have been aged in the presence of air but will utilize biotin solutions that have remained sealed in glass vials. The vitamin requirements of six species of ruminal bacteria have been determined and previous findings for two others confirmed. The ability of sterilized fluid from ruminal contents to stimulate the growth of seventeen species of ruminal bacteria has been studied. Data indicates that factors which stimulate ruminal bacteria other than the water soluble vitamins are present in ruminal fluid. These factors were not present in the defined media used in vitamin deletion studies.

Vitamin production studies of the ruminal flora indicate that vitamins produced by the growth of cells are not released by species showing growth in the defined media, but remain within the cells until autolysis or digestion by the host animal in the abomasum.

REFERENCES

1. Hungate, R. E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Revs. **14**:1-49.
2. Moore, W. E. C. 1966. Techniques for routine culture of fastidious anaerobes. International J. Of Systematic Bacteriol. **16**:173-190.
3. Prévot, A. R. 1966. Manual for the Classification and Determination of the Anaerobic Bacteria. (translated by Victorian Fredette) 402 pp. Lea and Febiger, Philadelphia.
4. Bryant, M. P. 1959. Bacterial species of the rumen. Bacteriol. Revs. **23**:125-153.
5. Fulghum, R. S., Baldwin, B.B, and Wang, C-.L. 1967. Vitamin requirements of, and vitamin production by, ruminal bacteria. Bacteriol. Proc. 1967, Paper No. G128.

THE ELECTROPHORETIC MOBILITY AND TITERS OF FLUORESCENT LABELED RABIES ANTISERA

Patric K. McIlwain

Department of Veterinary Science
North Dakota State University, Fargo, North Dakota

ABSTRACT

Precipitation of globulins with a 50% ammonium sulfate solution is utilized by most concerns producing fluorescent antibody reagents commercially. In addition to gamma globulins, some beta and alpha globulins are also precipitated by this method. It was felt that greater purification of such reagents would allow greater dilutions to be used for comparable fluorescence. Fluorescent labeled anti rabies sera prepared by the Sylvana Co.[1] was the reagent chosen. This, in the routine diagnostic procedures employed, gave a 4+ fluorescene against rabies virus at a 1:20 dilution. The material was passed through a 35 cm column having a diameter of 2 cm. The column was charged with Sephadex G-25 gel[2] and the vehicle was 0.85% sterile saline. The eluents were collected in 5 ml quantities. The fluorescent antisera concentrations were determined with a Beckman DU2 Spectrophotometer. The mobility of the eluents were ascertained with a Spinco Model R Electrophoresis apparatus. The greatest dilutions of the fractions giving a 4+ fluorescene were determined using brain tissues containing rabies virus. The 20 to 25 ml collected gave 4+ fluorescene at a 1:256 dilution and had the mobility of a gamma globulin.

MICROBIOLOGIC INDICATORS OF THE EFFICIENCY OF AERATED LAGOONS

John W. Vennes and Otmar O. Olson

Department of Microbiology
University of North Dakota, Grand Forks, North Dakota
and
Department of the Interior,
Federal Water Pollution Control Administration

ABSTRACT

The City of Harvey, North Dakota treats its domestic wastes with two aerated (1½ acres/cell) lagoons. Overflow manholes in the two cells allow them to be operated at 10-foot depths with a continuous discharge to the Sheyenne River. The aeration system consists of

[1]Sylvana Co., Millburn, New Jersey.
[2]Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey.

two 15 hp Sutterbilt blowers, each capable of providing 270 cfm at 9 psi. Air is distributed to the two cells through 78 diffuser lines distributing air into the primary cell and 40 lines into the second cell.

Microbiologic determinations on monthly samples, taken from raw city sewage and effluent from the primary and secondary cells, included assays for BOD, total bacterial population, enterococci and coliform organisms. Preliminary results indicate that BOD reductions averaging 90% are obtained. Although *total* bacterial numbers differed little (when comparing the raw, primary and secondary samples) their does exist significant differences in coliform and enterococci poulations. When comparing the raw waste with the secondary effluent it can be noted that during the winter months up to 2 log decreases and during the summer months up to 3 log decreases occur in enterococci numbers. Coliform decreases ranged from up to 3 logs during the winter months and up to 4 logs during the summer months.

STABILIZATION OF CHEESE MANUFACTURING WASTES

John W. Vennes, Raymond P. Rolshoven and David J. Hoss

Department of Microbiology

University of North Dakota, Grand Forks, North Dakota

and

North Dakota State Department of Health, Bismarck, North Dakota

ABSTRACT

At the present time there are at least twelve cheese manufacturing plants in North Dakota. The major waste from these plants is whey, which consists of primarily the protein, casein, and the sugar, lactose. Waste strengths from these plants may vary from a population equivalent of about 5,000 to better than 20,000.

It was the goal of this project to determine an economical method (or methods) which might be used in the state of North Dakota for the stabilization of these wastes. At the present time the study is related to final incorporation of the treated waste into a sewage lagoon. Probably the most economical method for disposal of these wastes would be their impoundment in small holding ponds. However, if one does not add air to these ponds, anaerobic conditions prevail with incomplete stabilization. At Selfridge, North Dakota, an anaerobic pond receiving cheese manufacturing waste has shown reductions in the waste strength.

