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VOLUME XX

1966

Published jointly by the University of North Dakota, the North Dakota State University of Agriculture and Applied Science and the Jamestown College

April, 1967

GRAND FORKS, NORTH DAKOTA

Printed by
THE UNIVERSITY OF NORTH DAKOTA PRESS
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SUGGESTIONS TO AUTHORS OF PAPERS FOR THE NORTH DAKOTA ACADEMY OF SCIENCE

Deadlines.—Both student and professional abstracts are due April 1. Student competition manuscripts are due on April 10. If the professional paper is to be published in complete form, it is to be submitted at the time of presentation. If these deadlines are not met, the paper will not be published.

Abstract.—Abstracts must be submitted on the prescribed form or an exact facsimile available from the Office of the Secretary. If this space is inadequate the Editorial Board suggests that a full manuscript be submitted even though the paper may be short. References in abstracts should be avoided if possible. If not possible, incorporate these in the running text within a set of parenthesis.

General.—Manuscripts must be typed double spaced with wide margins on one side using 8½" × 11" white paper. The manuscripts should be submitted in duplicate.

Authors must pay strict attention to details of presentation so that the final manuscript is a clear, concise, neat, orderly, and *craftsmanlike* product with consistency of style. Corrections in galley proof ordinarily can be made only for errors in typesetting. Consequently the author's manuscript should be in proposed final form when submitted. Changes after the galley proof is set can be made only with the approval of the Editor.

Headings.—Center headings are typed above major divisions, centered, and in capitals.

Side headings.—Material under one major division of the paper may be subdivided by side headings. These are indented for paragraphing, underlined once for italics, and followed by a period and dash (two hyphens).

Carefully organized papers commonly employ the following examples of center headings: INTRODUCTION, METHODS, RESULTS, DISCUSSION, SUMMARY, REFERENCES; or, INTRODUCTION, DISCUSSION, EXPERIMENTAL, ACKNOWLEDGMENTS, REFERENCES.

Figures.—Maps, drawings, graphs, complex tables (see below), and structural chemical formulas cannot be set in type and must have line cuts made separately by the printer. Therefore these must be submitted on separate sheets following the typescript as India ink drawings on high quality white paper or tracing paper or linen. In no case can figures or structural formulas be accepted on the same sheet with text material.

Make your figure (drawings or photographs) the exact size or

multiple thereof that it is to appear in the *Proceedings*. Therefore a full page figure is 4⅛ inches wide by 6¾ inches high (printed page area). A one-half page illustration is 4⅛ inches wide by 3⅜ inches high. Allow adequate space for the caption at the base of the figure.

Remember that line drawings must fit on a *Proceedings* page. Therefore make all lettering, dots, lines, etc., bold enough to stand appriate reduction, if any. Photographs should have a glossy finish; the picture must be unblurred and show sharp contrast between light and dark areas. *Plan your figures carefully!*

Figure captions should be typed and included on a separate page(s) with the manuscript (*not* as a part of the figure). Figures should be numbered consecutively in Arabic numerals and their approximate position indicated in the margin of the running text. Each original drawing must be identified on its back with the figure number, the author's name and a designation as to the top of the figure. Multiple illustrations within a given figure must be clearly identified.

Tables.—Simple tables are normally run in the text. Complex tables are difficult to compose on a linotype machine. Therefore tables with vertical lines, too many repeating characters per horizontal line, or unusually extensive tables should be drafted with India ink and submitted separately as figures for printing as line cuts (see instructions under figures, above). The caption should be above the table, in capital letters and designated with Roman numerals.

Sample table caption: TABLE I

THE EFFECT OF pH ON REACTIVITY
OF CHYMOTRYPSIN

References.—The list of references cited at the end of the paper (or abstract) must have the center heading—REFERENCE, or REFERENCES. The style of the individual references should be in the style of the best journals or authorities in the field of the author. The principal points are (a) *consistency* within the author's list and (b) complete citations (both book and journal). The references may be arranged (a) numerically in order of citation in the text, or (b) alphabetically by author's last name.

Examples:

(chemist) 2. A. P. Krapcho and M. E. Nadel, J. Am. Chem. Soc., *86*, 1096 (1964).

(geologist) Freers, T. F., and Carlson, C. G., 1963, Geology along the Portal pipeline, Lake Agassiz Plain: N. Dak. Acad. Sci., Proc., v. 17, p. 86-95.

(biologist) Schwartz, R. J. 1955. The complete dictionary of abbreviations. T. Y. Crowell Co., New York. 211 p.

Footnotes.—Footnotes are costly and should be kept to a minimum[1]; parenthetical remarks can almost always be phased to fit the text. Do not put citations to the literature in a footnote.

Citations.—Citations should be included in the text by use of a number in parenthesis or by weaving the author's name with the date of work and page into the sentence structure (see examples). In the former situation, the references at the end will be arranged numerically; in the latter case the references will be arranged alphabetically (see *References* above).

Examples:

> Jones (2) stated that science education is improving. However, this is a controversial issue according to others (3-6). *Ed. Note:* 3-6 means references 3, 4, 5 and 6.
>
> Jones (1962, p. 22) stated that science education is improving.
>
> It has been stated that science education is improving (Jones, 1962, p. 22).

It is usually preferable to refer to figures and tables in the text. This should be done according to the following example.

Hence Table III shows the data derived with this equipment (Figure 2). In Figure 3, the data of the previous figure and tables are summarized.

Acknowledgments.—If grants are not acknowledged in the text (as they probably would not be when only the abstract is to be published) they may be included as a footnote. Authors should check with the granting agency for preferred wording (if any); the title and number of the grant should be exact, as the Editorial Advisory Committee has no way of checking this information. In a full paper the acknowledgments are put in as a separate paragraph under the heading, ACKNOWLEDGMENTS, preceding the REFERENCES.

Miscellaneous.—Words underlined in the text mean "put in italics" to your editors and the linotypist.

All generic and specific names should be underlined.

All metric system abbreviations will be considered as symbols and thus will **not** be followed by periods unless occurring at the end of a sentence (mm, ml, cm, cc, g, mg, and μg).

Special instructions to the editors can be included as notes in the margin, e.g., suggested location of figures and tables. Any other

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[1]Notice how this footnote breaks your thought; however, it serves as a model of method of insertion if you *must* use a fotnote.

questions of form in format or writing should be in the style of the best journals or authorities in the field of the author.

Charges to Authors.—The cost of publishing the *Proceedings* has continually increased. For this reason, the Editorial Advisory Committee has been empowered by the Academy to assess charges to authors to help meet publication costs. Papers in excess of five printed pages will be assessed $9.00 for each page over five; figures in excess to two per paper will be charged at $9.00. Exceptions can be granted in unusual cases at the discretion of the Editorial Advisory Committee.

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1. Title page (separate sheet).
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3. Tables (separate sheets, if too large to fit the text).
4. Footnotes (separate sheet(s)).
5. Captions (legends) for figures (separate sheets).
6. Figures.

Each sheet of submitted material must be identified.

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F. D. Holland, Jr.
Virgil I. Stenberg
Revised, 1967
Edwin M. Anderson
Alan M. Cvancara
F. A. Jacobs
Virgil I. Stenberg
Warren Whitman

Additional copies of these "Suggestions to Authors" are available from the Secretary of the Academy.

THE SYNTHESIS AND POLAROGRAPHIC ANALYSIS OF SUBSTITUTED 6,6-DIPHENYLFULVENES

Gary Norton and Jerome Knoblich

Department of Chemistry

Jamestown College, Jamestown, North Dakota

First Place Winner
A. Rodger Denison Student Research Competition

INTRODUCTION

6,6-Diphenylfulvenes are highly conjugated hydrocarbons having

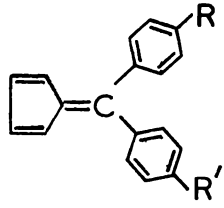


FIGURE 1

a deep red color. The structure of a 6,6-diphenylfulvene is indicated in Figure 1, where R and R′ represent various substituents.

Wawzonek and Wang Fan have shown that 6,6-diphenylfulvenes undergo reduction at the dropping mercury electrode(1). The purpose

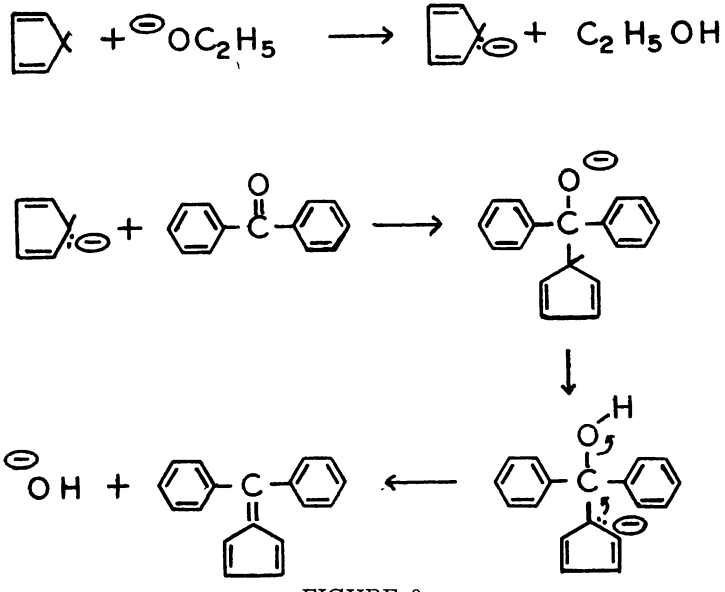


FIGURE 2

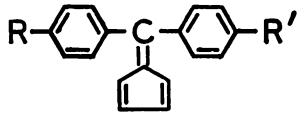
of this study is to correlate the ease of polarographic reduction and the substituents on 6,6-diphenylfulvene. The polarographic half-wave potential was used as a convenient measure of this ease of reduction.

DISCUSSION

The synthesis of substituted 6,6-diphenylfulvenes was accomplished by reacting freshly distilled cyclopentadiene with substituted benzophenone in the presence of sodium ethoxide. The mechanism for this reaction is believed to be as indicated in Figure 2.

Table I shows the analysis values, melting points, and polaro-

TABLE I



R	R′	M.P.	$-E_{1/2}$ vs. S.C.E.	CALCD. % C	CALCD. % H	FOUND % C	FOUND % H
Cl	Cl	113-115[2]	1.47				
Cl	H	74-75[2]	1.51				
Br	H	91-94	1.51	69.92	4.24	70.21	4.56
F	F	98-100	1.53	81.12	4.55	81.30	4.91
F	H	86-87	1.54	87.07	5.28	87.08	5.30
H	H	79-80[2]	1.55[1]				
CH_3	H	67-69	1.58	93.41	6.60	93.61	6.66
CH_3	CH_3	104-106[2]	1.60				
OCH_3	H	84-86	1.60	87.67	6.20	87.57	6.30
OCH_3	OCH_3	109-111	1.63	82.71	6.25	82.36	6.37

graphic half-wave potentials for the substituted 6,6-diphenylfulvenes investigated by the authors.

Wawzonek and Wang Fan(1) have indicated that two electrons are involved in the polarographic reduction of diphenylfulvene and that the process is similar to that involved in the reduction of numerous other unsaturated hydrocarbons. Electron-withdrawing substituents should decrease the electron density in the region of the double bond, thus making electron addition to the double bond less difficult and, consequently, lowering the half-wave potential. Similarly,

[1]Wawzonek and Wang Fan(1) reported: $-E_{1/2} = 1.57$

[2]Elemental analyses and melting points for these compounds are reported in ref. (2).

electron-donating substituents should increase the electron density around the double bond, thus increasing the voltage necessary to force electrons into the comparatively electron-rich region. This reasoning is supported by the data compiled for all substituted 6,6-diphenylfulvenes investigated, with the exception of the fluoro-substituted 6,6-diphenylfulvenes.

A large depression of the half-wave potential might be expected for 6-phenyl-6-(*p*-fluorophenyl)fulvene and 6,6-bis-(*p*-fluorophenyl) fulvene since fluorine is highly electronegative. Our results show that the fluoro compounds do not exhibit this expected behavior. This deviation is not entirely surprising as contributions from resonance structures of the type shown in Figure 3 should be greatest for fluorine. Indeed, a good correlation exists between the half-wave

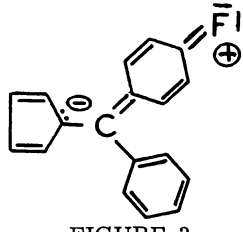


FIGURE 3

potentials and Hammett σ values for a number of *p*-substituents as as shown in Table II.

TABLE II

p-Substatuted Diphenylfulvene			Hammett σ Values (3)	
R	R′	E½	Group	σ
Br	H	—1.51	*p*-Br	+0.232
Cl	H	—1.51	*p*-Cl	+0.226
F	H	—1.54	*p*-F	+0.062
H	H	—1.56	H	0.000
CH_3	H	—1.58	*p*-CH_3	—0.170
OCH_3	H	—1.60	*p*-OCH_3	—0.268

I. *Apparatus.*—The polarographic reductions were performed with an Electrochem Auto-Scan Polarograph in connection with a Lab-Line Graphicorder. An H-cell containing a saturated calomel reference electrode and a dropping mercury electrode was used.

II. *Materials.*—Commercially available benzophenones were used in the preparation of the 6,6-diphenylfulvenes, with the exception of 4,4′-difluorobenzophenone, which was prepared by the reaction of 4-fluorophenylmagnesium bromide with 4-fluorobenzonitrile. Subsequent hydrolysis of the imine yielded 4,4′-difluorobenzophenone, which melted at 103-105°C.

An efficient fractionating column was found necessary to obtain pure cyclopentadiene from dicyclopentadiene.

A standard procedure was used to purify mercury(5).

After the dioxane was refluxed with sodium for several hours, it was filtered and distilled. The dioxane was stored in a frozen state to maintain purity.

III. *Method of Synthesis.*—The fulvenes were prepared by the general method of Kresze(2). A few modifications of the general procedure were made such as reaction time, reaction temperature, and the use of chromatography in purification.

Freshly distilled cyclopentadiene, benzophenone, and sodium ethoxide were dissolved in ethanol under a nitrogen atmosphere and allowed to react for twenty-four hours at 15°C. Higher temperatures appeared to cause polymerization.

Chromatography of the reaction mixtures on acid-washed alumina effected good separations while basic alumina effected poor separations. Further purification was obtained by recrystallization from low-boiling petroleum ether.

The melting points were taken in a capillary tube with a Thomas-Hoover Uni-Melt and are uncorrected.

The elemental analyses were done by Midwest Microlaboratories, Inc., Indianapolis, Ind.

IV. *Polarography.*—The same capillary, mercury head. and temperature (25.0±0.1°C.) was used for all measurements. The solutions used had the following composition: 0.175 M tetrabutylammonium iodide, 0.001 M fulvene, 75% dioxane. The half-wave potentials were determined by a graphical method outlined by Delahay(4).

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 6,6-diphenylfulvenes more difficult. Two substituents on the molecule approximately doubles the effect of one substituent.

ACKNOWLEDGMENT

The authors are indebted to Vernon Feil for his assistance in the completion of this project.

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ADSORPTION OF CARBON DIOXIDE ON H-HUMATE AND ON PURIFIED LYOPHILIZED HUMIC ACID FROM LIGNITE

Dennis S. Ferche[1] and Wasyl Hnojewyj

College of Chemistry and Physics

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

Second Place Winner

A. Rodger Denison Student Research Competition

ABSTRACT

Isotherms for the adsorption of carbon dioxide on vacuum-dried H-humate, prepared from lignite, have been established at 25° and 35°. In the range of 0-500 mm of equilibrium pressure, these isotherms show five and four slopes, respectively. The relationship of the observed slopes to the presence of various active sites in the complex H-humate molecule(s) is discussed.

Values of the calculated isosteric heats of adsorption are presented and correlated to the behavior of the adsorption of carbon dioxide on the active sites; these values were found to vary in the range of 5-9 kcal/mole.

Adsorption isotherms for carbon dioxide at -78° and 25° were established on a humic acid sample derived from the H-humate as described by Johnson and Hnojewyj (1). This sample, because of lyophilization, had a highly accessible surface, which enabled more complete exposure of the active sites to gases during adsorption. The isotherms performed at 25° on the lyophilized humic acid and on the H-humate are compared; increased adsorption and greater ease of attaining equilibrium were noted for the lyophilized humic acid.

INTRODUCTION

Previous investigations by Hnojewyj (2) have indicated that high-vacuum techniques can be applied to the characterization of the complex humic acid molecule(s) in a manner similar to that for proteins (3). Hnojewyj's initial study employed the isothermal adsorptions and desorptions of water vapor on Baroid H-humate samples. The results suggested the presence of at least three different sites (functional groups), which were accessible and distinguishable under the conditions employed.

To clarify these sites, Hnojewyj encouraged further studies utilizing a humic acid sample obtained by extraction of H-humate with base followed by dialysis for purification and lyophilization for surface development (1). With lyophilization performed, the attainment of equilibrium conditions during isothermal studies was

[1]Graduate Student, Physical Chemistry, North Dakota State University of Agriculture and Applied Science.

expected to be enhanced. The desired goal was to enable adsorption to occur at the molecular level rather than on highly associated "molecular clusters," which could cause shielding of active sites.

It is noteworthy that neither the H-humate nor the lyophilized humic acid samples were involved in any strong chemical or physical treatments. Because of this, any drastic effects in the total functionality were unlikely.

Other attempts have been made to expose the surface structure of substances similar to humic acid. Ihnatowicz et al. (4) employed chemical treatment which resulted in further opening of the structure; however, they noted drastic changes in the functionality.

Previous to experimentation, thoughts on what to expect during adsorption were influenced by the number and nature of the functional groups reported for humic acids and related materials. Wood and Moschopedis et al. (5, 6) have investigated the distribution of oxygen containing groups in the Baroid H-humate. The information they reported is entered in Table I.

TABLE I
FUNCTIONAL GROUPS PRESENT IN HUMIC ACID

Groups	Amount mequiv/g
methoxyl	1.7
phenolic OH	2.7
total carboxyl	4.3
carboxyls close enough to form anhydrides	3.5
isolated carboxyl	0.8
quinones	0.8

In addition, Bhaumik (7) reported evidence for some aromatic-aliphatic ethers in low rank coal.

Keeping in mind the possibility of our sample having any or all of the above functional groups (sites), it was decided to determine adsorption isotherms with carbon dioxide as the adsorbate. This was done in order to clarify the nature of the adsorbents. Carbon dioxide, as the adsorbate, is of interest because although CO_2 has no permanent dipole, the CO_2 molecule is polarizable (8). This characteristic of carbon dioxide led us to expect that the adsorption isotherms may indicate slopes resulting from different degrees of association between carbon dioxide and the active sites.

EXPERIMENTAL

Apparatus and Procedure.—The gravimetric, high-vacuum, apparatus utilized and its manipulation were similar to those described earlier (2). The apparatus provides a sensitivity of better than 1.5×10^{-5}g. Procedures for degassing of the samples are also described under (2).

The procedure for adsorption was to introduce an increment of carbon dioxide into the sample area, which was at the constant temperature, and then the pressure and the amount of adsorption on the sample were noted until equilibrium was reached. The data at each equilibrium gave one point of the isotherm, which is a plot of amount adsorbed versus equilibrium pressure.

Materials.—H-humate were supplied by the Baroid Division of the National Lead Company of Houston, Texas. This commercial material was obtained by leaching lignite with 5% HCl solution in an attempt to remove inorganic salts and to exchange alkali metal ions for hydrogen ions. The lignite was further leached with demineralized water to remove residual HCl and then tray dried at 125°F; the resulting material is referred to as H-humate.

Humic acid, symbolized by $H\text{-}Ac_{Na}$, was prepared from H-humate by the procedure described by Johnson and Hnojewyj (1).

Carbon dioxide used throughout this study was obtained from the Matheson Company with a purity of 99.9% by volume.

Adsorption isotherms of carbon dioxide on H-humate at 25° and 35°.—Desorption treatment to remove gases and prepare the sample for adsorption was performed as described previously (2). The adsorption isotherm of carbon dioxide at 25° was then determined. After complete desorption at 35°, causing a slight loss of sample, which seems due to further dehydration (anhydride formation), the isotherm of CO_2 adsorption at 35° was obtained in the same manner as that at 25°. These two isotherms are presented in Figure 1.