Laboratory pilot experiments using equal mixtures of whey and domestic sewage has resulted in findings which can be used in the construction of larger pilot experiments. These small scale experi-

ments have been related to the amount of air, ambient temperature, and holding time required for the utilization of the large concentration of lactose and casein and their final removal by mocrorganisms. The results of these experiments and their ultimate use in stabilization ponds will be discussed.

THE USE OF DIFFERENTIAL MEDIA IN DETERMINING THE BAUCH TEST FOR *USTILAGO HORDEI* (PERS.) LAGERH

Dennis H. Lade and Larry L. Jensen

Department of Plant Pathology
North Dakota State University, Fargo, North Dakota

ABSTRACT

The Bauch test is a method of determining compatible mating types in the smut fungi by colony morphology on an agar medium. A positive test means that sporidial fusion has taken place and the two mono-sporidial lines used are of different mating types designated (+) and (−).

Standard preparations of oat meal, corn meal, lima bean, nutrient, V-8 juice, 2% potato dextrose (PDA), and a modified 2% potato dextrose medium containing 80 gm dextrose, 40 gm malt extract and 5 gm peptone were used to test mating types of sporidia from 5 isolates of *Ustilago hordei.*

V-8 juice agar and PDA gave better results than did the other media tested. The morphology of the isolates clearly indicated mating types when using V-8 juice or PDA media. Those of the same mating type were glossy and smooth while those of opposite mating types were frequently dull and coarse. The growth on nutrient agar was not as profuse as that on V-8 or PDA but compatible matings were identified. Lima bean, corn meal, and oat meal agars did not give consistent results. The growth on these media was too sparce, reflecting lower sugar concentrations. The results on malt-peptone PDA were not consistent because the high sugar content produced a glossy-watery appearnce in most isolates.

Employment of the Bauch test saves time and greenhouse space, since incompatible matings are quickly determined prior to plant inoculations.

DETERMINATION OF THE VIREMIC PERIOD FOR RAUSCHER LEUKEMIA AGENT USING INSECTS OF THE GENUS *TRIATOMA*

M. F. Bobo and R. G. Fischer

Department of Microbiology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The viremic period for BALB/c mice infected with Rauscher leukemia was determined by allowing *Triatoma infestans* to feed on members of a group of donor mice, which were infected on day 0 of the test period. One hour after feeding, the inescts were homogenized for intraperitoneal injection into normal assay mice. The infectivity of triatoma-held blood was compared with syringe collected blood and spleen homogenate of equivalent concentration obtained from the same donor animals used for insect feeding. This procedure was repeated over a period of 18 days, using 2 or 3 donor animals on each day tested.

Donor mice were viremic on day 1 and throughout the 18-day test period, with the exception of day 7. Spleen recovered from donor mice was infective on the first day and through day 18. The intensity of the leukemia induced in assay mice varied with the source of the infective material (spleen or blood) and with the post-inoculation age of donors. There was no apparent difference in the infectivity of venosu blood collected by syringe and blood ingested by *T. infestans*, indicating that the insect has no immediate inactivating effect on the Rauscher virus. Spleen homogenates consistently produced a more intense response in recipients than did blood, as indicated by a decreased time until death and both histological and hematological manifestations of Rauscher disease. This may be due to a sequestration of infectious particles in the spleen. The infectivity of both blood and spleen preparation increased steadily over the 18 day test period. However, an apparent temporary regression of the viremia occurred on day 7.

INSECTS AS EXPERIMENTAL VECTORS OF TWIEHAUS AVIAN LEUKOSIS

Kenneth D. Thompson and R. G. Fischer

Department of Microbiology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Triatoma infestans were studied as possible vectors of Twiehaus avian leukosis. Experiments were designed to establish the viremic period of chicks infected with Twiehaus virus. One group of Tria-

tomas were allowed to feed on infected chicks at each 24-hour interval until the chicks died of Twiehaus avian leukosis. One hour after feeding, the Triatomas were homogenized and injected into test chicks. The Triatomas were able to extract virus from the infected chicks from day 4 through day 11. To determine the length of time the Triatomas could harbor the virus, groups of insects were allowed to feed on Twiehaus infected chicks. One group of insects was homogenized at each 24-hour interval and injected into test chicks. Seventy-two hours after feeding on the infected chicks the Triatomas still harbored the virus. It was determined that the viremic period of Twiehaus infected chicks begins on the 4th day and continues at least through the 11th day. These studies indicate the co-existence at certain times of a viremia and establish characteristic pathology. The length of time between injection of donors and subsequent feeding by *Triatoma infestans* is, within limits, inversely proportional to the length of time until death of the test chicks receiving Triatoma homogenate.

EFFECTS OF ORGANO-PHOSPHOROUS INSECTICIDES ON MICROBIOLOGICAL PROCESSES AFFECTING SOIL FERTILITY

Devaray Naik, B. R. Funke and J. T. Schulz

Department of Bacteriology and Entomology
North Dakota State University, Fargo, North Dakota

ABSTRACT

Three organo-phosphorous insecticides· Thimet, Diazinon, and Bayer 25141 (Dasanit) were tested for possible toxicity for soil processes.

Nitrification: No significant accumulation was found with Thimet and Diazinon at applications up to 100 times field rates. The Bayer compound inhibited nitrification markedly at 10 and 100 times field rates but not at field rates. This depression was recovered by the 11th day. Apparently no lasting effects were produced.

Populations of nitrifying organisms were estimated by most-probable-number methods under field conditions. In general there was some decline in population early in the experimental period for these organisms at higher applications but it did not persist to the 30th day.

Nodulation of legumes: Soybean nodulation was sharply decreased by all insecticides at 100 times field rate, particularly by Thimet. Sweetclover nodulation was also inhibited at 100 times field rate by Thimet. Alfalfa nodulation was less at 100 times field rate with both Diazinon and Thimet. With few exceptions there was little decrease in nodulation observed up to 10 times field rates. Only when

the application was in the range of 100 times field rates were decreases commonly observed. Judging from the appearance of the plants Thimet was the most potent phytotoxically. Plants were definitely stunted and chlorotic when Thimet was applied at 10 and 100 times field rates.

Disc inhibition studies on nodulating bacteria *in vitro* showed a high degree of inhibition against several strains of *Rhizobia* by the Bayer compound, Thimet to a lesser degree, and Diazinon not at all. Similar results were recorded with other common soil organisms.

AMINO ACID DISTRIBUTION IN SELECTED CEREAL GRAINS

J. C. Crandall and F. A. Jacobs

Department of Biochemistry
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

The nutritional value of cereal grain is lysine-limited. Since a high-lysine containing genetic strain of corn has been discovered recently, a similar potential exists for the discovery and genetic development of strains of barley and wheat with higher lysine levels. Since our interests have been related to the amino acid distribution found in natural food protein, these studies were undertaken. In this initial study, special attention is focused upon the lysine content of selected samples of several varieties of barley and wheat. The intent is to find varieties of wheat and barley exhibiting significantly greater relative concentrations of this essential amino acid, as well as others.

The amino acid distributions of selected cereal grain samples are reported. Milled samples of varieties of barley and wheat from North Dakota were subjected to a sealed acid hydrolysis procedure as outlined by D. H. Spackman. The hydrolysates were analyzed using an automatic amino acid analyzer employing ion-exchange chromatography. Significant differences were noted in the relative proportions of some amino acids with respect to the total protein in the grain. (Supported in part by N.I.H. Grant AM 2023).

MASS SPECTROSCOPY OF THE 3-PHENYL-5-METHYL-ISOXAZOLE-4-CARBOXYLIC ACID AND OF ITS METHYL AND ETHYL ESTERS

Franz H. Rathmann and John Watkins

College of Chemistry and Physics
North Dakota State University, Fargo, North Dakota
and
The Hormel Institute, University of Minnesota, Austin, Minnesota
Science Department

ABSTRACT

The mass spectra of the phenyl methyl isoxazole carboxylic acid and its methyl and ethyl esters show typical mass spectra in which the middle portions of the three spectra are nearly identical. The methyl and ethyl ester spectra extend fourteen and twenty-eight units further to the parent peaks at the high end, and also yield the typical breakdown patterns of methyl and ethyl groups, respectively, at the low end of the spectrum. The electron bombardment breakdown involves primarily a loss of carboxyl and of phenyl groups, accompanied by a fission of the isoxazole ring.

5-METHYL-3-PHENYL-ISOXAZOLE-4-CARBOXYLIC ACID; PREPARATION OF ESTER, AMIDE, ANILIDE AND SUBSTITUTED AMIDE AND ANILIDE DERIVATIVES

W. E. Anderson and Franz H. Rathmann

Hillsdale High School, Jeromesville, Ohio
and
North Dakota State University, Fargo, North Dakota

ABSTRACT

The preparation of 5-methyl-3-phenyl-isoxazole-4-carboxylic acid and the corresponding 4-carboxaldehyde was carried out by Quilico and Fusco (1) and by Quilico and Panizzi (2). Further work by Quilico, Rathmann, et al. (3,4) resulted in the preparation of a number of other 3-aryl-acids as well as some derivatives of both the carboxaldelyde as well as of the carboxylic acid (5).

The present paper, the result of an N.S.F. Research Participation Program for High School Teachers during the summer of 1966, is a report on the extension of previous work to the preparation of a more extended series of esters, amides, anilides, and substituted anilides.

The following compounds were prepared by the reaction of 5-

methyl-3-phenyl-isoxazole-4-carboxylic acid chloride with suitable alcohols, amines, anilines, hydrazines etc.; -methyl ester, 75°; ethyl ester, 49°; amide· 210°; N-methyl amide, 151°; N-butyl amide, 96°; N-benzyl amide, 139°; *m*-bromoanilide, 143.5; *p*-chloroanilide, 175°; *p*-nitro-anilide, 190-200° (decompn.) hydrazide, 219°; dipiperazide, 250-270° (decompn.). A number of other substituted anilides, hydrazides, etc., were prepared, but still require further purification.