In order to check the experimental values, the 25° isotherm was rerun after desorbing CO_2 at 35° and then lowering the sample tem-

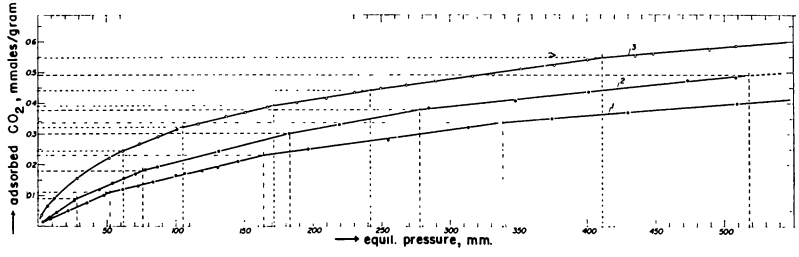

FIGURE 1—Adsorption isotherms of CO_2-gas on H-humates and lyophilized humic acid; experimental results: (1) on H-humates at 35°; (2) on Humates at 25°; (3) on lyophilized humic acid at 25°.

perature to 25°. At low pressures the two isotherms coincided within experimental error; however, at pressures above 100 mm, the repeated isotherm indicated a slight increase of adsorption. This effect could be caused by the further dehydration at 35°; dehydration would change the amount of hydrogen bonding, which may affect adsorption.

The results presented in Figure 1 are somewhat similar to the initial portions of many sigmoid isotherms; a unique feature, however, is the presence of a series of slopes in each isotherm. In the pressure range of 0-500 mm, the 25° isotherm shows the presence of five slopes whereas the 35° isotherm indicates only four. Beyond 520 mm the 25° run indicates a new slope while at 35° no change is noted and the same slope continues to the end of the isotherm, which is at 730 mm of pressure. The appearance of the slopes suggests that adsorption occurred stepwise; first either the most active or the most accessible sites reacted and later the less reactive or less exposed groups adsorbed.

It is interesting to note that Moschopedis et al. (5, 6) and Bhaumik (7) claim to have identified six different oxygen-containing functional groups in H-humate and related materials. Since the numbers of observed slopes and reported functional types are about the same, possibly there is a correlation between the amount of carbon dioxide adsorbed for each slope and the amount of the particular functional group which may adsorb under the conditions provided.

Isosteric heats of adsorption on H-humate at 25° and 35°.—The isotherms, 1 and 2, of Figure 1 have been used to calculate the isosteric heats of adsorption by the Clausius-Clapeyron method. In Figure 2, the calculated values are plotted versus the amount adsorbed. In the range of 0.0 - 0.5 mmoles adsorbed per gram of dry H-humate,

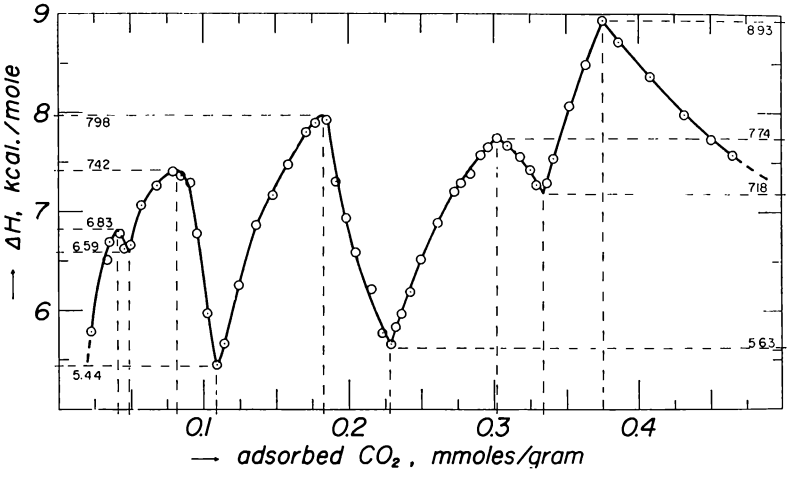

FIGURE 2—Isoteric heats of CO_2 absorption on H-humates at 25-35°.

the isosteric heats of adsorption were found to vary from approximately 5 to 9 kcal/mole indicating five different maxima. Approximately this number of maxima was expected because of the number

of slopes observed and the possible number of functional groups present.

It is important to note that the largest isosteric heat value, of approximately 8.9 kcal/mole, appears last in the plot. For other adsorbates investigated, H_2O and NH_3 on H-humate, the largest heats of adsorption occur first (2). A likely reason for our CO_2 adsorption being different is that the complex functional grouping may require a certain orientation before some sites can adsorb; this would correspond to large initial change of entropy. Hydrogen-bonding is possibly the controlling factor here; this should be investigated further.

Adsorption isotherms of carbon dioxide on lyophilized humic acid at -78° and 25°C.—The lyophilized humic acid sample was introduced into the system and desorbed at 60°. The sample temperature was then lowered to -78° using a slush of ethanol and dry ice. Thirty hours were allowed for equilibration of temperature; after this, the carbon dioxide adsorption isotherm at -78° was measured. Then CO_2 was completely desorbed at 60° and the sample temperature was lowered to 25°. The isotherm at 25° was then determined.

The two isotherms are shown in Figure 3. As plotted, the -78° and 25° isotherms are each characterized by initial curvature followed by two regions where constant slopes are noted (the scale of Figure 3, however, does not distinguish all the slopes for the 25° isotherm, which were apparent in Figure 2).

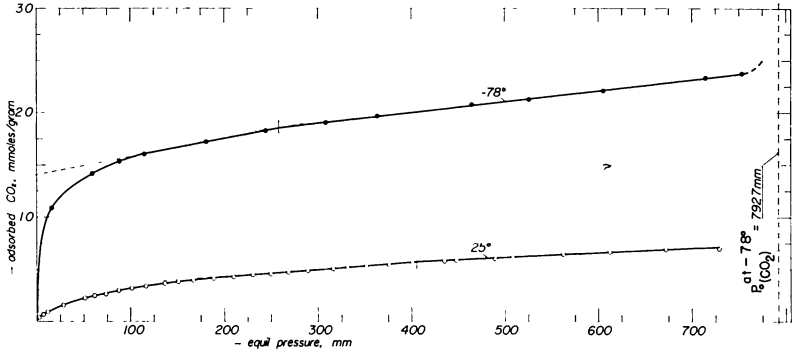

FIGURE 3—Adsorption isotherms of carbon dioxide on lyophilized humic acid at -78° and 25° C.

The low temperature adsorption was performed in order to investigate the region of maximum adsorption of CO_2; this was done for comparison with adsorption at 25° where less coverage is noted but greater selectivity of the CO_2 for the various active sites may be possible. The maximum amount of adsorption at -78°, before attaining the saturation pressure and condensation, is 2.42 mm/g. With this value, and utilizing the cross-sectional area of 17 A^2 for CO_2 (9), the "surface area" was calculated to be 248 m^2/g.

Comparison of the carbon dioxide adsorption isotherms obtained on H-humate and lyophilized humic acid at 25°.—Figure 1 allows a comparison to be made of the 25° isotherms for carbon dioxide adsorption on H-humate (curve 2) and on lyophilized humic acid (curve 3). One immediately recognizes the increased amount of adsorption on the lyophilized humic acid.

For the lyophilized sample, in the pressure range of 0-500 mm, four or possibly five slopes were observed. Initial curvature was also more pronounced in the humic acid isotherm. Possibly the preparation affected the molecular orientations in such a way that certain groups became more equal in reactivity. This could also be the reason why possibly one less slope is noted for the isothermal absorption on humic acid.

Another important feature on the lyophilized humic acid sample is that, at 25°, equilibrium was rapidly obtained. Approximately 85% of the weight-increase occurred within two minutes after the increment of CO_2 was added; on the average, after three hours, no further adsorption was observed. In comparison, the H-humate adsorbed CO_2 gradually, and ten to twelve hours were needed for attainment of equilibrium.

SUMMARY

The results have illustrated that H-humate and humic acid from lignite have at least four and possibly five types of sites reactive to carbon dioxide under the conditions provided.

The calculated heats of adsorption show that H-humate has unique adsorption characteristics which may be influenced by hydrogen bonding.

Lyophilization aided the attainment of equilibrium and is the suggested reason for the increased adsorption noted for the humic acid sample.

Through experiments now in progress, attempts are being made to clarify the low pressure regions of the lyophilized humic acid isotherms. This will provide data to be used in further clarification of adsorption.

Attempts to further reduce or eliminate the ash content of the humic acid are planned; ion exchange resins will be employed for this purpose. If the ash content is affected, the sample will be lyophilized and the adsorption procedures will be repeated for comparison to those presented above.

ACKNOWLEDGMENTS

This work was supported by a North Dakota State University Research Grant and also by a National Defense Graduate Fellowship awarded to D. S. Ferche. The authors are grateful for this support.

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SYNCHRONOUS DETECTION OF AM SIGNALS

David L. McFarland[1] *and E. G. Anderson*
Electrical and Electronics Engineering Department
North Dakota State University of Agriculture and Applied Science,
Fargo, North Dakota

Third Place Winner
A. Rodger Denison Student Research Competition

INTRODUCTION

In a modern communications receiver the operations performed on an amplitude-modulated signal are the following: multiple frequency conversion, with the final selectivity determined by a suitable filter inserted in the last intermediate frequency amplifier chain; rectification and filtering of the signal in a diode detector to recover the conveyed intelligence; and finally audio amplification of the resulting waveform to obtain sufficient output to drive a speaker or headphones. While this system is satisfactory for many uses it has a number of shortcomings. These include overloading and cross-modulation due to strong adjacent-channel signals, images, and "birdies" caused by the heterodyning of harmonics of the local oscillators.

The use of synchronous detection can greatly reduce the effects of adjacent-channel signals and, by proper design, completely eliminate images and "birdies"; moreover, it is far more efficient for weak signal detection.

It is the purpose of this paper to analyze the operation of a system of this type and compare its performance with that of a superheterodyne receiver with comparable amplifier gain.

METHODS

In the following treatment square-law detection is assumed;

[1]Senior, Engineering, North Dakota State University.

although this method is seldom used in practice it is fairly straightforward and the results are compatible with those obtained in actual use. It is further assumed, as stated above, that the two systems have the same total voltage gain, excluding that of the detector stage.

RESULTS

The input waveform may be characterized by

$$\begin{aligned} e' &= E(1 + m \cos w_m t)\cos w_c t \\ &= E \cos w_c t + E\, m \cos w_m t \cos w_c t, \end{aligned} \tag{1}$$

and the local oscillator signal by

$$\begin{aligned} e'' &= E_1 \cos((w_c + w)t + \&) \\ &= E_1 \cos(w_c t + (wt + \&)). \end{aligned} \tag{2}$$

In these equations m is the modulation index of the AM wave, w_m is the angular modulation frequency, w_c is the angular carrier frequency, w is the frequency difference between the local oscillator and carrier frequencies, t is the instantaneous time, E and E_1 are maximum amplitudes of the two signals, and & is the phase difference between the signals at time $t = 0$.

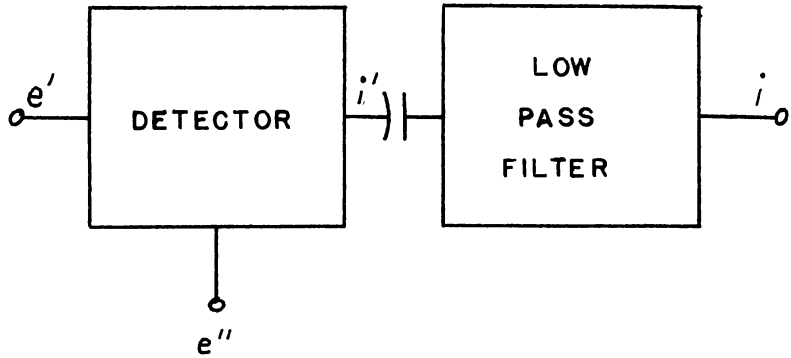

FIGURE 1—Square-law detector with low-pass filter.

When the waveforms are added and run through a square-law detector, as in Figure 1, the output current becomes

$$\begin{aligned} i' &= a(e' + e'')^2 \\ &= a(E \cos t\, w_c t + E\, m \cos w_m t \cos w_c t \\ &\quad + E_1 \cos((w_c + w)t + \&))^2 \end{aligned} \tag{3}$$

Expanding equation (3) results in

$$\begin{aligned} i' = a(&E^2 \cos^2 w_c t + E^2 m^2 \cos^2 w_m t \cos^2 w_c t + {E_1}^2 \cos^2((w_c + w)t + \&) \\ &+ E^2 m \cos^2 w_c t \cos w_m t + E E_1 \cos w_c t \cos((w_c + w)t + \&) \\ &+ E^2 m \cos^2 w_c t \cos w_m t \\ &+ E E_1 m \cos w_c t \cos w_m t \cos((w_c + w)t + \&) \\ &+ E E_1 \cos w_c t \cos((w_c + w)t + \&) \\ &+ E E_1 m \cos w_c t \cos w_m t \cos((w_c + w)t + \&). \end{aligned} \tag{4}$$

By trigonometric expansion and collection of terms,

$$i' = (a/2)(E^2 + E_1^2) + a E^2 m^2/4 + a E E_1 \cos(wt + \&)$$
$$+ a E^2 m \cos w_m t + a E E_1 (m/2)(\cos((w_m + w)t + \&)$$
$$+ \cos((w_m - w)t - \&)) + a E^2 (m^2/4) \cos 2w_m t \cos 2w_c t$$
$$+ a E E_1 \cos((2w_c + w)t + \&) + (a E_1^2/2)\cos(2(w_c+w)t + 2\&)$$
$$+ a E E_1 (m/2)(\cos((2w_c + w_m + w)t + \&) + aE^2(m^2/4)\cos 2w_m t$$
$$+ \cos((2w_c - w_m + w)t + \&) + (a E^2/2)(1 + m^2/2)\cos 2w_c t.$$

This output current is then passed through a low-pass filter by capacitive coupling to eliminate all DC and high frequency components. The output current, i, is then

$$i = a E^2 m(\cos w_m t + (m/4)\cos 2w_m t) + a E E_1 \cos(wt + \&)$$
$$+ a E E_1 (m/2)(\cos((w_m+w)t+\&) + \cos((w_m-w)t-\&)). \quad (5)$$

In practice the local oscillator component E_1 is usually much larger than the signal component E. With this assumption equation (5) becomes

$$i = a E E_1 \cos(wt + \&) + a E E_1 (m/2)(\cos((w_m + w)t + \&)$$
$$+ \cos((w_m - w)t + \&)). \quad (6)$$

Equation (6) may be used to determine the characteristics of the system under various relationships of signal and local oscillator inputs.

(a) $w = 0, \& = 0$

From Equation (6),

$$i = a E E_1 + a E E_1 (m/2)(\cos w_m t + \cos w_m t)$$

Eliminating the DC component,

$$i = a E E_1 m \cos w_m t \quad (7)$$

(b) $w = 0, \& = 90^\circ$

$$i = a E E_1 \cos 90^\circ + a E E_1 (m/2)(\cos(w_m t+90^\circ) + \cos(w_m t-90^\circ))$$
$$= - a E E_1 0 + a E E_1 (m/2)(-\sin w_m t + \sin w_m t)$$
$$= 0. \quad (8)$$

The above two relations are very important as they describe the operation of the system with a properly synchronized signal, as will be shown later.

It is also interesting to observe the output of the detector when the input signal is not properly synchronized with the local oscillator.

(c) $w \neq 0,\ \& = 0$

$$i = a\,E\,E_1 \cos wt + a\,E\,E_1(m/2)(\cos(w_m + w)t + \cos(w_m - w)t)$$

$$= a\,E\,E_1 \cos wt + a\,E\,E_1\,m \cos w_m t \cos wt$$

$$i = a\,E\,E_1(1 + m \cos w_m t)\cos wt \qquad (9)$$

(d) $w \neq 0,\ \& = 90^\circ$

$$i = a\,E\,E_1 \cos(wt + 90^\circ) + a\,E\,E_1(m/2)(\cos((w_m + w)t + 90^\circ) + \cos((w_m - w)t - 90^\circ))$$

$$= -a\,E\,E_1 \sin wt - a\,E\,E_1(m/2)(\sin(w_m+w)t - \sin(w_m-w)t)$$

$$= -a\,E\,E_1(1 + m\cos w_m t)\sin wt. \qquad (10)$$

In the above derivation it is assumed that w is much less than w_m.

(e) $w = 0,\ \& = \&'$

$$i = a\,E\,E_1 \cos \&' + a\,E\,E_1(m/2)(\cos(w_m t+\&') + \cos(w_m t-\&'))$$

$$= a\,E\,E_1 \cos \&' + a\,E\,E_1(m/2)(\cos w_m t \cos \&' - \sin w_m t \sin \& + \cos w_m t \cos \&' + \sin w_m t \sin \&')$$

$$= a\,E\,E_1 \cos \&' + a\,E\,E_1 m \cos w_m t \cos \&'$$

Since &′ is constant,

$$i = a\,E\,E_1 m \cos \&' \cos w_m t$$

$$= A \cos w_m t \qquad (11)$$

(f) $w = 0,\ \& = 90^\circ + \&'$

$$i = a\,E\,E_1 \cos(90^\circ + \&') + a\,E\,E_1(m/2)(\cos(w_m t + 90^\circ + \&') + \cos(w_m t - 90^\circ - \&'))$$

$$= -a\,E\,E_1\,m \sin \&' + a\,E\,E_1(m/2)(-\sin(w_m t + \&') + \sin(w_m t - \&'))$$

$$= -a\,E\,E_1\,m \sin \&' \cos w_m t$$

$$= B \cos w_m t \qquad (12)$$

Notice in equation (12) that the sign of the phase angle &′ determines the instantaneous polarity of the output voltage while in equation (11) it has no effect whatever. This phenomenon is highly useful in synchronizing the local oscillator with the input.

DISCUSSION

The output of a conventional diode detector is (1)

$$i = a\,E^2\,m(\cos w_m t + (m/4)\cos 2w_m t). \qquad (13)$$

Here, as in the previous derivations, a is a constant of proportionality.

Notice that equation (13) is the first term in equation (5). For the case of exact synchronization, as described by equation (7), the output is proportional to ($E E_1$) while for the diode it is proportional to E^2. Since for weak signals E_1 is much larger than E, it is readily apparent that a tremendous amount of gain is available at the detector with synchronous detection.

A system utilizing synchronous detection is shown in Figure 2.

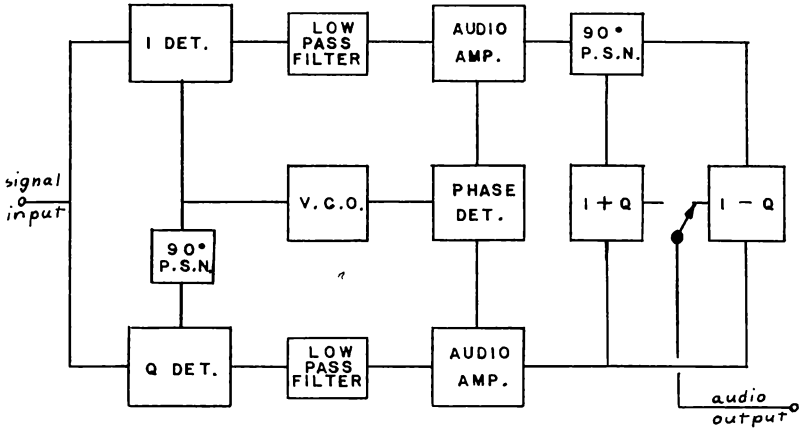

FIGURE 2—Block diagram of a synchronous receiver.

The local oscillator input to the I, or in-phase detector locks onto the incoming signal with exactly the same frequency and phase as the carrier, while the local oscillator is ninety degrees out of phase with the incoming signal in the Q, or quadrature detector. After passing through the low-pass filters and audio amplifiers the outputs are compared in a phase detector. If the signal is not properly locked there will be an output from the phase detector which is fed into a voltage-controlled oscillator. The frequency of the VCO then changes in such a manner as to cause it to synchronize with the incoming signal. With proper synchronization the output of the phase detector is zero and the VCO frequency is constant.