Infrared and NMR spectra showed the peaks to be expected. Mass spectra on the free carboxylic acid and on the methyl and ethyl esters (6) show similar break-down patterns, along with the specific methyl and ethyl patterns respectively for the two esters.

REFERENCES

1. Quilico, Adolfo, and Raffaello Fusco, *Gazzetta Chimica Italiana* **67,** 589-603 (1937).
2. Quilico, Adolfo, and Luigi Panizzi, *Gazzette Chimica Italiana* **68,** 411, 625 (1938).
4. Rathmann, Franz H., and Jacqueline Kay Wollan, *Proc. No. Dak. Acad. of Science,* **XIII,** 23-7 (1958).
5. Falaas, Dennis, and Franz H. Rathmann, *Proc. No. Dak. Acad. of Science,* **XVIII,** 47-50 (1963).
6. Rathmann, Franz H., and John Watkins, *Proc. No. Dak. Acad. of Science,* **XXII,** 208 (1967).

A GENERAL MECHANISM FOR THE ENZYME-CATALYZED HYDROLYSIS OF IONIZED SUBSTRATES

J. K. Tseng and J. A. Stewart

Department of Chemistry
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The pH-dependency of the chymotrypsin-catalyzed hydrolysis of ionizable substrates differs from that of non-ionizable substrates. The pH-optimum for the catalysis shifts to a more acidic value.

A general mechanism for the enzyme-catalyzed hydrolysis of ionizable substrates has been developed which can be used to explain the different pH-dependencies obtained experimentally. This mechanism is shown in Figure 1.

Mathematical solutions for the general mechanism have been derived. Using these solutions to plot pH-profiles, it is possible to compare the theoretical and experimental, and thereby, arrive at a specific mechanism. This procedure will be illustrated using chymotrypsin systems.

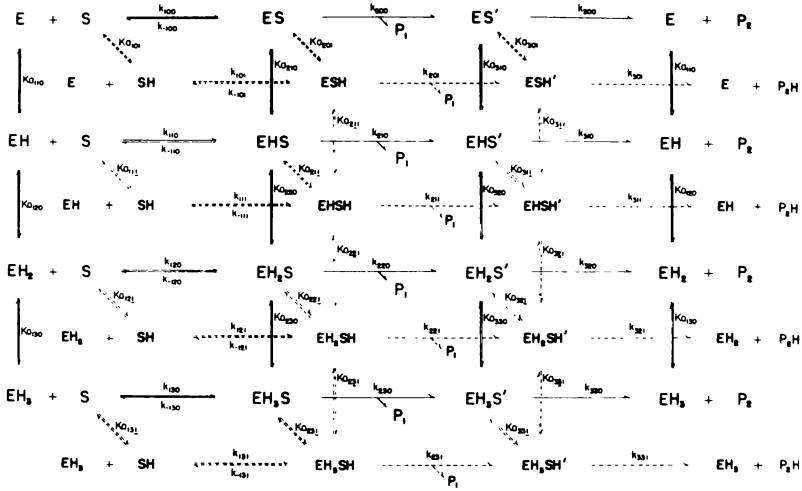


FIGURE 1—General scheme for the enzyme-catalyzed hydrolysis of ionizable substrates. E, EH, EH_2, and EH_3 are the different protonated forms of the enzymic site; S and SH are the different protonated forms for the substrate; ES, ESH, EHS, EHSH, EH_2S, EH_2SH, EH_3S, and EH_3SH are the different forms for the enzyme-substrate complex; ES′, ESH′, EHS′, EHSH′, EH_2S', EH_2SH', EH_3S, and EH_3SH' are the different forms of the acylated or alkoxylated etc., enzyme-intermediate; and P_1, P_2, and P_2H are the products of hydrolysis. The specific rate constants, K_{000}, are designated by three-digit subscripts, the first digit refers to the stage of catalysis; the second digit to the number of ionizable protons on the active site; and the third digit to the number of ionizable protons on the substrate. In a similar manner, three-digit subscripts are used to designate the various acid-base equilibrium constants, Ka_{000}, except that the last two digits refer to the number of protons in the associated form.

HYDROGEN ⇆ DEUTERIUM EXCHANGE EFFECT IN LYOPHILIZED INSULIN, HEMOGLOBIN AND HUMIC ACID

Wasyl S. Hnojewyj

College of Chemistry and Physics
North Dakota State University, Fargo, North Dakota

ABSTRACT

The D_2O vapor was adsorbed and desorbed in successive amounts on the lyophilized and vacuum dried insulin (acid form), hemoglobin (human) and humic acid (1) in a high vacuum system provided with McBain balance. An increase of weights, due to exchange of labile hydrogen of specimens-Sp(H) by deuterium from adsorbed D_2O according to the final equation $Sp(H)_n + nD_2O \longrightarrow Sp(D)_{2n} + nDOH$, were gravimetrically determined as described earlier (2).