This action is possible because of the characteristics of the detection process. The phase detector is designed so that there is no output if its inputs are ninety degrees out of phase or if one of them is zero, as in equations (7) and (8). If the carrier frequency shifts slightly its inputs are then in phase or 180 degrees out of phase, depending on whether the shift is above or below the local oscillator. This is shown in equations (9) and (10). (It should be noted here that although the amplitude variations of the waveforms of equations (9) and (10) are ninety degrees out of phase the waveforms themselves are not; thus there will be an output from the phase detector).

Similarly, if the phase relationship is not correct, in-phase signals are again fed into the phase detector, as shown in equations (11) and (12). Thus the only case in which there is no output from the phase detector is when the signal has the proper frequency and phase relationships with the local oscillator.

Audio filters may be readily designed to have a cutoff of three kilocycles or less. Thus the incoming AM signal could be fed directly to the detectors; no heterodyning of the signal or RF selectivity are necessary. Further, the detector could be FET mixers or something similar with high signal handling capability. Then there would be no "birdies" and very little, if any, adjacent channel signal overloading or cross-modulation. Also, since the "image" in this system is actually used in the detection process, that difficulty would also be completely eliminated.

With a slight modification of this system another advantage may be realized. It is apparent that any signal with its band of frequencies wholly on one side or the other of that of the local oscillator would result in an I detector output ninety degrees out of phase with that of the Q detector. The I output would lead or lag the Q output, depending on the relation of the signal frequency to the local oscillator frequency (2). Thus if a 90° phase shift network were placed in the I output the two signals would be 0° or 180° out of phase. Then these signals may be added or subtracted, causing them to enhance or cancel each other; since there is no output in the Q channel for the desired signal the latter is not affected. This system could be used to eliminate interference in one or the other of the sidebands of the desired signal with no effect on the signal itself. Interference suppression of up to 30 db may be obtained in this manner. The output taken in this fashion is shown in Figure 2.

SUMMARY

A synchronous detection system actually consists of two receivers. In one such system the incoming signal is fed into two mixers with the local oscillator phase-shifted ninety degrees between them. Since the local oscillator frequency is exactly that of the carrier, the output of these mixers consists of only the sidebands of the signal. It can be shown that the output of one detector (the Q detector in Figure 2) is zero when the incoming carrier has the same frequency and phase as the local oscillator, and that it is ninety degrees out of phase with the output of the I detector when the two signals are well apart in frequency. If the carrier is only slightly different in frequency than the local oscillator, or only different in phase, the signals from the detectors contain components that are in phase, or 180° out of phase with each other, depending on the direction of change. This difference is sensed by a phase detector, which then sends an error signal to the local oscillator; the signal causes the oscillator to synchronize with the incoming carrier. Since

the Q-channel output is zero, the audio used to drive the headphones or speaker is taken from the I-channel.

The output of the mixer section alone in this system is proportional to the product of the carrier and oscillator voltages, while for a conventional diode detector it is proportional to the square of the carrier voltage. Since the carrier voltage is much less than that of the local oscillator this system is capable of tremendous gain over a superheterodyne with a diode detector, assuming the total active gain of both systems is the same. If the synchronous detection system uses mixers of high signal handling capability and low intermodulation distortion followed immediately by steep-skirted low-pass audio filters, it is possible to completely eliminate "birdies" and images, and reduce greatly the effects of overloading and cross-modulation caused by strong adjacent-channel signals.

The primary disadvantage of synchronous detection is its greater complexity. However, its numerous advantages easily outweigh this consideration if the ultimate in weak signal reception is desired.

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1965 HAILSTORMS IN A COMMERCIAL HAIL SUPPRESSION PROJECT

Lawrence J. Hagen

Agricultural Engineering Department
North Dakota State University of Agriculture and Applied Science,
Fargo, North Dakota

INTRODUCTION

In the United States hailstorms cause more damage than tornadoes (1). The most serious hail damage occurs east of the Rocky Mountains in a north-south band extending from northeastern Montana to southeastern New Mexico. Insurance loss ratios in this area are generally above 8. That is, for each 100 dollars worth of crop insurance written, 8 or more dollars are lost to hail damage. Currently, property damage due to hailstorms in the United States is estimated to exceed 100 million dollars annually (2).

DISCUSSION

Hail and hail data in North Dakota.—For a 10-year period ending in 1953, North Dakota had an average loss to hail in excess of 3

million dollars annually. The loss ratio increases approximately 10 times from east to west in North Dakota, with loss ratios of 8 and above in the western portion of the state (1). If this loss ratio holds true equally well for uninsured farmers, then in western North Dakota the gross income of 1 crop in 12 is lost to hail. As crop yields increase, the losses due to hailstorms will also increase.

Despite these large losses, there is a lack of hail data to determine the extent and amounts of hail received in many areas. Current data sources include crop-hail insurance statistics and reports compiled by the Weather Bureau from newspaper clippings (2).

Hailstorms and cloud seedings.—Hailstorms begin as convective currents which rise due to heating of the earth's surface. If the atmosphere is moist, the water vapor in these rising parcels of air condenses as a result of adiabatic cooling. Condensation releases the latent heat in the water vapor and further increases the bouyancy of the air parcels. The cumulonimbus clouds which result from this process may penetrate the stratosphere and reach altitudes in excess of 60,000 feet (3).

Hailstones begin in the supercooled parts of a cumulonimbus cloud. The hail embryo is composed of translucent or transparent ice and grows as water vapor condenses on it. When the embryo exceeds .1 mm in diameter, accretion and collision with unfrozen droplets become the chief growth mechanism.

As the hailstone grows, it will acquire about 5 concentric, alternate layers of clear and opaque ice. The translucent layers are formed when air dissolved in the water droplets is trapped on the hailstone. Clear areas of ice result when slower freezing occurs and the air is forced out of the ice.

There are two factors in a cumulonimbus cloud which make growth of layered hailstones possible below freezing temperatures: (a) the variation in liquid water content (1.1 to 5 g/m^3) and (b) the variation in cloud temperatures at which growth can take place (0°C to -40°C). If the liquid water content is high, the rate at which the latent heat of fusion (80 cal/g) is transferred to the stone and surrounding air may not be sufficient to freeze the water upon contact. The result is slow freezing and clear ice. If the liquid water content is low it is easy to visualize small droplets of liquid water freezing upon collision and forming translucent ice.

From all the known facts, Ludlam (4) has proposed a hailstorm model which is illustrated in Figure 1. A typical medium sized hailstone might follow the path "B" indicated in the figure. The stone will grow at an increasing rate as it sweeps up supercooled droplets in a steady updraft. It may eventually attain a size and fall-speed which will allow it to fall through the updraft. It is possible for hailstones to cycle in this manner because the wind velocity increases with height.

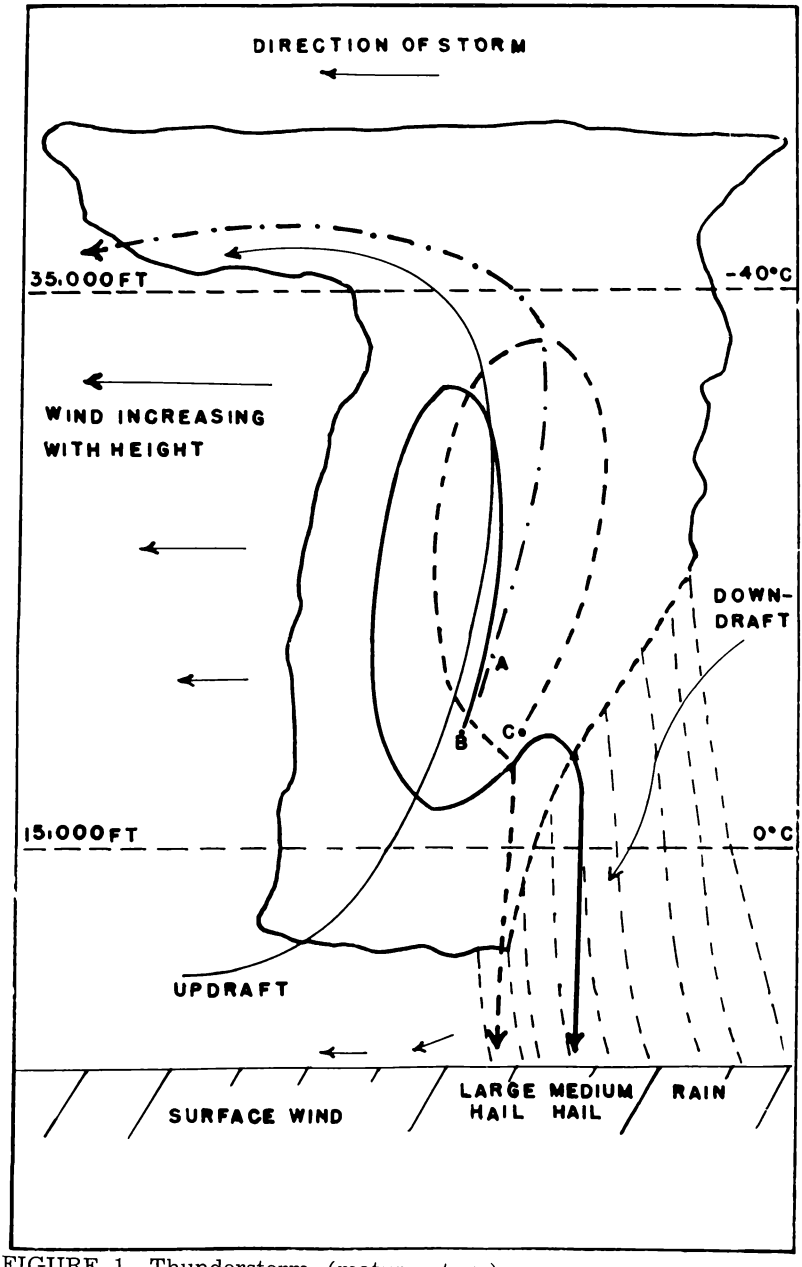

FIGURE 1—Thunderstorm (mature stage).

If the embryo is too small when it reaches the zone of maximum updrafts, it may follow the path "A" and be thrown out near the top of the cloud. Under optimum conditions, a hail embryo may follow the path "C" very slowly and a large hailstone will be formed. It should be noted that the draft structure in this model is in a constant state of generation and hence tends to sustain the life cycle of the thunderstorm.

Between 0°C and -40°C a thunderstorm is composed of both supercooled water droplets and ice crystals. It has been suggested that hailstorms could be suppressed by "seeding" the developing storm and, thus, cause the supercooled water droplets to form ice crystals. This would increase the number of potential hail embryos which would compete for the available water supply and, thus, decrease the size of hailstones. Since smaller stones fall slower, a reduction in the fall speed would permit more of the precipitation to melt on its way to the ground. In addition, these smaller stones might be slushy due to poor cohesion of the ice crystals and cause less damage upon impact (5).

EXPERIMENTAL

In view of the heavy hail damage and cloud seeding as a possible solution, a group of farmers in southwestern North Dakota formed the Bowman-Slope Hail Suppression Association. During the summer of 1965, hail data were gathered in Bowman County where seeding operations were taking place.

One of the methods of investigation used was a form which was mailed to various selected farmers in Bowman County. Approximately 112 farmers received forms with a distribution goal of at least one

TABLE I
CHART FOR DETERMINATION OF ENERGY NUMBER WITH HAIL ACCUMULATION AND WIND VELOCITY AS KNOWN QUANTITIES*

"Most Common" Stone Diameter	Wind in M.P.H.				
	10	20	40	60	80
	ft-lbs/ft²				
¼"	25	31	55	97	150
½"	62	74	123	202	314
¾"	134	152	226	350	525
1"	249	264	362	525	760
1½"	505	543	695	954	1325
2"	1132	1180	1370	1688	2124
3"	2074	2145	2445	2960	3695

*This chart figured for 100% ground coverage. If coverage is less than 100%, multiply the chart number by the actual percent coverage. If ground is 100% covered and "X" inches deep see Table II.

farmer per township. Included in the collected data were values of most common hail size, percent coverage, wind velocity and depth of hail (when the ground was covered). On the basis of Tables I and II compiled by Danielson (6), the reports were analyzed for energy values in foot-pounds per square foot.

TABLE II
CHART FOR DETERMINATION OF ENERGY NUMBER WITH 100% GROUND COVERAGE AND A GIVEN HAIL DEPTH*

Stone Diameter	Wind in M.P.H. 10	20	40	60	80
	ft-lbs/ft²				
¼"	100	124	220	388	600
½"	124	148	246	404	628
¾"	179	203	302	467	700
1"	249	264	362	525	760
1½"	336	362	463	635	884
2"	566	590	685	844	1062
3"	692	716	815	987	1230

*This chart figured for a one inch hail depth. If depth is more than one inch, multiply chart number by actual depth. If ground is less than 100% covered see Table I.

Because many of the values are relative estimates rather than precise measurements, the results were divided into three groups. Energy values of 0 to 10, 10 to 100 and greater than 100 foot pounds per square foot were considered. Schleusener (7) has indicated that these energy values will cause slight, moderate and severe crop damage, respectively. Using energy levels, however, has the advantage of giving a truer picture of hail fall early in the season when

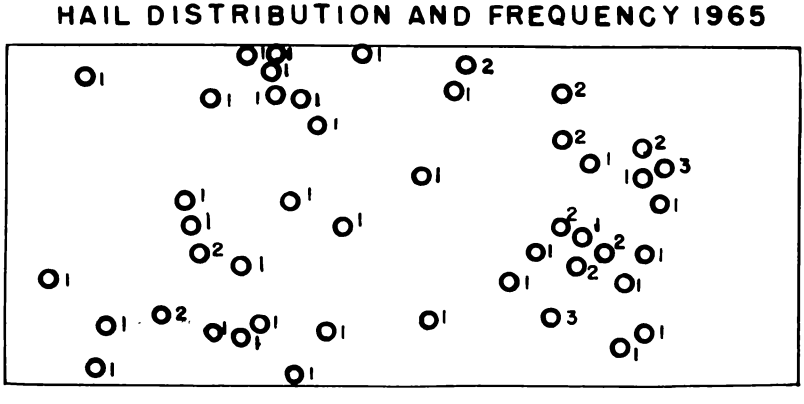

FIGURE 2—1965 hail distribution in Bowman County.

crop damage is negligible. It also avoids confusing crop damage and wind damage in the investigation of hail.

From 112 farmers who received the forms in Bowman County, 46 reported some hail fall during the summer of 1965. Figure 2 shows the distribution of farms reporting hail and the number of times each received hail. The hail distribution appears to be widespread, but more frequent in the eastern portion of the county. Some farmers reported as many as 3 hail days during the summer.

Table III gives the storm dates and energy values for each of the eight hail days recorded. There were 5 small hailstorms and 3 large hailstorms. Cloud seeding operations were carried out on all hail days with the exception of June 13. This storm affected the largest area and had the highest energy values. In addition, the hailstones tended to be larger during this storm. In the smaller storms, the energy values also tended to be lower on the individual farms.

TABLE III

ENERGY VALUES FOR 1965 HAIL DAYS OBTAINED FROM FARMER QUESTIONNAIRES IN BOWMAN COUNTY, NORTH DAKOTA

Date	No. of Farms Reporting	Energy Values (ft-lbs/ft^2) 0-10	10-100	100
June 1	3		3	
13	22	7	9	6
18	4	2	2	
25	2	2		
29	1	1		
July 7	12	5	4	3
Aug. 4	1	1		
23	13	6	6	1

CONCLUSIONS

It appears obvious that more data are needed on hailstorms to establish their climatology. This information would be valuable in trying to evaluate the effects of hail suppression efforts currently being made.

From the data it appears possible to distinguish between storms of various magnitudes using the procedure outlined in this paper.

In addition, it is possible that hailstorms occur more often in such places as Bowman County than even crop insurance reports indicate. This view is further substantiated by Henderson's 1965 report on the Shadehill project (8) in northern South Dakota. From a total of 66 hail recording indicators (9) in a 30 mile square area centered at Bison, South Dakota, not one indicator escaped some hail damage.

ACKNOWLEDGMENTS

The author would like to thank the farmers of Bowman County, J. D. Latham, County Agent, and the Bowman-Slope Hail Suppression Association for their aid in collecting this data. The suggestions of Dr. Allen Butchbaker, and the assistance of other members of the Agricultural Engineering staff of North Dakota State University were also greatly appreciated.

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THE GEOGRAPHY OF RIPARIAN COTTAGE OWNERSHIP ON LAKE MELLISSA, COTTON LAKE, AND ROUND LAKE, BECKER COUNTY, MINNESOTA

Donald J. Berg

Geography Department

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

INTRODUCTION

That riparian cottages (1) are a significant feature on the landscape of the Upper Lakes States (2) can't be disputed. According to one recent estimate, there are over 330,000 summer cottages most of which are located on the shores of some 10,000 inland lakes and the Great Lakes (3).

The lake shores of Becker County in northwestern Minnesota are no exception as attested by the fact that there are at least 2,200 cottages in that county alone (4). These cottages or lake homes range from buildings that are little better than shacks to structures in the $30,000 or higher category, and taken together they represent a substantial total investment.

Not to be overlooked is the proposition that these cottages and the recreational activities carried on in conjunction with them are an important part of the leisure time pursuits of the people in the lakeless Red River Valley region to the west. The cottages as real estate and the recreational activities associated with them are also significant to the economy of nearby towns, such as Detroit Lakes, and other service centers.

The basic geographic concepts of site and situation come into play in an examination of the location of certain representative lakes with respect to surface geology and population concentration, of distribution patterns of cottages around the lakes, and of the home addresses of the owners.

The lakes selected as representative are Lake Mellissa, which has been mapped in detail, Cotton and Round Lakes. Data for this study were obtained by intensive investigation of county tax records and inspection in the field during the summer of 1965.

LOCATIONAL AND OTHER CHARACTERISTICS

An examination of the map in Figure 1a will aid in understanding the location of the lakes under consideration in relation to the population clusters of the Red River Valley, to surficial deposits, and to each other. The three lakes are located generally within a belt of glacial end moraine, which is actually part of a large system which runs through Minnesota and is the locale for most of that state's lakes. It is significant, however, that Lake Mellissa does not lie directly in a moraine deposit but rather on a tongue of glacial

outwash, a fact which helps to explain that more cottages have been built around that lake than either Cotton or Round Lakes, whose basins lie in end moraine (5). Both the sand of the outwash plain and its subdued relief favor cottage building in the case of Lake Mellissa.

Driving distances from Fargo-Moorhead, the main urban area of the southern Red River Valley, and the characteristics of the road network as well as development through time are also influential on lakeshore developments. Lake Mellissa, for instance, has been favored by relative nearness to Fargo-Moorhead (47 miles) over good roads (and on a main line railroad prior to the automobile era). The road network serving the lake shore on Mellissa is also more complete than those of the other two lakes.

Cotton Lake lies about 12 miles northeast of Mellissa on a straight line distance, and is about 56 miles from Fargo-Moorhead by road. Unlike Mellissa, Cotton Lake is somewhat removed from the main highway. However, satisfactory county and township roads serve the perimeters of the lake.

Round Lake seems considerably more remote in comparison to the other two lakes even though only about 12 miles northeast of Cotton Lake. The driving distance to Round Lake from Fargo-Moorhead is about 73 miles. However, the lake is situated between two state highways, about 18 miles apart, and is reached over several miles of usually rough gravel roads. Much of the lakeshore is inaccessible by car.

All three lakes are within the Red River of the North drainage into Hudson Bay. Lake Mellissa, the largest with 1,791 acres in surface, is one of the Pelican River's chain of lakes. Cotton Lake, with the next largest surface area of 1,668 acres, drains through a short stream into the Otter Tail River, and Round Lake, 1,087 acres in surface area, is one of the headwaters lakes on the Otter Tail system.