RESULTS OF INVESTIGATION

a) *Insulin:* Referring to the amino-acid sequences together with the accepted structure of its molecule, as elucidated (3) for its smallest unit equal to 5733 of M.W. with the content of 87 labile hydrogen, the expected increase in wt. for complete H→D exchange equal to 1.517 mg/100 is in good agreement with our experiments.

At a higher temperature, however, observed some loss of original sample during high vacum desorption due to probably sublimation.

b) *Hemoglobin:* Referring to amino-acid composition (4) the M.W. could be estimated equal to 64,204 with 4 H_2O bound to hemes for "the deoxygenated form" and 64,259 for "the oxygenated form," with the number of labile H-atoms present equal to 912 and 904 respectively.

The theoretically expected increase of completely vacuum dried sample equal to 1.420 and 1.406 mg/100 seems in agreement with experiments in a relatively wide temperature range, which do not cause any "sublimation."

c) *Humic Acid:* Increase of weight due to $H \longrightarrow D$ exchange was found equal to 0.781 mg/100 for $H\text{-}Ac_{(Na)}$ (5). It depends, however, upon the preparation method (1) of the humic acid sample.

The observed instability of humic acid (loss of wt.) progresses with rising temperature during high vacuum desorption, indicating lower molecular wt. Data are discussed related to results obtained with the above mention protein.

REFERENCES

1. Johnson, S. O., and Hnojewyj, W. S., Proc. N. Dak. Acad. Sci., **20,** 180 (1966).
2. Hnojewyj, W. S., and Reyerson, L. H., J. Phys. Chem., **65,** 1694 (1961).

3. Ryle, A. P., Sanger, F., Smith, L. F., and Kitai, R. Bioch. Jour., **60,** 541-556 (1955).
4. Branitzer *et al.,* and Konigsberg *et al.,* Advances in Protein Chemistry **16,** 297 (1961), Acad. Press, Inc., New York and London.
5. Ferche, D. S., Ph.D. Thesis: Surface Studies of H-humates and Humic Acid(s) derived from Lignite, North Dakota State University, 1967.

GRAN'S METHOD FOR LINEAR PRESENTATION OF TITRATION DATA IN SEVERAL POLYBASIC ACIDS, GLUTAMIC AND CARBONIC ACIDS, HISTIDINE, CYSTEINE AND ASPARAGINE

W. S. Shore and P. A. Setness

Department of Chemistry
Concordia College, Moorhead, Minnesota

ABSTRACT

This paper simplifies and applies Gran's method to the above acids. The scheme leads to more accurate equivalence points and indicates where acid or base is an impurity or whether the titrand is contaminated. Utilizing all the data points, even in the buffer region, the treatment provides the dividend of pK values consistent with these points. Though pK values separated by as few as two units may be resolved (cysteine), the method applies only to $3<pK<11$. That is, no way is proposed for correction of hydrogen or hydroxyl ion.

AMINONITRILE COMPLEXES OF SOME TRANSITION METAL IONS

James M. Olson and Howard L. Haight

Department of Chemistry
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Several coordination compounds involving aminonitriles as ligands with Cu(I), Cu(II), Zn(II), Ag(I) and Cd(II) have been synthesized. The ligands used were *p*-aminobenzonitrile, ω-aminocapronitrile and aminoacetonitrile.

Preparation of these complexes was accomplished by dissolving the ligand in methanol and mixing with a suitable solution of the metal salt. In most cases a solid compound crystallized out within 24 hours.

P-aminobenzonitrile gave crystalline compounds with Cu(I), Ag(I) and Cd(II). The cadmium complex was nearly colorless and had the composition $CdCl_2L_1$ ($L = C_7H_6N_2$). It crystallized in the orthorhombic system with cell constants determined by X-ray diffraction to be 7.16 Å, 21.1 Å and 23.5 Å. The measured density of 2.27 g/cc indicated there were 16 formula weights per unit cell.

The Ag(I) complex with *p*-aminobenzonitrile gave rather poorly defined yellowish crystals and preliminary data indicate a complex containing three moles of ligand per mole of $AgN0_3$.

When the above procedure is used to prepare the Cu(I) complex, $CuCl_2$ is reduced to CuCl by bubbling SO_2 through the solution before the ligand is added. Upon addition of the solution containing the ligand, black crystals precipitate. These crystals have the composition $Cu_3Cl_4L_2$ ($L = C_7H_6N_2$). This stoichiometry suggests that copper is present in both the +1 and +2 oxidation states with two moles of CuCl per mole of $CuCl_2$. Preliminary data indicate this compound crystallizes in the monoclinic system with β approximately equal to 91° and having cell constants of 6.9 Å, 11.7 Å and 11.9 Å. The measured density is 2.07 g/cc and there are two formula weights per unit cell. Systematic absences when $1 \neq 2n$ for h01 data and when $k \neq 2n$ for 0k0 data lead to the selection of the unique space group $P2_1/c$.

The ω-aminocapronitrile gives compounds with Cu(I), Cu(II), Zn(II), Ag(I) and Cd(II). Characterization of these compounds is currently in progress.

Studies using aminoacetonitrile as ligand indicate that complexes are formed but they are too soluble to precipitate from solution.

PRELIMINARY INVESTIGATION OF NORTH DAKOTA BENTONITES FOR USE IN TACONITE PRODUCTION

Charles F. Metzger

Department of Geology

North Dakota State University, Fargo, North Dakota

ABSTRACT

In the last two years the Minnesota mining companies have made rapid steps in developing a process for concentrating their low grade ores, making them suitable for commercial steel use. This concentrating of the low grade ores is called the taconite process. Because of the fine powder consistency of the concentrated ore, it was necessary to develop a binder in order to make the taconite acceptable for blast furnace use. Certain bentonites were found to be excellent as a binding material.

In light of this new application of bentonites a more extensive look at our North Dakota resources was initiated. Random sampling was carried out in a 150 square mile area which was located from

the North Unit of Theodore Roosevelt National Park, north to route 85. This encompassed portions of Long X Divide, Sperati Point, Teepee Buttes and Stocke Butte Quadrangles. Seventy spot-samples were taken and analyzed by x-ray diffraction for their mineralogical content. The samples are from the Big Blue bed which is a bentonite layer in the Tertiary Sentinel Butte member of the Tongue River Formation. The Big Blue is a shelf forming bed, generally exposed on the sides of the buttes found in this area. It is generally eight to fifteen feet thick and composed of alternating blue and light gray layers. Samples were taken from both sequences.

The mineralogy of the bentonites is fairly homogeneous with the major variations occurring between the blue and the light gray sequences. The predominant clay mineral in the blue sequence is a sodium montmorillonite with minor amounts of illite and kaolinite. The non-clay components are quite consistently quartz, cristobalite, plagioclase, calcite, dolomite, mullite, and chlorite.

In the light gray sequence greater variation occurs in the major clay mineral. Some samples showed a definite sodium montmorillonite composition, while others definitely developed a sodium-calcium montmorillonite. Illite and kaolinite were still present. The non-clay mineral distribution was similar to the blue sequence, only the occurrence of dolomite, calcite and mullite was not as consstent. The x-ray diffraction patterns also indicated that the actual clay mineral content of the blue sequence was higher than that of the light gray sequence.

The presence of a large quartz percentage is understandable since the bentonites are genetically related to volcanic ash.

Clay mineralogically the North Dakota bentonites have possible commercial value, but because of the relatively large number of non-clay components in the bentonites, further investigations on the physical properties such as viscousity, water holding capacity, and pelletizing ability must be carried on in order to definitely determine the commercial value of these deposits.

STUDIES *IN VITRO* ON THE EFFECT OF THIOBARBITURATES ON VASCULAR SMOOTH MUSCLE

H. S. Richmond, D. L. Pastoor and B. DeBoer

Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Previous investigation in this laboratory has shown that *in vivo* perfusion of hind limbs of dogs or cats resulted in vasoconstriction when thiobarbiturates were added to the perfusate and no change or slight vasodilation when oxybarbiturates were perfused. In order to study this effect in more detail, rabbit and beef arterial strips

were tested in a tissue bath using a Casella automatic bioassay apparatus. This method makes it possible to treat the same preparation with several drugs and also to pretreat with blocking agents.

In these experiments barbiturates 5.0 mg/ml in physiological saline were the test drugs. The oxybarbiturates pentobarbital and secobarbital were compared with their thioanalogs thiopental and thiomylal, respectively. Since these substances would not remain in solution at a pH of less than 9 artery strips were also tested in physiological saline at this pH. In order to compare the effects of the sulphur moiety, the non-barbiturate thiouracil was tested at the same pH. Arterial strips showed contraction with thio-barbiturates and thiouracil. Tissue treated with oxybarbiturates showed no change in the contractile activity. Tissue bathed in physiological saline at a pH of 7.4 or saline at a pH of 9 showed no contraction. Artery strips pretreated with phenoxybenzamine, an alpha adrenergic receptor blocking agent, did not respond to either thio or oxybarbiturates.

This confirms the *in vivo* observations and lends support to the explanation previously suggested by workers in our laboratory that perfusion with thiobarbiturates causes the release of norepinephrine from the arterial wall resulting in vasoconstrction. Oxybarbiturate perfusion does not result in norepinephrine release. The constrictor effect of thiouracil lends support to the theory that the sulphur moiety is essential to the reaction. The blocking action of phenoxybenzamine also suggests that the constrictor effect is not due to direct action of the sulphur or to the pH change but is mediated by an adrenergic agent, presumably norepinephrine released from the arterial wall.

PURIFICATION AND PROPERTIES OF ARYLAMINE N-GLUCOSYLTRANSFERASE FROM SOYBEAN

D. Stuart Frear

U. S. Department of Agriculture

Metabolism and Radiation Research Laboratory, Fargo, North Dakota

ABSTRACT

A major pathway in the metabolism of several herbicides by plants now appears to be the biosynthesis of N-glucosylamines.

A soluble enzyme system from soybean acetone powders, which catalyzes the synthesis of N-glucosylamines, has been purified several fold and partially characterized. The enzyme was found to be specific for uridine diphosphate-5'-glucose (UDPG), but exhibited a rather broad specificity for acceptor anilines. The partially purified

enzyme was used in studies of its optimum pH, Km values and inhibition.