SUMMARY OF LAKE COTTAGE DEVELOPMENTS

Of the three lakes discussed in this study, the construction of riparian cottages was begun by far the earliest on Lake Mellissa. Although the records indicate that the first subdivision of real estate was platted thereon by Thomas Corbett in 1888, it is probable that some adventurous people might have spent their summers on the lake a few years before that date. With the initiation of channel improvements on the Pelican River in the late 1880's and the placing in service of the Pelican River Navigation Company's boat, *Lady of the Lakes,* cottage building received considerable impetus (6).

During the early 1890's several additions to the original Corbett plat were made, and two new subdivisions were platted. There is a break of 18 years between the filing of the last plat in the 1890's (1892) and the filing of the first plat in the twentieth century (1910). A few more plats were recorded in 1911-1919, but it wasn't until

the 1920's and early 1930's that lake shore development on Lake Mellissa entered a renewed period of activity. Seven new subdivisions were made and six additions filed. The greatly increased use of the automobile undoubtedly contributed to the growing popularity of lake shore summer places during the period.

After a decline suffered during the late 1930's and through World War II, lake shore developments were resumed in 1946 and continued through the mid-1950's. Since 1957 there have been no new subdivisions or additions of lake shore property on Lake Mellissa. There are a few locations where construction of new cottages is going on, and a few of the vacant lots where no lake homes existed before are being filled in. Within not too many years hence, the virtual saturation point will be reached in the number of cottages that can be built on the lake.

Although riparian cottage building wasn't begun much before the early 1920's on Cotton Lake, there were probably a few people who visited the lake and who maintained hunting camps there. It appears that the two factors of the increased use of automobiles and the construction of improved roads into the back country stimulated developments on Cotton Lake. Lake shore subdivisions were platted on the lake in the early and late 1930's, but there is a 15 year break in the filing of plats through the 1940's and into the early 1950's. From 1953 through 1959 Cotton Lake appears to have undergone a period of considerable new cottage construction as four subdivision plats were filed. Today there is a substantial amount of lake shore which has not been built up, but not much of the unused frontage is suitable for cottage construction. As can be seen in Figure 2a the zones where cottages are found on Cotton Lake are fairly well distributed around the lake.

On Round Lake the first lake shore subdivision plat was filed in 1925, although there were undoubtedly a few hunting camps on the lake prior to the influx of summer time residents. Since the lake is relatively remote from the main roads, it seems certain that the building of passable roads and the use of automobiles were the principal factors that aided the development of the lake. It is worth noting that the four lake shore subdivisions on Round Lake were all filed prior to World War II. Since the end of the war period one minor addition was established in 1963. This state of affairs tends to suggest that most of the developable lake shore on the lake has already been built up. The author's observations confirm this impression.

RIPARIAN COTTAGE OWNERSHIP ANALYSIS

It would appear from observing the gross aspects of cottage distribution on Lake Mellissa, shown in Figure 1f, that there are about four major elements or divisions. Our purpose here, then, is to briefly look at each one and describe the nature of it (7).

Ringing the northern and western shores of the lake is a band

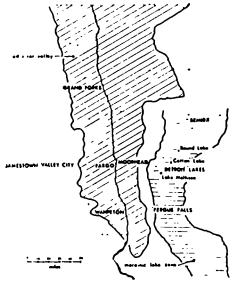
of almost continuous lake homes. The breaks present represent such features as commons, unoccupied lots, and public access points. Along the 2¾ mile stretch there are 172 lots with as many cottages on them. The nine subdivisions and 13 additions were platted in the years ranging from 1888 to 1947. In this northwestern division of the riparian cottages 95 (55%) of the units are owned by Fargoans. Red River Valley people, other than Fargoans, are a weak second with 28 (16%) of the cottages. Closely following, however, is the Intermediate Place category with 23 (13%). Detroit Lakes people about half of whom live nearly the year around on the lake (8), and the Distant Place group supply the remainder with 17 (10%) and nine (5%), respectively.

In the southwestern corner of Lake Mellissa is a rather different situation as contrasted with the northwest. Along this approximately one mile length of lake shore are 47 cottages. Three subdivisions and two additions are present which were filed in the years 1910 to 1946. One of the subdivisions, unlike any of the rest about the lake, is separated from actual connection with the lake by a county road. Perhaps the most unusual feature to be found on the bay is an area unofficially called Roosevelt Beach. The beach fronts for over ¼ mile along the lake and there are about eight cottages present. The beach is unique because of the fact that the land is owned under one name. In all, Fargoans constitute the largest single group along the bay and slightly to the east with 18 (38%) of the units. Following very closely behind Fargo is the Red River Valley complex with 16 (34%) of the cottages. Detroit Lakes people, Intermediate Place people, and Distant Place people constitute the remainder with six (13%), three (6%), and four (8.5%), respectively.

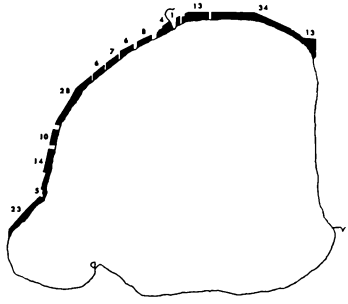
The third major division on the lake is made up of what can be termed the virtually undeveloped south shore. This ⅞ mile stretch of beach has no subdivisions and only six cottages were present when the study was made. Two Fargoans, two Red River Valley people, one Detroit Lakes individual, and one Distant Place person constitute the group. There is one cabin resort along the shore at the outlet of the lake. Some of the lake shore is not developable at the present because it is too low lying, and the rest is probably being held for speculative purposes.

The east shore of Lake Mellissa or fourth division composes the final element in the analysis. This one and one-half miles of lake shore is somewhat diverse as there are three groups of cottages on it which are separated by tracts of unoccupied and sometimes unplatted land. At present there are four subdivisions and four additions. Of the 58 cottages on the east side, Fargoans make up the largest single group of owners with 22 (38%) of the units. This is followed quite closely with the Red River Valley composite group with 19 (33%). The Detroit Lakes contingent is prominent with 10 (17%) of the cottages. Intermediate and Distant Place people complete the

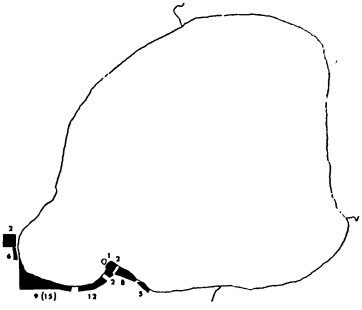
complement with five (8.6%) and two (3.4%), respectively. The east shore of Lake Mellissa is the only part where any substantial recent cottage construction has occurred and undoubtedly in the next few



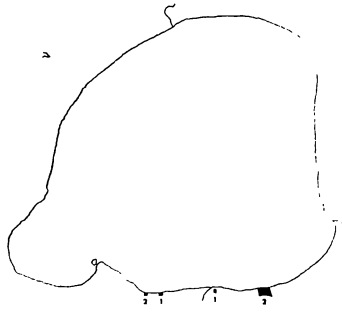
A. ORIENTATION MAP



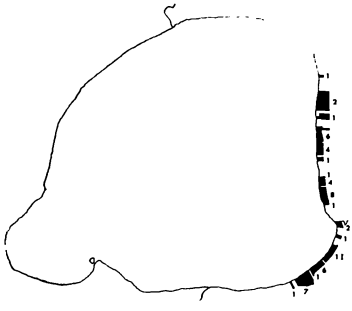
B. NORTHWESTERN DIVISION



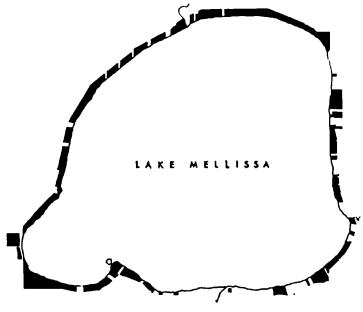
C. BAY DIVISION



D. SOUTH SHORE



E. EAST SHORE



F. COMPOSITE

FIGURE 1—Orientation map and the four divisions of Lake Mellissa.

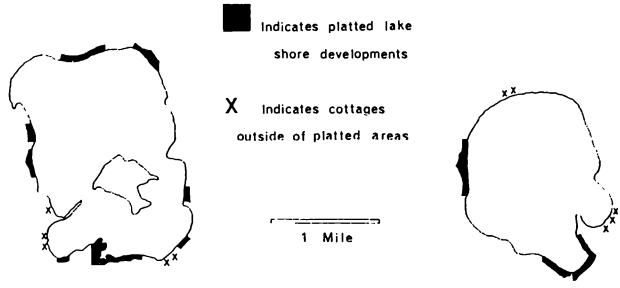
years all of the suitable lakeshore will have been taken up if the present trends continue.

If one views Figure 1f the complete pattern of riparian cottage development on Lake Mellissa can be seen. What is observed is a land use pattern that has been termed linear recreational (9). The cottages on the lake form with their lots a band of land use that is quite distinct from the surrounding countryside and is found almost exclusively on the shores of lakes and the banks of streams where predominately water-based recreational activities are carried on. Surrounding most of Lake Mellissa is land on which agricultural activity is the primary use. The linear type of recreational land use can be contrasted with the areal type of recreational land use in such areas as the wilderness of the Canadian shield or the Canoe Country in northern Minnesota (10).

If one summarizes the ownership characteristics of the riparian cottages on Lake Mellissa in the five owner-location categories already alluded to, as has been done in the pie diagram in Figure 2c, a graphic picture of the home addresses of the cottage owners can be seen. The principal observation to be made here is the fact that Fargoans own almost one-half (48%) of the 283 cottages on the lake as of June 1, 1965. Although Fargoans are found in every division on the lake shore, the majority have summer homes on the north and west shores. After Fargo the Red River Valley class has the second highest number with 65 (23%). This grouping includes villages that range from the size of Davenport, North Dakota, to cities larger than Moorhead and from Wahpeton to Grand Forks in geographic extent. The local or Detroit Lakes people make up the third section with 34 (12%). They are distributed sparsely around the lake. Following the Detroit Lakes category is the Intermediate Place group with 31 (11%) of the units. Although distributed about most of the beaches, the Intermediate Place cottages are mainly found on the northern and western shores. Lastly there is the Distant Place division with a small (16), but still statistically important 4.6% of the number.

With the main themes of the geography of cottage distribution and ownership of Lake Mellissa in mind we can now turn to a brief discussion of those phenomena on Cotton and Round Lakes. Upon looking at Figure 2a one can see the discontinuous placement, in comparison with Lake Mellissa, of the eight subdivisions and one addition on Cotton Lake. In these nine areas are to be found most of the 146 cottages on the lake. Round Lake, of the three, is the least populated and, as can be seen on the map (Figure 2b), there are only four subdivisions and one addition in which the majority of cottages are located.

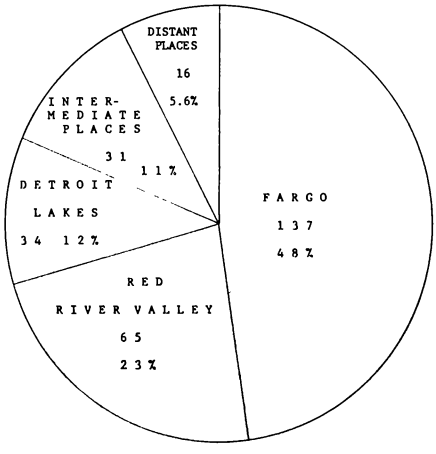
Since detailed mapping was not done for Cotton and Round Lakes we can proceed directly to the summary diagrams in Figure 2c. Here can be seen pie diagrams which are proportional in relation to the number of cottages on each lake. The different percentages



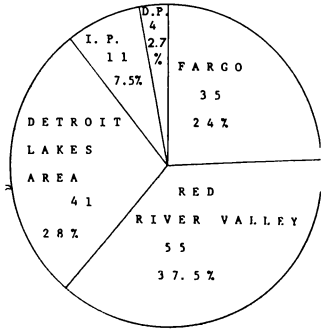
A. COTTON LAKE

B. ROUND LAKE

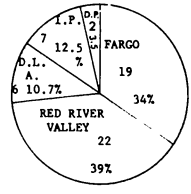
C. OWNER'S RESIDENCE ANALYSIS



Lake Mellissa 283



Cotton Lake 146



Round Lake 56

D. CUMULATIVE TOTALS FOR THREE LAKES

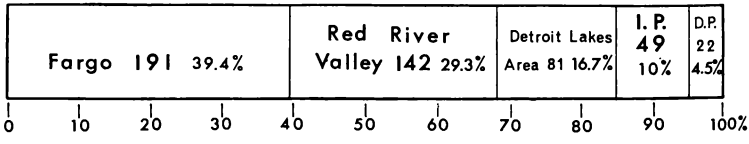


FIGURE 2—Cotton Lake (A), Round Lake (B), and owner's residence analysis of these plus Lake Mellissa.

of owners in each of the five divisions are shown. Although the range of the numbers of Fargo people vary for each lake, they are still the largest single city group. The rest follow in a succession of declining values although in some instances a category combining a group of towns and cities may be larger than Fargo's number for a particular lake (i.e., Red River Valley people on Cotton Lake).

CONCLUSION

In conclusion it can be observed from the sample of the three lakes presented in this paper that the lake shores in western Minnesota are important features on the landscape to the people of the Red River Valley. This is evidenced by the fact that these individuals own 333 or 68% of the lake homes on Lake Mellissa, Cotton and Round Lakes. On the basis of the author's field observations, a similar percentage of ownership probably applies to most of the other lakes with cottages on them which lie eastward from the parallel to the Red River Valley (11). The interpretation and understanding of the settlement geography of eastern North Dakota and northwestern Minnesota is not complete without some concept of the riparian cottage developments and their association with the people in that area.

ACKNOWLEDGMENTS

The author would like to take this opportunity to thank Mrs. Harold Rahn, Becker County Deputy Auditor, and Dr. Warren D. Kress, Associate Professor of Geography at North Dakota State University, whose time and assistance made this study possible.

NOTES

1. The term "riparian cottage" as used in this report refers to a dwelling that is built on a lot that abuts a body of water. The majority of cottages are used as summer homes and although most are substantial buildings, the term "cottage" is used as a matter of tradition and convenience. Some of the cottages on the lakes described in this paper are lived in almost all year around and therefore aren't strictly speaking seasonal units as are about 85% to 95% of the cottages. For purposes of statistical convenience and in order to show a more complete picture of the cottage developments around the lakes, the lake homes, permanently occupied, are included. Such terms as summer homes, seasonal homes, and seasonal residences are used interchangeably in the literature.
2. The Upper Lakes States region includes Michigan, Wisconsin, and Minnesota.
3. Derek Thompson, "Riparian Cottages in the Upper Lakes States," Hamilton, F. E. Ian, editor. *Abstracts of Papers: 20th International Geographical Congress.* London: Nelson, 1964, p. 259.
4. This figure has been estimated by the author after examining the

topographic map coverage of Becker County and counting the number of cottages around the various lakes. (Extensive research on cottages on Minnesota lakes is being carried out by the Minnesota Outdoor Recreation Resources Commission, but no results pertaining to the main points of the present paper have been published as of this writing. A specific project, being investigated by Mr. George W. Orning, is now in progress under the auspices of the North Central Forest Experiment Station in St. Paul and will undoubtedly yield considerable information pertaining to certain lakes in north central Minnesota.)

5. Frank Leverett and Frederick W. Sardeson. *Quaternary Geology of Minnesota and Parts of Adjacent States.* U. S. Geological Survey Professional Paper 161. Washington: U. S. Govt. Printing Office, 1932, plates I and III.
6. Alvin H. Wilcox. *A Pioneer History of Becker County Minnesota,* St. Paul, Minn.: Pioneer Press Company, 1907, p. 477.
7. For the purposes of this paper a five-fold classification system was devised to bring some order out of the numerous locations in which the riparian cottage owners live. After tabulations were begun the nature of the data suggested that since Fargoans turned out to be the largest single group (i.e., being from one city), they would represent a single category. Second, all the owners living in the Red River Valley (other than Fargo) were included together. The third grouping of people were the locals, those who listed Detroit Lakes or a few of the nearby towns as their addresses. Fourth came those people who would have to drive between 100 and 300 miles to get to their lake homes. Lastly were the distant place people who live in cities outside either North Dakota or Minnesota.
8. According to one paper on the subject, these units are termed "dispersed urban units." By this it is meant that the occupants of these homes are essentially urban dwellers, (i.e., their income is derived from working in either a town or city and they have amenities associated with city life) but they live out in the country. See John R. Borchert et. al. *Urban Dispersal in the Upper Midwest.* Urban Report Number 7, Minneapolis: Upper Midwest Economic Study, June, 1964, 24 pp.
9. The term "linear recreational" land use pattern has been adopted from Roy I. Wolfe, "Summer Cottagers in Ontario," *Economic Geography,* 27 (January 1951), pp. 10-32.
10. Wolfe, *ibid.,* p. 15.
11. It can be noted in this connection that in the case of resort occupancy the Minnesota counties paralleling the Red River Valley are generally favored by vacationers from North Dakota. See Minnesota, Department of Business Development, Research Division. *A Survey of the Minnesota Resort Industry,* St. Paul, July, 1964, p. 8.

THE FORT UNION GROUP

Steven P. Lund

Bismarck Senior High School, Bismarck, North Dakota

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

ABSTRACT

The Fort Union Group of geological formations in western North Dakota was studied to determine the age relationship between the Cannonball and Tongue River Formations. This was accomplished by studying 40 faunal and 20 floral genera which I collected and classified, and by studying the relationships between outcrops in western North Dakota. Much of this study concerned the geological history and the ancient landscapes of the Cannonball and Tongue River Formations in western North Dakota.

MICROCLIMATIC GRADIENTS IN MIXED GRASS PRAIRIE[1]

Warren C. Whitman and Gale Wolters

Department of Botany, Agricultural Experiment Station North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota

ABSTRACT

Microclimatic gradients in prairie grassland in the range from the soil surface to 5 feet above the surface and from the surface to a depth of 4 feet in the soil were measured during the growing seasons of 1962, 1963, and 1964. The studies were made on a protected portion of a 30-acre piece of native grassland at the Dickinson Experiment Station in southwestern North Dakota. Factors observed included soil moisture, soil and air temperatures, evaporation, relative humidity, and wind movement. Precipitation was measured with standard rain gauges.

The existence of microclimatic gradients in the atmosphere near the surface of the earth and in the soil itself has been established by a number of investigators, but practically nothing is known of the nature and magnitude of these gradients in the native grassland of the Northern Great Plains.

Soil moisture is a critical factor in the growth and development of the grass cover, and the available moisture over the three seasons of observation at the Dickinson Station showed a trend from relatively high in the early part of the season to near exhaustion by the end of July.

Most of the atmospheric temperature gradient was in the foot above the soil surface, with maxima being much higher and minima appreciably lower near the soil surface than at points 18 inches to 5 feet above the surface. During the summer season the soil temperature gradient is from warmer near the surface to cooler downward with average temperatures showing about a 15° F. difference between the surface and the 4-foot depth.

Total miles of wind increased markedly with increase in height above the soil surface. Miles of wind at 5 feet averaged about 10 times the wind mileage recorded at 6 inches. The curve of evaporation from Livingston bulbs corresponds somewhat with the curve for miles of wind, but the evaporation at 5 feet is only about 80 percent more than the evaporation at 6 inches. A gradient in relative humidity exists at nearly all times with generally higher relative humidity values at 6 inches than at 5 feet above the surface.

It is apparent that the grass plants of the native prairie do create a microclimate with considerably different parameters than those of the surrounding macroclimate. Most of the modification in climatic gradients in this type of grassland is in the first two feet above the soil surface. The degree of climatic modification is such that it is apparent that different portions of the grass plants exist under markedly different environmental conditions at different times during the day and throughout the growing season.

[1]Study jointly supported by the National Science Foundation and the North Dakota Agricultural Experiment Station. Abstract published with the approval of the Director, N. Dak. Agr. Exp. Station.