The possible role of this enzyme in the metabolism of several herbicides in plans will be discussed.

RELATIONSHIPS BETWEEN PHYSICAL AND CHEMICAL SORPTION WITH REGARD TO FUMIGANT RESIDUES IN FOODS

Ben Berck

Canada Department of Agriculture
Research Station, Winnipeg, Manitoba

ABSTRACT

Fumigant residues, defined as the amount of chemical product remaining in or on foods after application of the fumigant, are of two types, namely, physically bound residues (pbr) and chemically bound residues (cbr). Criteria for distinguishing pbr from cbr are outlined. The influence of exposure period (equivalent to contact time) must be recognized, since a given pbr level may be transitory, and may diminish due to (a) continued volatilization and diffusion to the outside atmosphere, or (b) conversion to cbr. The rate of pbr $\longrightarrow$ cbr transformation may vary, depending on the type of fumigant and conditions of use. A low pbr value may also be due to low affinity of the substrate for the fumigant molecules, and a low cbr value may be due to weak chemisorption. The influence of fumigation temperature and the nature, moisture content and particle size of the substrate on cbr formation are illustrated with examples from the author's current research with phosphine, PH_3.

EFFECT OF HYPERBARIC HELIUM ON RED BLOOD CELL FRAGILITY

R. E. Thompson and T. K. Akers

Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Recent observations on human red blood cells subjected to two and three atmospheres of pressure indicate an alteration, either mechanical or chemical, within the RBC, leading to distortion and hemolysis. The hemolysis, checked by the RBC fragility test with hypotonic saline, is apparently concomitant with the degree of pressure used and more specifically with the gaseous composition of the atmosphere.

This preliminary study using dog blood is concerned with quanti-

tative and qualitative dog RBC changes at 0 psig (1 atm.), 68 psig (5 atm.), and 135 psig (10 atm.), using 90% helium and 10% ambient atmosphere mixtures. Results on RBC subjected to the above respective pressures for predetermined times and drawn from the chamber have shown that increased helium pressures make dog RBC susceptible to hemolysis when placed in NaCl solutions ranging from 0.50% to 0.28% concentration. Observations have shown that a shift of the normal range curve occurs at higher than atmospheric pressures under the influence of hyperbaric helium. However, the exact nature of the cause for hemolysis changes is not understood.

UREA EXCRETION IN THE HIBERNATING COLUMBIAN GROUND SQUIRREL

John C. Passmore and E. W. Pfeiffer

Department of Zoology
University of Montana, Missoula, Montana

ABSTRACT

Ground squirrels (*Citellus columbianus*) avoid stressful external conditions of food and water shortage by hibernating, but in doing so may become faced with stressful internal conditions. Since the kidney appears to excrete little or no urine during this time it is reasonable to ask if there is urea buildup in the blood of the hibernating ground squirrel.

The squirrels (*C. columbianus*) were placed in cages in a refrigerator and observation was made to determine when each squirrel began hibernating. Then, at the end of the cycle, which lasted from 7 to 15 days urine was collected under mineral oil and a blood sample was taken by heart puncture. After a second hibernation period of 1 to 3 days less than the first cycle the animal was sacrificed, a blood sample taken by heart puncture, and all urine was aspirated from the bladder.

Four non-hibernating summer ground squirrels were dehydrated in wire cages for 1 day with no food or water. Blood samples were taken at start and finish and all urine that was voided was collected.

Urea concentration and osmolality were determined for all samples collected.

The mean serum urea concentration (S. U.) of 7 of the 10 winter hibernation experiment animals at the first arousal was appreciably lower than the S. U. of these animals when they were sacrificed ($P<0.001$). Also when the total amount of urea in all the body fluids was calculated on the basis of what it was in the serum, all ten squirrels showed a significant increase between the mean body urea at entrance into and the mean body urea at sacrifice during hibernation. On the other hand the summer dehydrated squirrels under-

went a significant ($P<0.05$) drop in S. U. demonstrating that non-hibernating ground squirrels with functioning kidneys tend to clear their blood of urea.

In the winter sacrificed ground squirrels, neither the volume of bladder urine nor its total urea content differed between those squirrels sacrificed after 4 days of hibernation and those squirrels sacrificed after 9 days of hibernation. So it is impossible to hypothesize that urine is deposited in the bladder at any regular rate during hibernation. It was assumed that any urine found in the bladder at sacrifice was left there from the previous arousal.

The percentage of the osmotic concentration that was composed of urea was definitely higher in the urine of the winter animals ($P<0.05$) than in the summer dehydration experiments. This is evidence that perhaps the winter squirrel was excreting more urea with less water loss.

PITUITARY-ADRENAL REGULATION OF PLASMA CERULOPLASMIN IN THE RAT

Gary W. Evans and R. E. Wiederanders

Harmon Park Research Laboratory, Williston, North Dakota

ABSTRACT

Copper metabolism studies in this laboratory have shown that hypophysectomy and bilateral adrenalectomy cause a significant rise in ceruploplasmin oxidase activity in the rat. In contrast, ACTH in gelatin (10 I.U. per day for 15 days) administered to intact animals caused a significant decrease in ceruloplasmin activity. Corticosterone (2..5 mg per day for 15 days) given to adrenalectomized rats lowered the oxidase activity significantly. The total plasma copper concentration increased or decreased correspondingly for each of the above groups, indicating that the oxidase activity is a measure of the ceruloplasmin concentration.