ANATOMY AND CYTOLOGY OF ONION ROOTS TREATED WITH KINETIN[1]

M. Arif Hayat

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

INTRODUCTION

The root tips of vascular plants have been widely used for the study of cell differentiation. Due to their rapidly developing cells, roots have also been used to study the effect of growth substances on cell metabolism. Kinetin (6-furylaminopurine) is known to delay senescence in detached leaves (Richmond and Lang, 1957) and to promote cell division in plant tissues (Miller and Witham, 1964). The recent work of Jensen et al. (1964) indicated that immediately after

[1]Supported by grants from American Philosophical Society and the Society of the Sigma Xi.

treatment with kinetin there was an increase in cellular RNA content of onion root tip, whereas the DNA content was not immediately affected. Very little information is available on the morphological changes in the cells treated with kinetin. In view of the importance of kinetin in cell morphogenesis, the present investigation was made to determine the specific changes in the structure of various cell components after treatment with kinetin. The results reported here are part of a broader study on the effects of growth hormones on the cellular organelles and their relationships to biochemical activities or physiological functions.

MATERIALS AND METHODS

Roots emerged within 48 hours after bulbs of *Allium cepa* var. White Globe were placed on vials filled with double distilled water in the dark at a constant temperature of 25°C. Two days after germination the bulbs and their intact root systems were transferred to vials containing 1 ppm kinetin in distilled water. Root tips 5 mm in length were collected at ½, 1, 4, 6, 12, 24, and 48-hour intervals after treatment. These were killed and fixed under vacuum using methods previously reported (Hayat and Canright, 1965). Fixed materials were dehydrated and embedded in Tissuemat according to standard procedures. Transverse sections 8 μ in thickness were cut on a rotary microtome, and these were stained in a combination of iron-hematoxylin and safranin.

For electron microscopy two types of fixation were used: i.e., gluteraldehyde-OsO_4 and $KMnO_4$. In the former, the tissues were fixed 2 hours in 3% gluteraldehyde at pH 6.8 at room temperature, washed in buffer, and placed in 2% OsO_4 for 1 hour at room temperature; in the latter, the tissues were placed in 2% $KMnO_4$ at 4°C for 4 hours. In both cases, the tissues were dehydrated in an acetone series and embedded in Epon (1:1).

Silver and gray sections were cut with glass knives on a Sorvall MT-1 ultramicrotome. Sections were picked up on uncoated 400-mesh grids and stained in Reynolds' (1963) lead citrate for 3-10 minutes. Observations and electron micrographs were made with an R.C.A. EMU3-C microscope at 50 kv, using Dupont Croner Ortho S Litho film (COS-7).

RESULTS

Kinetin caused an immediate effect on the rate of cell division. Roots treated for ½ hour showed a definite increase in the rate of cell division over the control. This increased rate was noticed in the cells of all 4 regions (epidermis, cortex, stele and cap) of the root. The most pronounced effect on the rate of cell division was observed in the derivatives of the apical initials. There was no apparent effect of kinetin treatment on the initials of all the roots treated for different periods of time with respect to cell division.

The greatest rate of cell division occurred in roots treated for 1

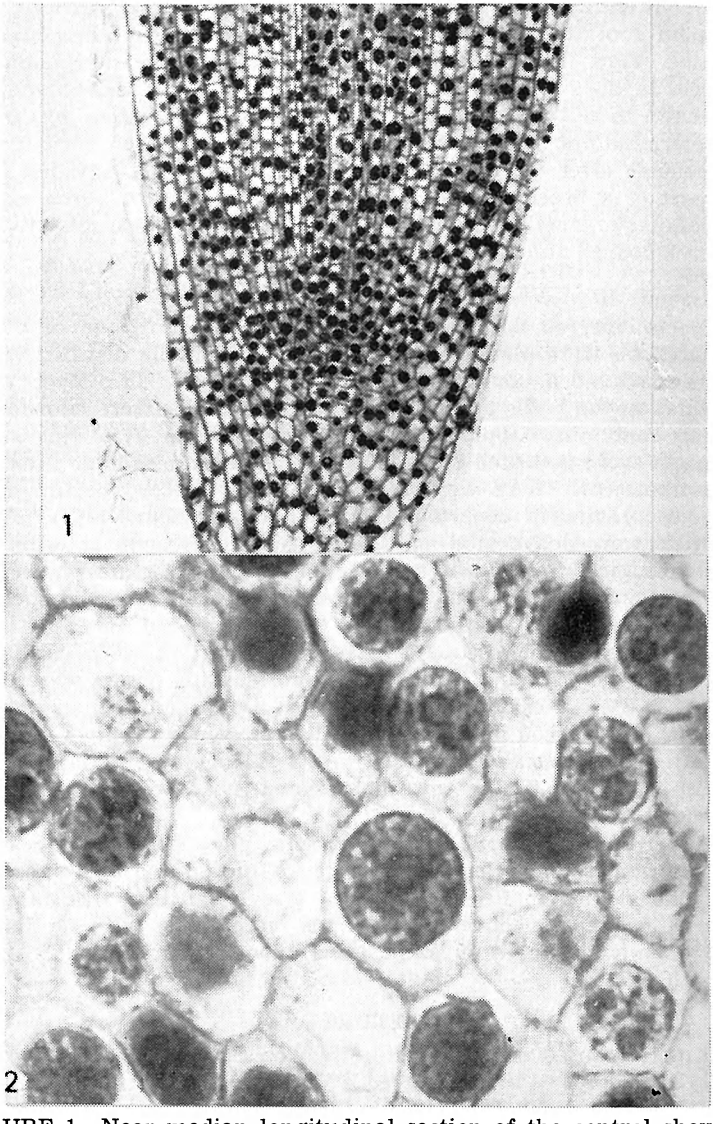


FIGURE 1—Near median longitudinal section of the control showing normal tissue pattern. X234.

FIGURE 2—Transverse section through the stelar derivatives of the apical initials of the control showing the normal shape and size of the nuclei. Note the prominent nucleoli and distinct chromatin material. X1800.

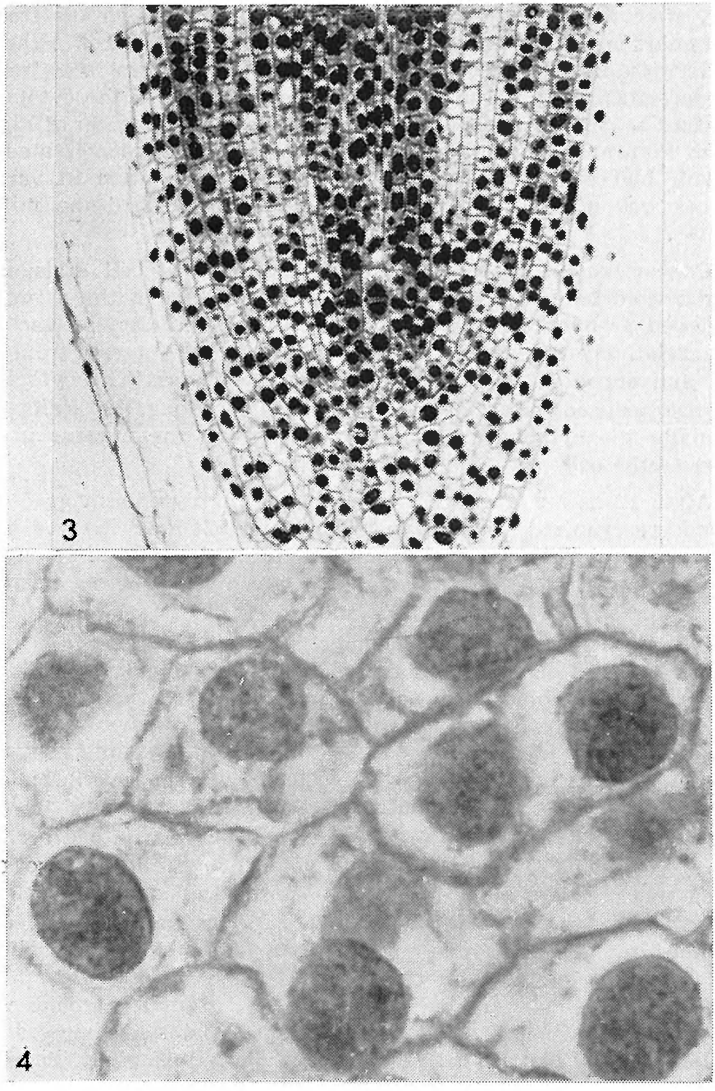


FIGURE 3—Near median longitudinal section of the ½-hr treated root illustrating the increase in vacuolation; mitotic figures are present. X276.

FIGURE 4—Transverse section through the stelar derivatives of the apical initials of the ½-hr treated root indicating distinct chromatin material. o changes in the shape or size of the nucleoli is apparent when compared with the control (Figure 2). X1800.

hour, after which there was a gradual decline. In the roots treated for 4 hours or longer, the rate fell below that of the control. Although the decrease in the rate of cell division was observed in roots treated for more than 1 hour, the changes in the structure of the cytoplasm appeared as early as ½ hour after treatment. A comparison of Figure 1 with Figure 3 indicates that the cytoplasm of most cells treated for ½ hour had begun to disorganize and that vacuolation in various degrees was present. No attempt was made to record the mitotic counts.

Concomitant with the decrease in the rate of cell division in roots treated longer than 1 hour most of the cells in the 4 regions developed pronounced vacuoles (Figures 5, 6). Initially the vacuoles were relatively small, but they increased in size away from the apex and appeared to coalesce into larger vacuoles. The cytoplasm was disorganized, shrunken and accumulated along the walls. Occasionally the cytoplasm was shrunken around the nucleus in the center of the cell.

After 12 hours of exposure to kinetin, the cytoplasm gradually became reorganized. The cytoplasm of the 24-hour treated cells stained evenly (Figures 7, 8), but was much less dense than that of the control. The reorganization was accompanied by a decrease both in the size and number of vacuoles.

Cells treated for ½ hour demonstrate that kinetin had a more immediate effect on the cytoplasm than on the nuclei. Figures 3 and 4 indicate cytoplasmic changes, but the nuclei were apparently unaffected. There was little difference in the nuclear structure (including nucleolar size) between the cells treated for ½ hour (Figure 4) and those of the control (Figure 2). The nuclei showed normal organization with distinct chromatin strands and one or more nucleoli.

Only after the 4-hour treatment were the nuclei and cytoplasm markedly affected. Such cells were alive, although nuclear disorganization was common (Figure 6). In contrast to all other treatments, both nucleoli and chromatin material were not visible, and it appeared as if meristematic activity stopped in most of the cells. After the 6-hour treatment the nuclei were found to gradually "reorganize". The nuclei of the 24-hour treated roots appeared normal in shape with one or more distinct nucleoli, but the chromatin material was not visible. There was no evidence of cell division in any of the roots treated for 24 hours.

With adequate destaining of hematoxylin, some cells in the root cap of the 4-hour treated roots were found to contain discrete particles (Figure 9). Typically these particles developed in most cells within a region of approximately 250 μ from the distal end of the root cap. However, not all cells in this region seemed to have the same amount of granules, and derivatives of the apical initials in the root cap were devoid of the particles.

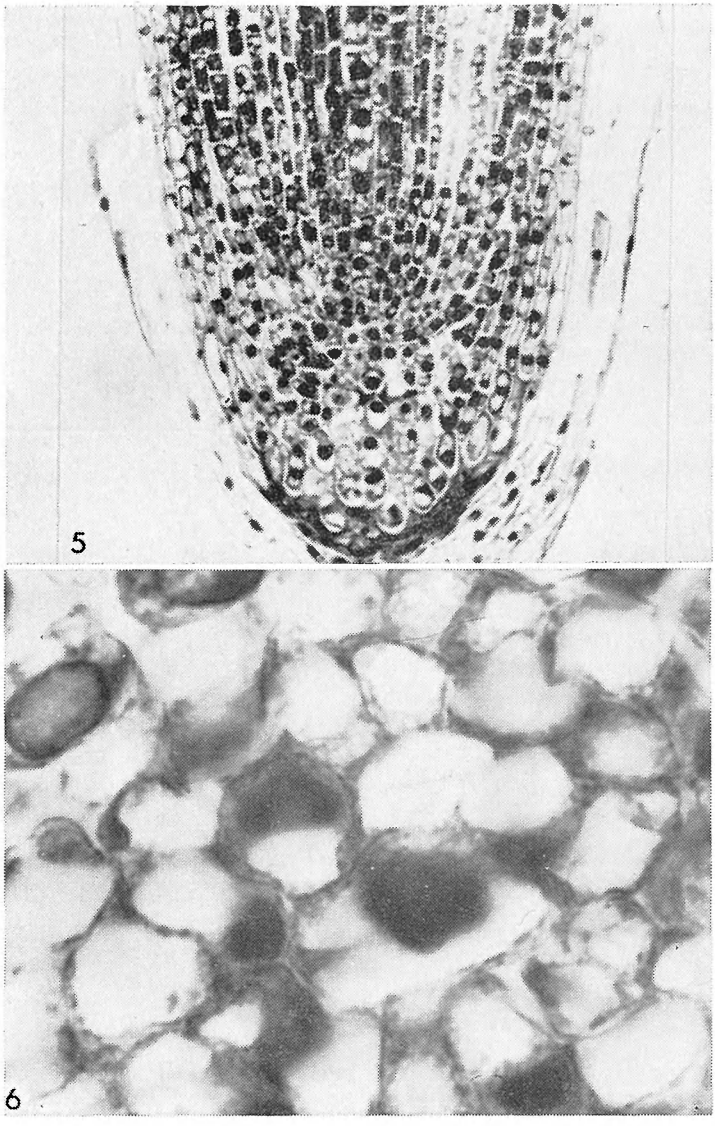


FIGURE 5—Near median longtitudinal section of the 4-hr treated root showing the general disorganization of cells. X244.

FIGURE 6—Transverse section through the stelar derivatives of the apical initials of the 4-hr treated root illustrating the extreme vacuolation, partial disorganization of the nuclei and cytoplasm; the nucleoli and chromatin material are not visible. X1800.

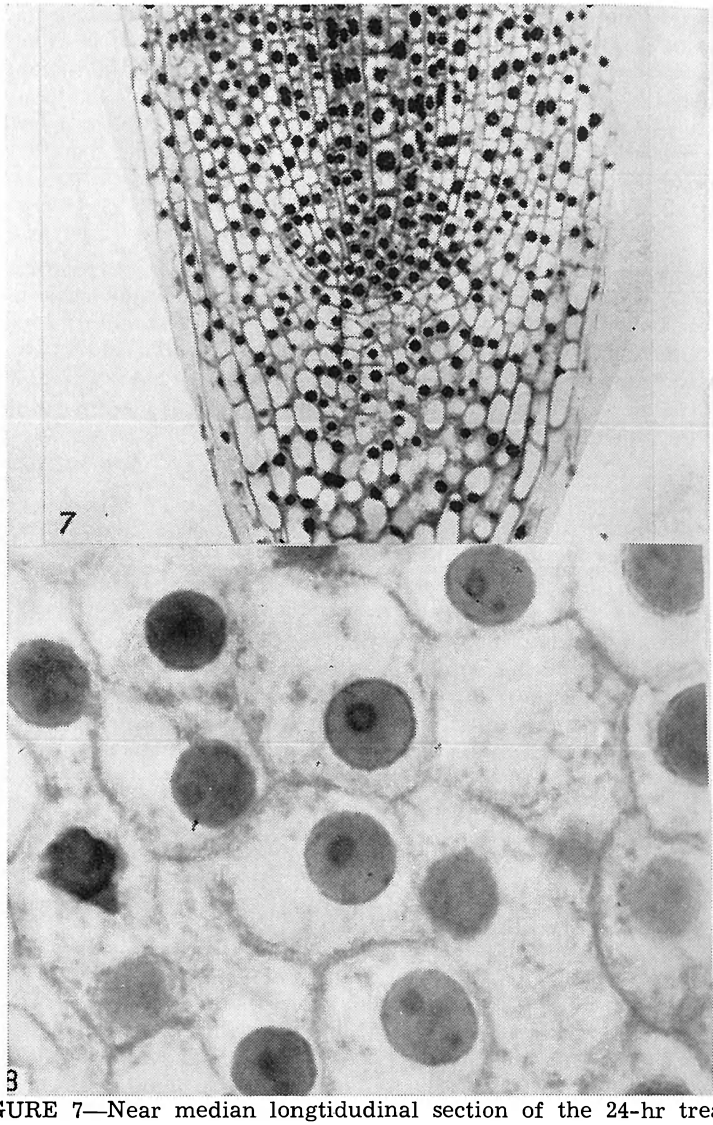
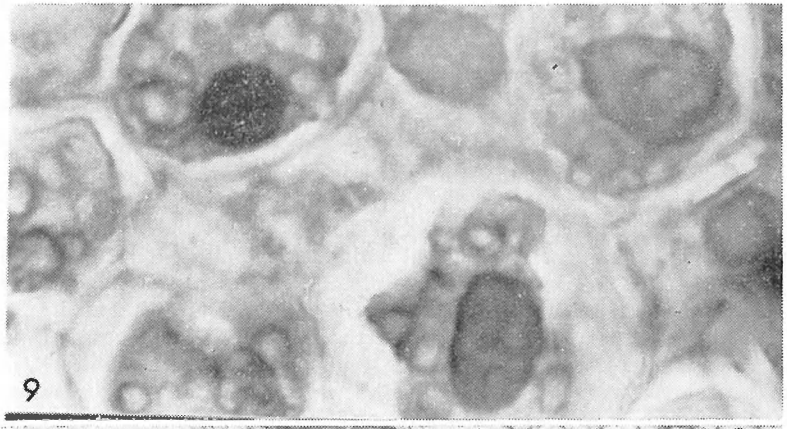


FIGURE 7—Near median longtidudinal section of the 24-hr treated root demonstrating the general "reorganization" of cells. X280.

FIGURE 8—Transverse section through the stelar derivatives of the apical initials of the 24-hr treated root showing more or less evenly-stained cytoplasm and normal shape of the nuclei. Note the presence of prominent nucleoli and indistinct chromatin material when compared with the control. X1800.



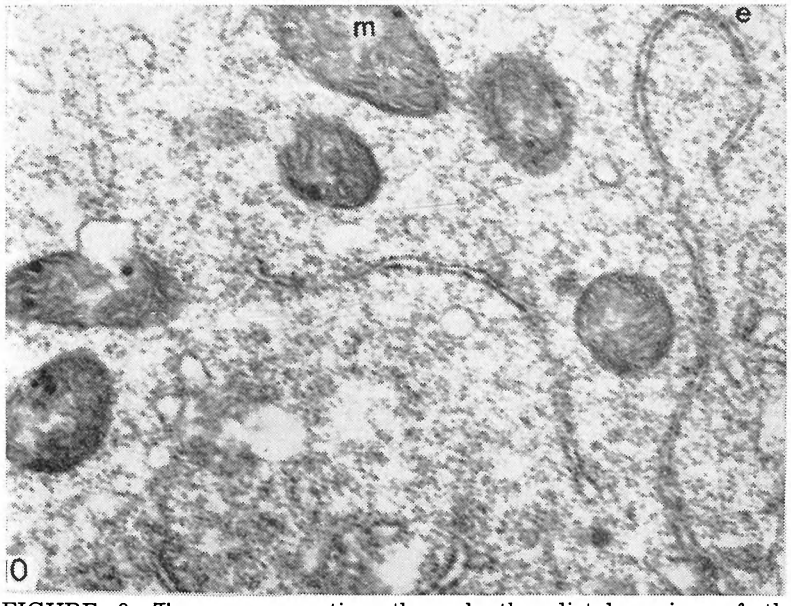


FIGURE 9—Transverse section through the distal region of the rootcap of the 4-hr treated root showing a drastic change in the appearance of the cytoplasm and abnormal nuclei. X2000.

The remaining figures are micrographs of the stelar derivatives. The key to the labelling is as follows: *e*, endoplasmic reticulum; *m*, mitochondrion; *c*, cell wall; *v*, vacuole; *p*, proplastid; *n*, nucleus.

FIGURE 10—Portion of a cell of the control showing the normal flattened cisternae profile of the endoplasmic reticulum and abundant ribosomes. Note the association of ribosomes with the surface of the endoplasmic reticulum. X45000.