Ceruloplasmin is a protein and as such depends upon RNA synthesis for its production. Recently, steroid hormones have been shown to alter protein synthesis by acting at the site of RNA synthesis. This implies that the level of circulating steroids could influence ceruloplasmin concentration. In the neonatal rat, ceruloplasmin activity is low until the 20th day, and thereafter rises slowly to adult levels. It has been shown that the level of corticosterone in the neonatal rat decreases from birth until adulthood. This indicates an inverse relationship between the corticosterone level and the level of ceruloplasmin.

Extirpation of the adrenal glands possibly removed an inhibitor of RNA synthesis thus allowing a rise in ceruloplasmin levels. ACTH administered to the intact animal provoked a decrease in ceruloplasmin by causing an increase in the level of adrenal steroids. ACTH

given to the adrenalectomized animals brought about no changes in ceruloplasmin levels while corticosterone, administered to adrenalectomized rats, lowered the ceruloplasmin significantly.

Our present data indicate the role of the pituitary-adrenal axis in regulating the ceruloplasmin level. Regulating the level of circulating steroids, the pituitary-adrenal system may control ceruloplasmin production at the site of RNA synthesis.

SOME EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF JUGLONE TO RATS[1]

John P. Ries and T. K. Auyong

Department of Physiology and Pharmacology
University of North Dakota Medical School,
Grand Forks, North Dakota

ABSTRACT

Juglone, 5-hydroxy-1,4-napthoquinone, was reported to be a depressant agent to a number of laboratory animals. It was also shown to be toxic to kidney and lungs when administered intravenously to dogs. This study was performed to further elucidate some of the properties of juglone. One week prior to injection of juglone, hematocrit, serum potassium and sodium as well as plasma protein levels were determined on rats. Juglone dissolved in corn oil was administered to 2 groups of rat intraperitoneally at doses of 2 mg/kg and 3 mg/kg respectively. Control animals received corn oil only. Twenty-four hours after injection the animals were sacrificed and the blood levels of the various parameters mentioned above were determined. The lungs of the animals were removed for measurements of their water content. Any fluid aspirated from the peritoneal cavity was measured and its electrolyte and protein content determined.

In both groups of animals that received juglone there was noted a rise in the hematocrit of about 30% and an accumulation of peritoneal fluids of about 30 ml/kg. Plasma potassium levels rose significantly in both groups that received juglone. Sodium levels were not significantly different in control as well as the experimental groups. The wet wt./dry wt. lung ratios revealed a significant decrease in water content of the lungs of animals that received juglone.

These results seem to indicate that juglone may affect the permeability of vessel walls permitting an efflux of fluids into the peritoneal cavity with subsequent changes in serum protein levels. This loss of fluid from the circulation into the peritoneal cavity may be

[1]Guy and Bertha Ireland Research Laboratory, School of Medicine. Supported in part by PHS Grant HE-09652-02.

reflected in the loss of water in the lung and hemoconcentration as evidenced by the rise in hematocrit. The increase in potassium level may indicate a change in cellular membrane permitting a loss of intracellular potassium.

THE EFFECT OF INTRAVENOUS ADMINISTRATION OF JUGLONE IN THE DOG[1]

James N. Boelkins, L. K. Everson and T. K. Auyong

Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Juglone (5-hydroxy-1,4-napthoquinone), a natural product of the walnut tree, has demonstrated fungistatic activity, *in vitro* depression of smooth and cardiac muscle, CNS depressant effects in fish, mice and rats, abdominal fluid accumulation following intraperitoneal injection in the rat, and toxicity to the lungs and kidneys when administered IV to dogs. This study was undertaken to determine the *in vivo* cardiovascular responses to juglone in the dog.

Juglone, in a 5% glucose and 47% ethanol mixture was injected IV, 5 mg/kg, to eleven "experimental" animals. A corresponding volume of the ethanol/glucose vehicle was given to seven "control" animals. Following a control period, juglone and/or the vehicle was injected and recordings were made hourly for six hours following injection. The parameters measured were: hematocrit, heart rate, respiration rate, electrocardiogram, blood and plasma specific gravity. At the end of 6 hours the animal was sacrificed and the right superior lobe of the lung was removed for wet-dry weight determination.

The results indicate a significant rise in blood specific gravity, hematocrit, respiration rate and the lung wet-dry weight ratios. The plasma specific gravity showed a significant decrease. The heart rate and electrocardiogram did not show significant changes. From these findings it appears that juglone acts at the capillary and tissue cell level with the lung being a primary target. This is manifested by the increased hematocrit, respiration rate and lung wet-dry weight ratio.

[1]Guy and Bertha Ireland Research Laboratory, School of Medicine. Supported in part by PHS Grant HE-09652-02.

AUTHOR INDEX

AUTHOR INDEX