Ultrastricural Observations.—The fine structure of the cellular organelles of control and kinetin-treated root tips were studied in detail. The endoplasmic reticulum and vacuoles exhibited the most striking response to kinetin. The number and size of vacuoles increased rapidly after kinetin treatment, and the endoplasmic reticulum showed pronounced changes in its shape, size and structure. In the ½ hour treated roots the endoplasmic reticulum was broken up into relatively small vesicles of various shapes (Figure 11). These vesicles were more or less uniformly distributed throughout the ground cytoplasm. The normal endoplasmic reticulum seen in the control (Figure 10) disappeared completely. Abundant ribosomes were found associated with the membranes of the vesicles and free in the adjacent cytoplasmic matrix. On the basis of limited observation, no differences were visible in the size and quantity of ribosomes seen in control versus treated roots. Further intensive studies are planned to analyze the possible qualitative and quantitative changes in the ribosomes after kinetin treatment.

The endoplasmic reticulum of cells treated longer than 1 hour exhibited a gradual pattern of reorganization. In roots treated for 4 hours, the start of reorganization was evidenced by a transformation of the vesicles into distended tubules (Figure 12). After the 12-hour treatment most of the vesicles and tubules had coalesced into cisternal profiles with distended areas still present (Figure 13). The endoplasmic reticulum membranes were studded with numerous, closely adherent ribosomes. Abundant ribosomes also were evenly distributed in the cytoplasm.

In the 24-hour treated cells the endoplasmic reticulum appeared as long, close-fitting membranes (Figure 14). It was apparent that the reorganized endoplasmic reticulum was structurally different from that of the control. Although numerous ribosomes were present in the ground cytoplasm, their association with the membranes was less prominent than in the untreated cells. Some vesicles were also present and their membranes showed comparatively more associations with the ribosomes than did the membranes of the reorganized endoplasmic reticulum.

DISCUSSION

The observation that kinetin induces an immediate increase in the rate of cell division agrees with the results of other studies (Das et al., 1956; Hayat and Salama, 1966). Normally the derivatives of apical initials divide more rapidly than any other tissue of the root tip. After kinetin treatment the most rapid replication of DNA is also shown by the derivatives. In other words, the cells which divide rapidly under normal conditions are also the cells most influenced by kinetin in terms of increased DNA replication. On the basis of these observations, it may be inferred that the catalytic effect of kinetin on DNA synthesis is dependent on the presence and concentrations of other substances. Kinetin may lose its effect when these

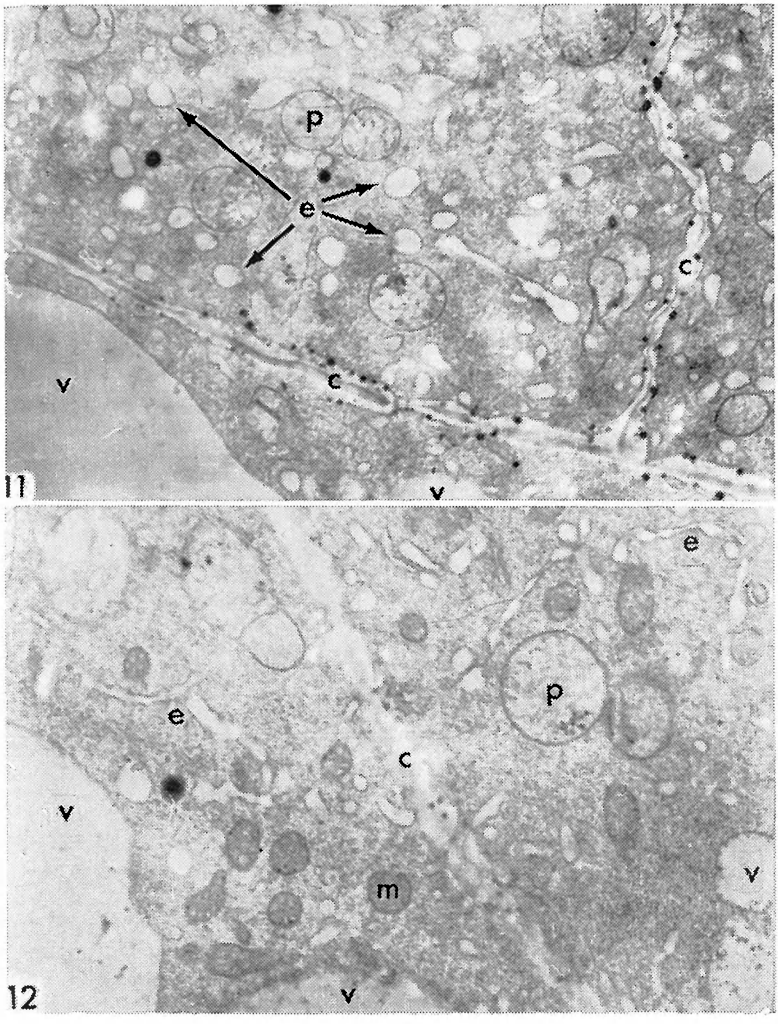
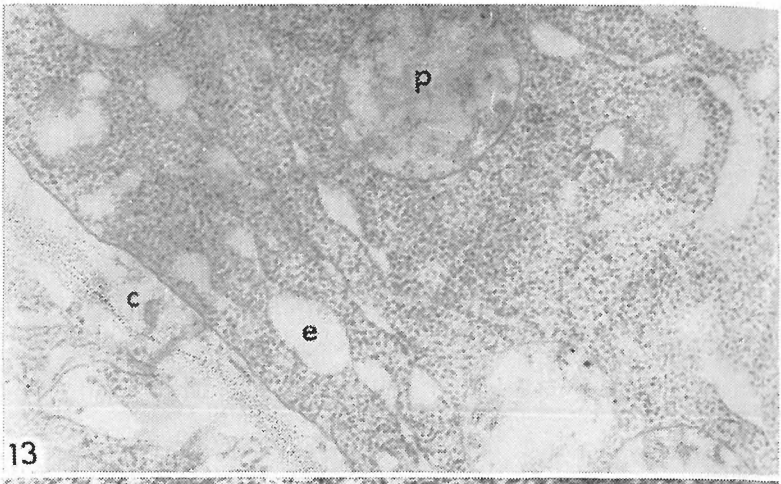


FIGURE 11—Portion of a cell of the ½-hr treated root showing the breakup of the endoplasmic reticulum into vesicles of various shapes and sizes. Abundant ribosomes are present throughout the cytoplasm. X8000.

FIGURE 12—Portion of a cell of the 4-hr treated root showing the beginning of "reorganization" of the endoplasmic reticulum. Fusion of individual vesicles into short bead-like structures is apparent. The ribosomes on the outer surface of the endoplasmic reticulum are not heavily stained in this preparation but they are as abundant as seen in Figure 13. X9000.



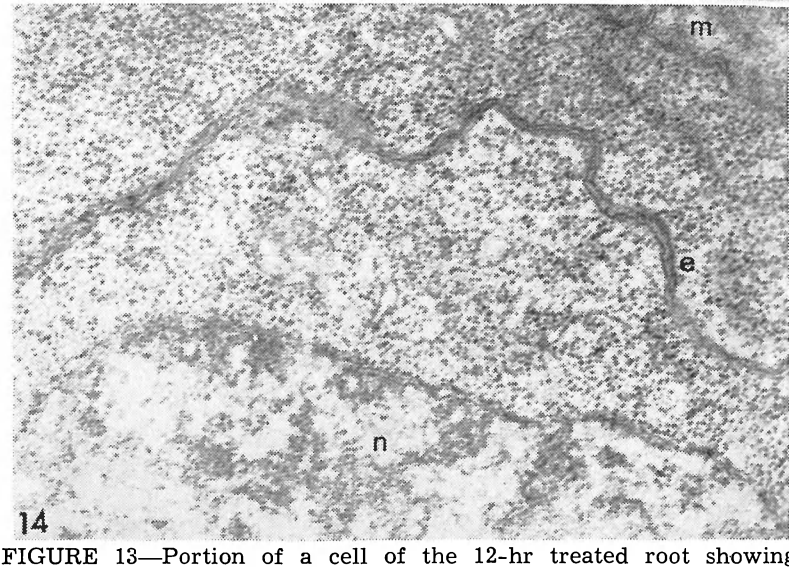


FIGURE 13—Portion of a cell of the 12-hr treated root showing further "reorganization" of the endoplasmic reticulum into long bead-like structures widened at intervals. Abundant ribosomes are present in the ground cytoplasm as well as in association with the surface of the endoplasmic reticulum. X45000.

FIGURE 14—Portion of a cell of the 24-hr treated root showing the "reorganized" endoplasmic reticulum which appears different in structure compared with the control (Figure 10). Note relatively scanty association of the ribosomes with the surface of the endoplasmic reticulum. X45000.

substances are depleted. Skoog and Miller (1965) have suggested that both kinetin and IAA are required for active DNA synthesis. It is possible that the depletion of IAA and/or other substances may cause a reduction in the rate of DNA synthesis after a period of active replication in the presence of high concentrations of kinetin.

The rapid development of vacuoles and the drastic modifications in the shape, size and structure of the endoplasmic reticulum in the ½-hour treated roots seem to have no immediate effect on the rate of DNA synthesis, for most of the cells in these roots continued to show rapid cell divisions. It is obvious that as long as the nucleus is undamaged, the DNA synthesis continues. It is not known whether the drastic changes in the cytoplasm (due to kinetin), or the prolonged treatment with kinetin, or both cause the eventual disorganization of nuclei and cessation of meristematic activity in the 4-hour treated roots.

In general it is recognized that actively dividing meristematic cells contain large nucleoli, whereas cells with declining protein synthesis possess nucleoli which are small or inconspicuous. In the present study no significant difference was found between control roots and those treated for ½ hour (which according to Jensen et al. (1964) show doubling of RNA content) with respect to the size or the number of nucleoli. Furthermore, the roots treated for 24 hours seemed to contain a higher percentage of nucleoli than either the control or the ½-hour treated roots. According to Jensen et al. (1964) the RNA content of 24-hour treated roots is below that of the control. It is, therefore, apparent that the increased RNA synthesis due to kinetin treatment may not be controlled by the nucleolus.

The ½-hour treated roots showed nuclei with prominent chromatin material, but no significant increase in the number of nucleoli. This may indicate a chromosomal (extranucleolar) role in the increased RNA synthesis due to kinetin stimulus. This deduction does not necessarily preclude the possibility that kinetin stimulates the nucleolus to actively synthesize RNA without any visible increase in the amount of nucleolar material. In this connection the role of nucleolar organizer of chromosomes cannot be omitted. However, it must be emphasized that kinetin can induce active synthesis of RNA and proteins only in the presence of prominent chromatin material. This was evident in the 24-hour treated roots which had prominent nucleoli but inconspicuous chromatin material, and therefore, contained considerably less RNA than the controls.

All roots treated with kinetin for different periods of time showed changes in the endoplasmic reticulum. No exceptions to this were noted, and these changes appeared to be a direct result of the kinetin stimulus. The reorganization of endoplasmic reticulum with prolonged exposure to kinetin indicates that the initial breakup of the endoplasmic reticulum did not represent degenerative effects. Structural differences in dimensions of membranes may indicate distinctive

properties despite their superficial resemblances. It is likely that slight modification in the structure of a membrane may result in a significant change in its function.

It is known that ribosomes are intimately associated with the surface of the granular endoplasmic reticulum, and it is also known that ribosomes are involved in activities concerned primarily with protein synthesis. It is likely that the surface of endoplasmic reticulum becomes a highly reactive region when coming in contact with ribosomes. The present study shows that the breakup of normal endoplasmic reticulum into numerous small vesicles results in an increased surface area. Thus a larger surface area is available for ribosomal attachment. Doubling of RNA content in ½-hour treated roots has previously been reported (Jensen et al., 1964). In the present study roots exposed to similar treatment showed a maximum surface area of endoplasmic reticulum. On the basis of these observations it is suggested that modifications in the structure of the endoplasmic reticulum may represent a response to an increased demand for protein synthesis due to the catalytic effect of kinetin. It is hypothesized that the increased association of ribosomes with lipoprotein membranes of the endoplasmic reticulum may play a vital role in the increased synthesis of proteins.

SUMMARY

As early as ½-hour after kinetin treatment all the primary tissues showed increased rates of cell division over the control. In this regard the apical initials remained unaffected. The number and size of cytoplasmic vacuoles increased rapidly after treatment. The endoplasmic reticulum broke up into relatively small vesicles of various shapes, thus causing an increased surface area of lipoprotein membranes for ribosomal attachment. It is hypothesized that the increased association of ribosomes with membranes of the endoplasmic reticulum may play an important role in increased protein synthesis due to the catalytic effect of kinetin.

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GREEN HERBAGE PRODUCTION OF NATIVE GRASSLANDS IN THE RED RIVER VALLEY—1965[1]

R. D. Ralston and R. L. Dix

Science Department, Mayville State College, Mayville, North Dakota
and
Department of Plant Ecology, University of Saskatchewan, Saskatoon, Canada

INTRODUCTION

The relative importance of environmental factors on green herbage production in the Northern Great Plains and adjacent regions has been evaluated by several investigators. Whitman (1954) and Cosby (1964) have reported on the influence of soils, Larson and Whitman (1942) and Quinnild and Cosby (1958) have measured yield on protected relict sites, and Dix (1960) and Vogl (1965) have studied the effects of fire on northern grasslands. However, no such studies appear to have been done on the grasslands of the Red River Valley in North Dakota, Minnesota and Manitoba. The study reported here was therefore initiated to provide quantitative data on green herbage yield of native grasslands within the Valley. Data are presented here on the total green herbage production as related to geographical section (macroclimate), physiographic position, dominance type and soil texture. This report is part of a general phytosociological study being made by the authors of the native grasslands of the Red River Valley.

STUDY AREA

The Red River Valley is a narrow triangular area, the apex of which is located approximately at the junction of the North Dakota, South Dakota and Minnesota State Lines, and extends directly northward until its base is lost in the vicinity of the southern shores of Lakes Winnipeg and Manitoba. The Valley describes an area that was inundated by an extension of Glacial Lake Agassiz into the

[1]This investigation was supported by funds made available by the National Science Foundation (Grant No. GB-672)

preglacial Red River basin (Elson, 1959). Well developed beaches and deltas consisting of silts, sands and gravels occupy the ancient shorelines at the periphery of the glacial lake basin. Relief is slight within the Valley proper and elevations center around 750-800 feet above sea level. Drainage is northward through the Red River of the North into Hudson Bay.

The climate is sub-humid with an annual precipitation of approximately 20 inches. The winters are cold (January average 6° F. in the south and -1° F. in the north), the summers cool (approximately 70° F.), while the growing season varies from 130 days in the south to about 100 days in the north.

The native vegetation of the Red River Valley is primarily Tall Grass Prairie (Weaver and Clements, 1938; Whitman, 1963), with gallery forests occupying stream and river banks.

The soils are largely chernozems, having been developed under sub-humid grasslands from lacustrine clays and silts (Anon, 1960).

The Red River Valley has been under heavy cultivation since 1875; much of it was first cultivated during the great bonanza farm operations of the early 1880's (Kazeck, 1956). Intensive cultivation has continued since that time and has greatly accelerated in recent years because the fertile, level land with little or no stone is ideal for mechanized farming. The native grasslands have now all but disappeared beneath the plow, but numerous isolated fragments remain along railroad rights-of-way, in remote corners of cultivated fields, in rural cemeteries and occasionally in school sections. These grassland fragments form the basis of the present study.

METHODS

The grassland remnants which are available usually have a history of disturbance. The stands selected for study, however, were considered to have a minimum of disturbance; i.e., they showed no signs of grazing or cultivation and were clearly dominated by native grassland species. The stands selected for use were mostly on railroad shoulders and are intentionally burned almost every year. It was necessary, therefore, to confine our study to green herbage production only, although the importance of the various mulch categories (Dyksterhuis and Schmutz, 1947) is recognized (Weaver and Rowland, 1952; Dix, 1960; Cosby, 1964; and Vogl, 1965).

The stands used in this study were first sampled for species composition by use of the frequency method, employing 30 quadrats of a size 0.25 x 0.25 m placed throughout the stand. The stands were sampled for species composition during the summers of 1963, 1964, 1965. While the phytosociological data are not presented in this paper, they were one of the major criteria used in selecting the stands for production measurements.

The green herbage production was sampled by methods first described by Dyksterhuis and Schmutz (1947). Stands were sampled on August 17, 18 and 19, 1965. At that time the plants were uniformly

mature and the dominant species were generally in a process of curing. The growing season of 1965, as well as the previous two seasons, were favorable for plant growth in all parts of the study area. The stands were sampled for green herbage production by placing 5 quadrats of a size 0.5 x 0.5 m at representative positions throughout the stands and clipping the plant material produced during the current growing season at ground level. The collected material was first air dried, and then placed in drying bins at 132° F. for 72 hours. The dried material was weighed to the nearest gram immediately upon removal from the bins. The data were then converted to pounds per acre to make them directly comparable to published data.

Soil samples were taken in each stand and textures determined according to Bouyoucos (1936). At the time of sampling, each stand was placed in a physiographic category.

The taxonomic nomenclature is according to Scoggan (1957).

RESULTS

The geographical distribution of the 40 stands which were sampled for green herbage production is shown in Figure 1. The northernmost stand is located near Warren, Manitoba and the southernmost near Fairmont, North Dakota. The north to south distance is 285 miles (between 46° N and 50° N latitude). All stands are within the Red River valley, with the exception of 6 stands from Nelson County, North Dakota, which are on the Drift Plain but which are included here to suggest possible differences in production from east to west. Production of green herbage throughout the Valley was analyzed according to geographical section, physiographic position, dominance type and soil texture.

The Red River Valley was arbitrarily divided into three geographical sections as shown in Figure 1. Eleven stands were included in the northern section, 14 in the central section and 15 in the southern section. Mean production of green herbage as related to these geographical sections and to physiographic position within the sections is presented in Table I. The highest overall production was clearly

TABLE I

MEAN PRODUCTION OF GREEN HERBAGE IN POUNDS PER ACRE OF STANDS GROUPED BY GEOGRAPHICAL SECTION AND PHYSIOGRAPHIC POSITION

		Physiographic Position			
		Dry	Mesic	Wet-Mesic	Mean
Geographic Section	No. Stands	8	23	9	
Northern	11	1863	2164	2458	2108
Central	14	3129	3679	4470	3713
Southern	15	3349	3920	5049	4213
Mean	..	2551	3433	4345	..

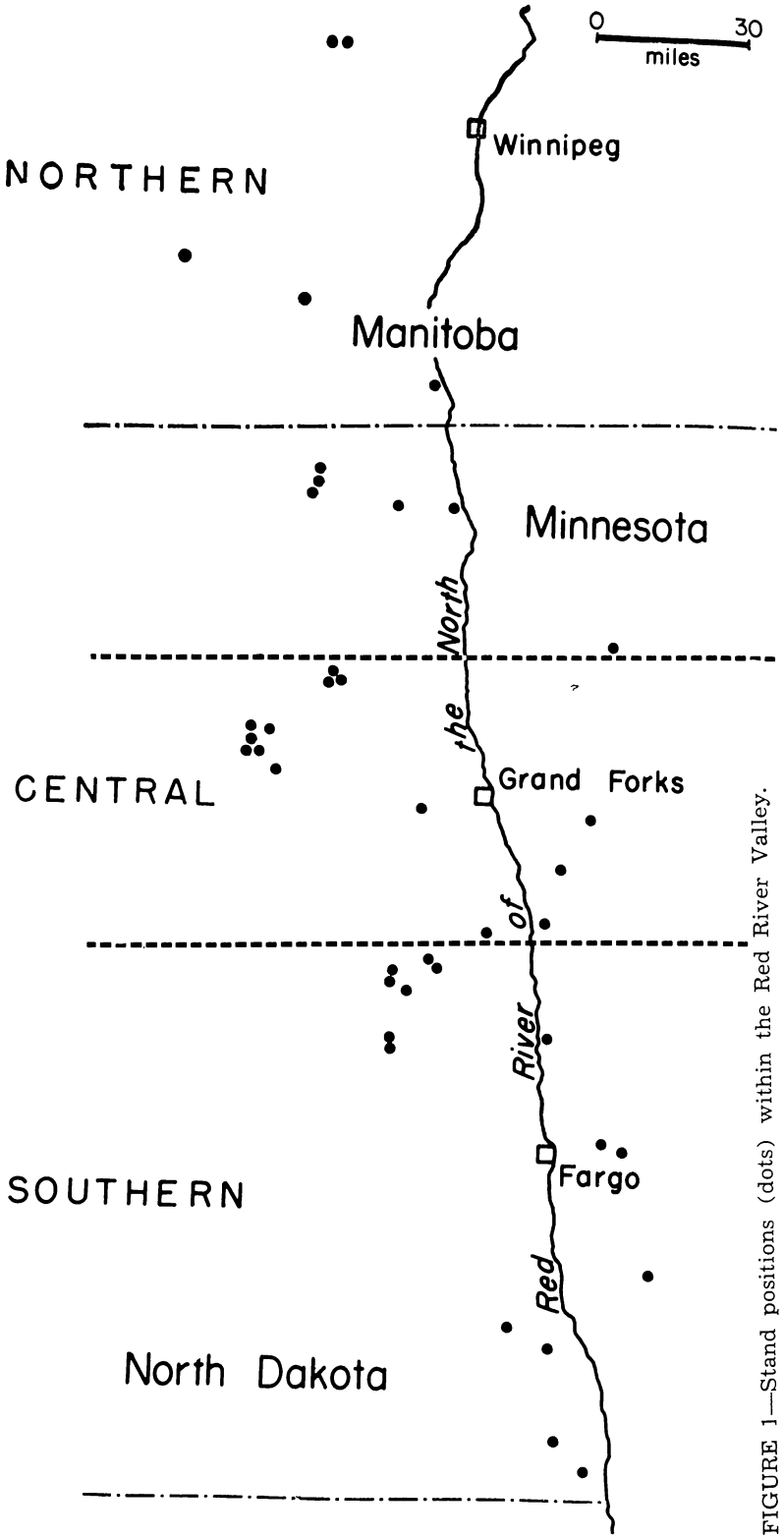

FIGURE 1—Stand positions (dots) within the Red River Valley.

in the south (4213 lb/acre), approximately 100 percent more than in the north. When physiographic positions within the geographical sections were considered, wet-mesic stands showed uniformly higher production than the mesic or dry stands in the same geographical sections. Within the same physiographic position, production in the south was again approximately 100 percent more than in the north. Comparisons in production between the 6 Nelson County stands and other Red River Valley stands at the same latitude failed to show significant differences; both produced an average of 3700 pounds per acre. Thus, the gradient of green herbage production within the Valley during 1965 seems clear: the highest production on wet-mesic sites in the southern part of the Valley with the least production on dry sites in the north.

In Table II, production was evaluated within and between geographical sections according to the dominance types of the stands.

TABLE II

MEAN PRODUCTION OF GREEN HERBAGE IN POUNDS PER ACRE OF STANDS GROUPED BY DOMINANCE TYPES IN THE THREE GEOGRAPHICAL SECTIONS

		Geographical Section			
		Northern	Central	Southern	
Dominance Types	No. of Stands	10	11	13	Mean
Andropogon gerardi	16	2415	3801	4765	3816
Andropogon scoparius	10	2197	3355	4210	3552
Stipa spartea	5	2175	3379	4148*	3051
Stipa comata	3	1551		2551*	1884

*One stand only.

Six stands had to be disqualified from this comparison because their dominance types did not occur in two of the geographical sections. Four dominance types were considered. Stands dominated by *Anropogon gerardi* had the highest (3816 pounds per acre) while stands dominated by *Stipa comata* had the lowest (1884 pounds per acre) production. *Andropogon scoparius* and *Stipa spartea* dominated stands were more similar in production to the former than to the latter. There was a notable difference in production between like stands from south to north. Within each dominance type, the southern stands produced approximately 100 percent more green herbage than similar northern stands. Within the same geographical section, however, differences in production between dominance types was not great, usually within 15 percent, with the exception of *Stipa comata* stands which were comparatively poor producers in all geographical sections.

The trends in green herbage production with regard to dominance types seems well defined: *Andropogon gerardi* stands were the best

producers, followed closely by *Andropogon scoparius* and *Stipa spartea,* while *Stipa comata* stands produced far less than the others. Table II suggests, however, that, with the exception of *Stipa comata* stands, dominance type is less important than geographical section in determining production. Even within *Stipa comata* stands, however, production in the southern section was significantly greater (60 percent) than in the north.

Table III was prepared to test the relative importance of soil texture in determining green herbage production. In order to include

TABLE III
MEAN PRODUCTION OF GREEN HERBAGE IN POUNDS PER ACRE OF ALL STANDS AND OF MESIC STANDS GROUPED BY TEXTURAL CLASSES

Soil Textures	No. Stands	Means All Stands	No. of Stands	Means Mesic Stands
Sandy loam-sandy clay loam	11	2652	4	3017
Loam-silt loam	6	3611	5	3501
Silty clay loam-silty clay	9	3635	7	3961
Clay loam-clay	14	4067	7	3160

sufficient stands for comparative purposes, textural values for the stands were grouped into 4 categories ranging from coarse to fine. When all stands were considered, production was shown to be highest on clays or clay loams and lowest on coarse textured soils. Ellis (1938) has pointed out, however, that the finer soil fractions tend to occur at lower slope positions, thus raising some doubt as to whether the high production on fine textured soils is attributable primarily to physiographic position or to soil texture. Fortunately, our mesic stands, as shown in Table III, had a wide range of textures. The mesic stands were separated into the textural categories given in Table III and their green herbage production within the categories averaged. These data showed that moderately coarse to moderately fine soils yield the highest production on mesic sites while both very fine and coarse soils yield less. Thus, it is suggested that soil texture is secondary to physiographic position in determining green herbage production within the Red River Valley.

Certain dominance types, of course, occurred more frequently in some physiographic positions than others. On dry sites, the most frequent leading dominant was *Stipa comata* with its associated species *Calamovilfa longifolia, Panicum perlongum, Koeleria cristata* and *Bouteloua gracilis.* Mesic sites were most frequently dominated by *Andropogon scoparius* and *Stipa spartea,* while associated species were *Sporobolus heterolepis, Sorghastrum nutans* and *Andropogon gerardi.* Wet-mesic sites usually supported stands dominated by

Andropogon gerardi with *Spartina pectinata, Calamagrostis inexpansa* and *Andropogon scoparius* as secondary species. Thus, dominance type, physiographic position and soil texture all seem interrelated.

All of the dominant types sampled in the study occurred throughout the Valley, although some dominance types were rare within a given geographical section. The species composition of the dominance types was essentially the same throughout the Valley, although a few differences were noted. For example, *Selaginella densa* occurred with fairly high frequency values in *Stipa comata* stands in the north but did not occur in these stands in the central or southern sections. Similarly, *Liatris pycnostachya* was important in *Andropogon gerardi* stands in the southern section, but was absent from these stands in the central and northern sections.

The data presented in Tables I, II and III clearly show the north-south gradient to be the principal factor in determining the overall production of green herbage in the Red River Valley. To examine the reasons for this, the mean values for several climatic characteristics were compiled, averaged by geographical sections, and presented in Table IV. The climatic data used were the long-term averages for

TABLE IV
MEAN CLIMATIC CHARACTERISTICS OF STANDS GROUPED BY GEOGRAPHICAL SECTIONS

	Northern	Central	Southern
No. of Stands	11	14	15
Length of growing season (days)	111	126	130
Avg. date of first killing frost	Sept. 18	Sept. 22	Sept. 24
Avg. date of last killing frost	May 27	May 19	May 14
January Avg. Temp. (°F)	-1	5	6
July Avg. Temp. (°F)	68	69	70
Avg. precipitation (inches)	19	19	21

the closest weather station to each stand. The most conspicuous climatic difference between the northern and southern sections was the length of the growing season; in the north, the growing season averaged 111 days as compared with 130 in the south. There was remarkably little variation between the average climatic values for other factors. Thus, it appears that much of the observed difference in green herbage production found in the present study to occur between the north and south is attributable to the length of the growing season. In all probability, it is the date of the last frost in spring, rather than the first frost in fall, which determines production. (Whitman, 1954).

DISCUSSION

Many of the values for green herbage production presented in this paper appear somewhat higher than data reported for comparable sites in adjacent areas of the northern grasslands (Sarvis, 1941;

Whitman, 1954; Dix, 1960; Cosby, 1964; and Vogl, 1965). These comparatively high values may be accounted for, for the most part, by the favorable growing seasons of 1964 and 1965. Throughout the Red River Valley, the period April through July, 1964 had approximately 16 percent more precipitation than normal, while the precipitation for the same period in 1965 was 35 percent above normal. Cosby (1964) found that herbage yields in *Andropogon gerardi - Calamovilfa longifolia* grasslands in the Souris River Area of North Dakota, some 150 miles west of Grand Forks (Figure 1), varied greatly in drought years (1340 pounds per acre) as contrasted with years of above normal precipitation (3420 pounds per acre). The latter figure seems comparable with the mean production value of 3801 pounds per acre given in Table II for the *Andropogon gerardi* dominance type in the central section of the Red River Valley. In normal years, or following a series of near normal years, the central section of the Red River Valley grasslands would probably yield production values only slightly higher, because of higher precipitation (16 in. for the Souris River area and 19 in. for the Red River Valley), than the 6 year averages given by Cosby (1964) for the Souris River area. Similarly, in the Red River Valley northern stands would be expected to produce substantially less and southern stands substantially more than his 6 year average. Since the production values given in the present paper reflect two exceptionally moist years, it seems likely that our values approach the maximum annual production possible for the Red River Valley grasslands.

Frequent burning of the Red River Valley grasslands contributes to their vigour by permitting a rapid turnover of mineral nutrients (Curtis and Partch, 1950), retarding a depressing accumulation of mulch (Weaver and Rowland, 1952; Dix and Butler, 1954), and inhibiting the development of woody species (Ewing, 1924). Thus, it seems likely that the surviving prairie fragments of the Red River Valley, because of their association with railroad right-of-ways and hence fire, are substantially similar to the prairies of presettlement time. Prairie is essentially a disturbance vegetation (Stewart, 1956), the major disturbance being fire, and the continuance of this close association through time in the Red River Valley has been a major factor in sustaining these prairie fragments.

The data presented in the present paper form an interesting parallel with a study by Weaver (1924) on plant production along a moisture gradient from eastern Nebraska to eastern Colorado. The decrease in yield of herbage production with the decrease in precipitation from east to west which he reported closely parallels our decrease in yield with a decrease in growing season from south to north. Weaver reported differences between dominance types, production and physiographic positions analogous to those found in the present study. Thus, a similar plant response appears to have been achieved in the two areas, although a totally different limiting factor

was involved in each. McMillan (1959) has pointed out the similarities in flower response of grassland species in the western and northern parts of the Central Grassland of North America, suggesting that the plants of both areas respond primarily to a short growth period. He has suggested a historical link between the grasslands of the west and north.

SUMMARY

Significant differences in production of green herbage were found between phytosociologically similar stands in the northern as contrasted with the southern sections of the Red River Valley. Southern stands yielded twice the amount of herbage as did comparable northern stands. Within any geographical section, stands located at wet-mesic physiographic positions out-produce stands at mesic and dry positions by 20 to 30 percent, respectively. The highest producing dominance type was *Andropoyon gerardi,* with *Andropogon scoparius* and *Stipa spartea* types following closely. Dry physiographic positions dominated by *Stipa comata* and associated species were the poorest producers of green herbage. The climatic factor playing the most important role in determining yield within the Red River Valley appeared to be the length of the growing season. The growing season in the north averaged 111 days while in the southern stands it averaged 130 days. The date of the last killing frost in spring appeared more important to plant production than the date of the first killing frost in fall.

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A COMPARISON BETWEEN MECHANICAL AND SULFURIC ACID SCARIFICATION OF DORMANT WILD BUCKWHEAT (*POLYGONUM CONVOLVULUS*) SEEDS

Paul C. Quimby, Jr., John D. Nalewaja and Allyn R. Bell
Department of Agronomy
North Dakota State University of Agriculture and Applied Science,
Fargo, North Dakota

INTRODUCTION

Wild buckwheat (*Polygonum convolvulus*) is a persistent nuisance in the small grain and flax fields of North Dakota. The authors were interested in conducting extensive greenhouse and field competition studies and herbicide trials in order to better evaluate crop

losses caused by the weed and methods of control. These studies required an adequate source of germinable seed. Freshly harvested mature seeds were dormant and highly variable in germination.

Seed dormancy in *Polygonaceae* can be overcome by scarification. As described by Woodcock (4), the seeds of the *Polygonums* are in fact single seeded fruits (achenes). The seeds are enclosed in a thick, hard, cutinized pericarp surrounded by a brown calyx layer (3). The testa is closely compressed and its crushed remains comprise a thin inseparable layer around the embryo and endosperm (4). Scarification of wild buckwheat seeds is the removal of the calyx, if present, and the pericarp from the seed. Justice (1) used the terms "intact seeds" for unscarified fruits and "naked seeds" for seeds which had been freed from the pericarp. His terminology is used in this paper.

Ransom (2) attributed dormancy in *Polygonaceae* to "dormant embryos which must undergo a period of after-ripening before germination can take place." Justice (1) wrote, "Some field observations . . . indicate that the relatively thick pericarp surrounding the seed in *Polygonum* may be responsible for the dormant condition in such seeds. The pericarp . . . controls the rate of after-ripening by restricting the exchange of gases. It retards the rate of water absorption by seeds to some extent, but this does not seem to be of great significance. The pericarp might offer a certain amount of mechanical resistance to germinating seeds, but fully after-ripened seeds were able to overcome this obstacle."

From anatomical work with *P. scandens,* Justice (1) concluded that the after-ripening of the embryo was physiological and not reflected in morphological changes. He also conducted trials on embryos excised from acid scarified seeds. The after-ripening period at 2 to 4° C required for development of excised embryos on nutrient agar was similar to that for germination of naked seeds stored between layers of moist cotton at the same temperature. However, embryos germinated immediately at room temperature if they were aerated in liquid nutrient solution or exposed for 7 days to approximately 50% oxygen.

Justice (1) used a 20-minute period for sulfuric acid scarification of *P. convolvulus* fruits. He removed the remnant pericarp and discarded visibly injured seeds. Three weeks of chilling at 2 to 4° C and subsequent exposure to 25° C gave 55% and 25% germination for the naked and intact seeds, respectively. In comparison, intact seeds germinated 75% after storage between layers of moist cotton at 2 to 4° C for 8 weeks. Ransom (2) reported that intact seed of *P. scandens* required three times longer for after-ripening than naked seeds obtained from fruits scarified with sulfuric acid for 5 minutes. Temperatures of 3 to 6° C were best for after ripening. Neither Ransom (2) or Justice (1) reported information on the seed: acid volume ratio used in scarification or whether the seed and

acid mixture was stirred. However, Porter and Koos as reported by Justice (1) obtained better germination from intact *P. convolvulus* seeds than from naked seeds obtained from scarification with sandpaper. Justice postulated that the embryos were injured by the sandpaper.

The objective of this study was to develop a practical method of scarifying and increasing germinability of dormant wild buckwheat seeds so as to ensure an abundant supply for various experiments.

MATERIALS AND METHODS

Acid scarification.—Preliminary studies in our laboratory indicated that 45 minutes of seed exposure to acid without stirring gave about 70% germination. Moreover, removal of pericarp remnants after scarification did not increase germination. In this study various volumes of seeds, viz., 5.0 cc, 3.3 cc and 2.5 cc were mixed with 10 cc of concentrated sulfuric acid to comprise seeds:acid volume ratios of 1:2, 1:3 and 1:4, respectively. The 3.3 cc of seeds was equal to about 400 seeds. Separate lots of each of the seeds:acid ratios were slowly agitated with a ½-inch magnetic stirrer in 20-ml beakers for 15, 30 or 45 minutes. The control was the same as the 1:3 ratio except water was substituted for acid. All seeds were washed for 30 minutes after exposure to the acid and then rinsed in a slurry of Arasan for fungus control. No attempt was made to remove remnants of the pericarp.

Mechanical scarification.—Intact seeds were mechanically scarified by one pass through a scarifier consisting of a carborundum wheel turning at high speed within a steel casing. The scarifier was designed by H. J. Gorz, Crops Research Division, Forage and Range Research Branch, Agr. Exp. Sta., Lincoln, Nebraska 68503 and built by the Fargo Foundry Company, Fargo, North Dakota. The scarifier removed the pericarp from about one-third of the intact seeds. Then naked seeds were separated from intact seeds by repeated passes through a Carter dockage tester. The intact seeds were then passed through the scarifier again and the separation repeated. No attempt was made to separate injured seeds from non-injured seeds.

Post-scarification storage treatments.—Seeds from each scarification treatment were subjected to various post-scarification storage treatments ranging from one week dry at 24° C to 20 days wet at -10° C (Table I). The wet treatment consisted of placing the seeds in a 9-cm petri dish with filter paper in the bottom plus 5 ml of distilled water. Germination tests were conducted at 24° C after the post-scarification storage treatment and percentages of germination were determined after 14 days.

RESULTS AND DISCUSSION

Wild buckwheat seeds germinated 60% from the 1:3 seeds:acid volume ratio with 15 minutes of stirring and after 10 days wet storage at 3° C, while unscarified controls treated similarly germi-

nated 8% (Table I). As the volume of seed decreased per 10 cc of acid, increased stirring times caused seed injury and reduced germination. Forty-five minutes stirring time caused nil germination of seed from the 1:3 and 1:4 seeds:acid volume ratios. Fifteen minutes of stirring resulted in better germination than 30 minutes over all ratios. Physical appearance of seeds within the 1:2 ratio indicated that 15 minutes stirring did not scarify adequately. However, longer stirring times for this ratio decreased germination probably because the seeds were in closer contact and injured by stirring.

Germination increased as post-scarification storage time increased from 3 to 10 days at 3° C. An additional 10 days, i.e., 20 days of post-scarification storage had little added effect on germination except on the unscarified controls which germinated 16.3% compared with 8% for the 10 day treatment.

A later trial indicated that germination percentages up to 80% could be obtained by using the 1:3 ratio with stirring for 10 minutes and by chilling for 10 days at 3° C.

About 35% of the embryos were injured by the mechanical scarification method, but the remaining 65% nearly all germinated regardless of refrigeration treatment except freezing decreased germination by two-thirds (Table I). In contrast to Ransom's (2) and Justice's (1) conclusions that dormancy partially resides in the embryo, our data suggest that the pericarp is the sole cause of dormancy in *P. convolvulus*. The so-called "after-ripening" cold treatment may be necessary only to overcome some effect of sulfuric acid on the seeds or to overcome the effect of a possible residual chemical inhibitor originating in the pericarp and not removed by acid scarification.

Mechanical scarification is easier and more efficient than acid scarification for obtaining large quantities of germinable buckwheat seeds because acid scarification is more limited in the amount that can be scarified at a given time and more dependent on added variables such as stirring time, volume ratios and post-scarification storage.

SUMMARY

Germination of dormant wild buckwheat seeds was influenced by the volume ratio of seeds:acid, time of exposure to acid, the presence or absence of stirring during acid exposure, and the duration of storage at 3° C under moist conditions prior to placement in the germinator at 24° C. Acid scarified seeds germinated 64% from a seeds:acid volume ratio of 1:3 (3.3 cc or 400 seeds + 10 cc acid, magnetically stirred for 15 minutes) and post-scarification storage at 3° C for 10 days. Unscarified fruits germinated 8%. Removal of the pericarp remnants after acid scarification did not increase germination.

Fruits were mechanically scarified by one pass through a scarifier consisting of a carborundum wheel turning at high speed within a steel casing. This removed the pericarp from about one-third of the fruits. Then naked seeds were separated from intact fruits with

TABLE I

PERCENT GERMINATION[a] OF DORMANT WILD BUCKWHEAT (*POLYGONUM CONVOLVULUS*) SEEDS AS AFFECTED BY ACID AND MECHANICAL SCARIFICATION AND VARIOUS POST-SCARIFICATION STORAGE TREATMENTS

Post-scarification storage treatment	Check[b]			Acid scarification: Volume ratio of fruits: concentrated H_2SO_4							Mechanical scarification[d]
				1:2			1:3		1:4		
	Stirring time (minutes)[c]										
	15	30	45	15	30	45	15	30	15	30	
Non-direct to germinator	3.0	1.1	2.8	13.1	1.0	2.7	14.9	4.2	20.4	1.7	60.7
7 days dry at 24° C	1.0	1.7	1.3	16.5	2.2	3.0	16.2	4.1	20.8	.9	57.0
3 days wet at 3° C	1.7	2.7	2.7	13.6	4.3	3.2	31.3	13.4	29.9	.5	52.1
6 days wet at 3° C	4.3	3.3	3.7	13.4	3.3	2.3	37.1	13.6	51.6	1.3	65.2
10 days wet at 3° C	8.0	7.1	4.5	23.3	2.5	4.6	64.0	21.5	54.5	2.3	60.9
20 days wet at 3° C	16.3	18.5	17.1	23.8	2.8	3.2	52.9	19.0	46.3	1.7	64.9
3 days wet at -10° C	1.7	0	0	8.4	1.1	1.3	11.3	3.8	27.2	1.5	27.1
20 days wet at -10° C	1.0	2.0	2.4	15.6	1.5	2.2	25.2	11.6	27.3	1.0	27.4
Mean		4.6	4.3	16.0	2.3	2.8	31.6	11.4	34.8	1.4	51.9

[a]Trials conducted in autumn of harvest year. Germination percentages were calculated after the post-scarification storage treatment and 2-weeks in the germinator at 24° C.

[b]Control seeds were treated the same as those having the seeds:acid ratio of 1:3 except water was substituted for the acid.

[c]The seeds-liquid mixture was agitated with a magnetic stirrer for the indicated time and then the seeds were rinsed with cold tap water for 30 minutes.

[d]Mechanically scarified by a carborundum wheel turning at high speed inside a metal casing.

a Carter dockage tester. About 35% of the embryos were injured by this scarification method, but the remaining 65% nearly all germinated without a refrigeration treatment. According to data presented, seed dormancy in *P. convolvulus* is probably caused solely by the pericarp. Mechanical scarification is easier and more efficient than acid scarification for obtaining large quantities of germinable wild buckwheat seeds.

ACKNOWLEDGMENTS

This study was supported in part by funds from North Central Regional Project NC-61, "The ecology of crops and weeds as related to control measures."

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SELECTED BIOLOGICAL COMMUNITIES OF THE WARWICK-MCVILLE IRRIGATION DISTRICT

Jean M. Toczek

Department of Entomology

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

ABSTRACT

During the spring and summer of 1964, a preliminary survey was conducted to describe the biological communities of the Warwick-McVille Irrigation District. A total of 11 plant communities were defined and qualitatively described together with their associated insect life, specifically the grasshoppers (Acrididae), blister beetles (Meloidae), and the mosquitoes (Culicidae). These plant communities included the quackgrass-slender wheatgrass community, the Kentucky bluegrass-little sage-wolfberry community, the Kentucky bluegrass-needle-and-thread community, the mixed grass-forb community, the *Salix-Carex* marsh community, the smooth brome meadow community, the cropland community, the woodland community, the bluegrass-wolfberry-shrub community, the roadside community, and minor communities. This study was continued during the summer of 1965 emphasizing selected insect groups of the smooth brome meadow community exclusively.

DIFFERENTIAL SPECTROPHOTOMETRIC DETERMINATION OF HAFNIUM IN THE PRESENCE OF ZIRCONIUM

Arnold R. Johnson, Jr., and Harry Freund

Departments of Chemistry

Minot State College,[1] Minot, North Dakota, and

Oregon State University, Corvallis, Oregon

INTRODUCTION

In general, the chemical methods of analysis for the determination of hafnium in the presence of zirconium have been indirect. An excellent review of these indirect chemical methods, as well as the available instrumental methods, is given by Goward and Jacobs (6). These chemical methods usually involve the determination of the total hafnium and zirconium by two independent chemical methods, one of which consists of weighing a mixture of the pure ignited oxide and the other a gravimetric, volumetric or spectrophotometric procedure. The composition of the mixture is then found by solving two simultaneous equations or determined on the basis of a calibration curve in which a fixed weight of known oxide mixtures is used. This paper describes a differential spectrophotometric method in which chloranilic acid is used as a complexing agent for zirconium and hafnium.

Thamer and Voigt (10) found that zirconium in perchloric acid solution forms soluble, colored complexes with chloranilic acid. They have also described the use of chloranilic acid for the analytical determination of small amounts of zirconium. The molar absorptivity at 330 mμ was reported to be about 1.8×10^4. This system was also studied by Frost-Jones and Yardley (5). However, they measured the absorbance at 525 mμ. At this wavelength the molar absorptivity is only about 1×10^3. Application of this complex to the analysis of zirconium in steel has been made by Hahn and Johnson (7), and to the analysis of zirconium in plutonium alloys by Bricker and Waterbury (3).

Since the zirconium-chloranilic acid complex seemed to have several desirable characteristics, its applicability to the determination of hafnium in the presence of zirconium by differential spectrophotometry was investigated. The results of this investigation are reported in this paper.

The differential spectrophotometric approach utilized in this research is based on the following considerations:

(1) Hafnium and zirconium will react in an identical manner with chloranilic acid.

[1] A. R. J., Jr.

(2) The intensity of the colored complex formed with chloranilic acid and mixtures of hafnium and zirconium will depend on their ratio when the total weight concentration is held constant.

(3) Differential spectrophotometry will provide sufficient gain in accuracy and sensitivity to permit accurate analysis by this method.

The differential method which was used is commonly known as the *Transmittance Ratio* method. For excellent discussions of this technique and other differential techniques see references 1, 2, 8, 9, and 11.

The paper of Freund and Holbrook (4) should be consulted for a discussion of the theoretical basis of the method developed in this paper. In this method, an absorbing reference solution of the mixed oxides is used to set the R = 100 end of the spectrophotometer scale. Solutions of greater absorbance are then measured relative to the reference solution. The absorbance which is read, is the difference in absorbance between the reference solution and the solution being measured and will be referred to as the relative absorbance.

Because of the chemical similarity of zirconium and hafnium, the only difference in absorbance between the zirconium and hafnium chloranilate complexes is due to their difference in atomic weight. This relationship is shown below:

$A_{Zr O_2} = \epsilon \times b \times C \times 1/ZrO_2$

$A_{Hf O_2} = \epsilon \times b \times C \times 1/HfO_2$

where A = absorbance of complex

ϵ = molar absorptivity of complex (same for both Zr and Hf)

b = cell path length

C = weight concentration of the oxide

From the above relationships, it can be seen that if the weight of the oxides is held constant, the absorbance of any mixture of hafnium and zirconium will fall between these limiting values set by the pure components. Since the weight of the total oxide (W_t) must be constant, it is necessary to establish what this weight should be. This can be accomplished by the method suggested by Bastian (2) and is based on the considerations outlined below.

If a series of complex solutions of varying amounts of ZrO_2 differing by some fixed weight increment is prepared, then the absorbance of each solution can be measured relative to the next lowest weight, beginning with the pure solvent. The slope (S) of the straight line obtained between each of these pairs of solutions can then be calculated from the relationship $S = \Delta A/\Delta C$ where ΔA is the minimum difference in absorbance (instrumental error) that can be detected and ΔC is the minimum difference in concentration, expressed in weight/volume, that can be detected. The slope relationship can be rearranged to give $\Delta C = \Delta A/S$ which on dividing both sides by C gives,

$\Delta C/C = \Delta A/S \times C$

Since $\Delta C/C$ is the relative error, we wish to minimize it. It can be seen from this relationship that $\Delta C/C$ will be at a minimum when S x C is a maximum, assuming that ΔA remains constant. A plot of S x C against the concentration of the reference solution will, therefore, be sufficient to establish the optimum concentration. The concentration corresponding to the maximum of this plot will be the optimum concentration of the ZrO_2 for the complex being measured. This maximum is reached because of deviation from Beer's law or because the reference solution has become so absorbing that the instrument is no longer linear.

Once the optimum conditions are established, a standard curve can be constructed. A series of standard solutions of varying $\%HfO_2$ and constant W_t (optimum concentration) is prepared. The R = 100 end of the spectrophotometer scale is set with the solution containing the highest $\%HfO_2$ since it will be least absorbing. The remainder of the standard solutions are then measured relative to this reference solution. If the relative absorbance is then plotted against $\%HfO_2$, a straight line calibration curve should be obtained.

EXPERIMENTAL

Apparatus.—A Beckman Model DU Spectrophotometer with Photomultiplier and matched 1 cm. silica cells was used for all measurements.

Reagents.—Perchloric acid, 4M. Dilute 684 ml of 70% (11.7M) perchloric acid, reagent grade, to 2 liters with distilled water.

Chloranilic acid, 0.10%. Dissolve exactly 0.5000 g of previously purified chloranilic acid (2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone), Eastman No. P4539, in distilled water and dilute to 500 ml in a volumetric flask. The chloranilic acid was purified by the precedure suggested by Thamer and Voigt (10). Eight grams of the acid was dissolved in a liter of distilled water at the boiling point and then filtered. The filtrate was extracted with 200 ml portions of benzene in a separatory funnel at 50°C. The benzene phases were discarded and the aqueous phase cooled in an ice bath, yielding bright red crystals of chloranilic acid, which were filtered off and washed with three 10 ml portions of ice water. The crystals were then dried at 110°C.

ZrO_2 solution, 2.624 mg ZrO_2/ml. About 3 g of $ZrOCl_2 \cdot 8H_2O$ were dissolved in distilled water and precipitated with ammonia. The precipitate was washed several times with distilled water and was finally dissolved and diluted to 500 ml with 2M perchloric acid. The solution was standardized gravimetrically by precipitating with cupferron and igniting to the oxide.

HfO_2 solution, 0.988 mg HfO_2/ml and 0.988 mg HfO_2/ml. This solution was prepared and standardized in the same manner as the ZrO_2 standard except that about 0.9 g of $HfOCl_2 \cdot 8H_2O$ was used. The

0.988 mg HfO_2/ml standard was prepared by diluting the more concentrated solution ten times with 2M perchloric acid.

Absorption Curves.—Absorption curves of chloranilic acid and its zirconium complexes were run in both the UV and visible region. The results confirmed those obtained by Thamer and Voigt (10) and by Frost-Jones and Yardley (5). On the basis of these spectra, 330 $m\mu$ was selected as the wavelength to be used for differential measurements.

Optimum Concentration.—A series of solutions containing from 0.05 mg ZrO_2/25 ml to 0.70 mg ZrO_2/25 ml was prepared. A five-fold excess of chloranilic acid was added to each solution, which was diluted to volume in such a manner that the final solution was 2M in perchloric acid.

The optimum concentration was then determined by measuring the absorbance of each of these solutions relative to the one of next lower concentration as reference. The slope of the straight line between the reference solution and standard solution was calculated by dividing the relative absorbance by the concentration increment. The slope value was then multiplied by the reference solution concentration to give S x C. The reference solution concentration at which S x C was greatest was taken as the optimum concentration. These data are summarized in Table I.

TABLE I

OPTIMUM CONCENTRATION OF ZrO_2

Solution mg ZrO_2/25 ml					
To set zero	To obtain reading	Relative absorbance	Slope (S)	Concentration (C)	S x C
Water	0.05	0.378	7.56	..	..
0.05	0.10	0.422	8.44	0.05	0.422
0.10	0.15	0.448	8.97	0.10	0.897
0.15	0.20	0.489	9.78	0.15	1.47
0.20	0.25	0.520	10.4	0.20	2.08
0.25	0.30	0.502	10.1	0.25	2.51
0.30	0.40	0.840	8.40	0.30	2.52
0.40	0.50	0.389	3.89	0.40	1.56
0.50	0.60	0.104	1.04	0.50	0.52
0.60	0.70	0.063	0.63	0.60	0.38

It can be seen from this table that the optimum concentration is about 0.30 mg ZrO_2/25 ml, i.e., the product of the slope and concentration (S x C) is a maximum when the 0.30 mg ZrO_2/25 ml solution is used as the reference.

Determination of Relative Absorbance Between Solutions of $f_{HfO_2} = O$ and $f_{HfO_2} = 0.20$ *at Different Total Oxide Concentrations.*—In order to further confirm the optimum concentration, several

pairs of solutions containg 0% and 20% HfO_2 were prepared at different total oxide concentrations. The difference in absorbance each pair of solutions was then measured. The total oxide level at which the absorbance is the greatest will be the value nearest the optimum concentration. For this portion of the investigation, the total oxide level was varied from 0.250 mg oxide/25 ml to 1.250 mg oxide/25 ml. The results are shown in Table II.

TABLE II

DIFFERENCE IN ABSORBANCE BETWEEN 0 AND 20% HfO_2 SOLUTIONS WITH CHLORANILIC ACID

Solution %HfO_2 To set zero	To obtain reading	Total oxide (mg/25 ml)	Relative absorbance
20	0	0.250	0.146
20	0	0.500	0.044
20	0	0.750	0.005
20	0	1.000	0.013
20	0	1.250	0.012

These data also indicate that the optimum concentration is low, as the maximum relative absorbance was observed at the lowest concentration. It should also be noted from the data in Table III, that the relative absorbance for solutions differing by 20% HfO_2 at the optimum concentration of 0.30 mg oxide/25 ml, is even greater than that obtained at the 0.250 mg oxide/25 ml level. This would, of course, be expected if the optimum concentration previously determined is correct.

Standard Curve for %HfO_2 in the Presence of ZrO_2.—A series of standard solutions containing 0% to 50% HfO_2 at the optimum concentration of 0.30 mg oxide/25 ml was prepared. A standard curve was then constructed by plotting relative absorbance *vs.* %HfO_2.

TABLE III

STANDARDIZATION CURVE FOR %HfO_2 IN PRESENCE OF ZrO_2

Solution, %HfO_2 To set zero	To obtain reading	Relative Absorbance
50.00	40.00	0.086
50.00	30.00	0.176
50.00	20.00	0.257
50.00	15.00	0.318
50.00	10.00	0.363
50.00	5.00	0.395
50.00	3.00	0.415
50.00	1.00	0.459
50.00	0.00	0.442

Each standard solution was measured using the 50% HfO_2 solution as the reference standard. These results are summarized in Table III and Figure 1.

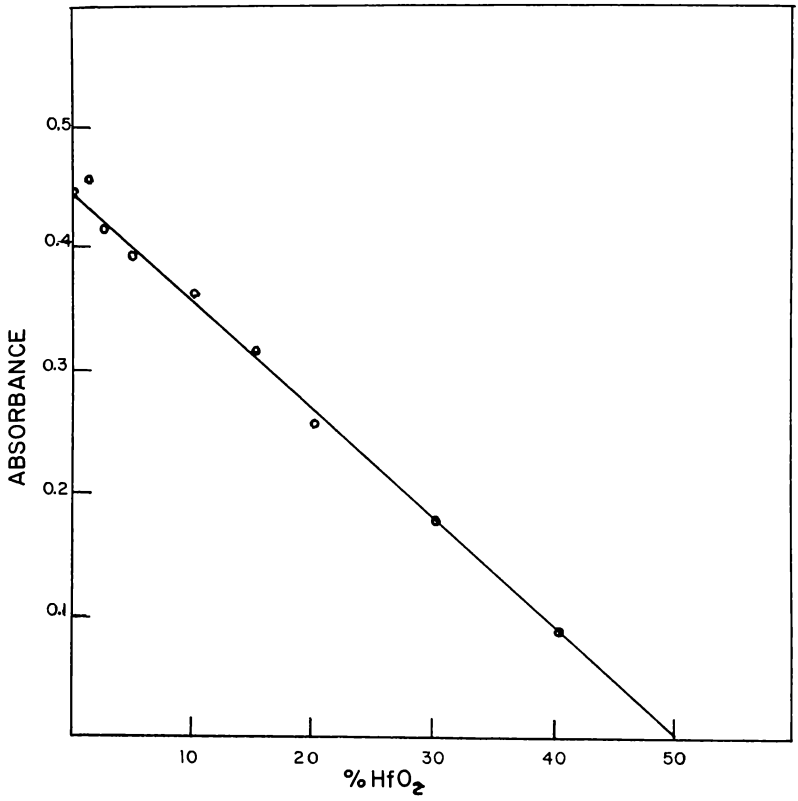

FIGURE 1—Standard curve for the determination of HfO_2 in the presence of ZrO_2.

The results of this research indicate that hafnium can be determined in the presence of zirconium by differential spectrophotometry using chloranilic acid as a complexing agent.

Although the method developed is for the 0 to 50% HfO_2 range, other ranges should also be feasible. For example, it should be possible to determine samples falling within the range 30 to 50% HfO_2, by constructing a calibration curve for standards falling between these values. It also seems likely that 0 to 10% HfO_2 or even 0 to 5% HfO_2 could be determined by constructing appropriate standard curves. These lower ranges should be feasible with an instrument such as the new Beckman DU-2 spectrophotometer which pro-

vides a tenfold scale expansion of the 90 to 100% T scale. The 0 to 5% HfO_2 range would be especially useful for the determination of HfO_2 in ZrO_2 at the levels generally found in zirconium-hafnium minerals and ores.

The method developed should, therefore, be applicable to the determination of hafnium-zirconium ratios in any type of sample falling within the range of analysis, provided the Zr and Hf can be quantitatively separated together as the pure oxides. A very useful reagent for this purpose is mandelic acid.

Similar differential spectrophotometric procedures, using both quercetin and mandelic acid, have been developed by the authors. These illustrate the applicability of this general approach to the problem.

Further research utilizing this approach, as well as others, is in progress.

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THERMAL NITROGEN LOSSES IN A 12-12-12 FERTILIZER MIXTURE

George H. Madany

Department of Chemistry

Minot State College, Minot, North Dakota

ABSTRACT

When a 12-12-12 fertilizer is heated above 100° C it loses nitrogen due to the decomposition of ammonium nitrate. This decomposition is catalyzed by the chloride and hydrogen ions in combination. The addition of 1% urea inhibits these losses by raising the pH of the mixture. Lower or higher urea concentrations are less effective. There is a pronounced peak in the rate of losses during the first hour of heating at 225° C. Acetanilid proved ineffective as an inhibitor.

This work was supported by the National Science Foundation.

KINETICS OF THE OXIDATION OF SECONDARY ALCHOHOLS BY DI-*TERTIARY*-BUTYL CHROMATE IN ANHYDROUS BENZENE

Bruce W. Farnum, Sylvia A. Farnum and William A. Mosher

Departments of Chemistry

Minot State College[1], *Minot, North Dakota, and*

University of Delaware,[2] *Newark, Delaware*

The mechanism of the oxidation of secondary alcohols with chromium trioxide in aqueous acetic acid has been studied by a number of authors, and a general review of the subject has been prepared by Stewart (1). Hillard (2) has measured reaction rates for a series of *para* substituted phenyl-*t*-butylcarbinols and obtained a Hammett rho value of -0.90 in 86.5% acetic acid. Kwart and Francis (3) measured the rates of oxidation of a series of phenylmethylcarbinols in benzene and obtained rho values ranging from -0.37 to 11.01 under a variety of conditions. The generally accepted mechanism formulated by Westheimer (4) for chromic acid oxidation involves a bimolecular decomposition of the chromate ester of the alcohol in the rate determining step. Kwart and Francis (3) proposed a unimolecular decomposition of the chromate ester with the hydrogen on carbon being transferred to the oxygen on chromium as a

[1]B.W.F. and S.A.F.
[2]W.A.M.

proton, rather than transfer of the proton to a molecule of water. The cyclic transition state has little to support it under aqueous conditions, where water is expected to be a stronger base than an oxygen atom in the chromate. However, under anhydrous conditions in neutral benzene, the cyclic transition state for decomposition of the chromate ester is a very logical pathway.

Substituent effects observed by prior workers in aqueous media have been contrary to what would be expected for decomposition of a chromate ester. The rate controlling decomposition of the chromate ester by proton removal should exhibit a positive rho value. Electron withdrawing groups should facilitate the loss of a proton from the carbinol carbon more than they discourage the loss of an electron pair from the more remote oxygen-chromium bond (1).

A series of *para* substituted phenyl-*t*-butylcarbinols were oxidized with di-*t*-butyl chromate in anhydrous benzene. The disappearance of the characteristic absorbance of chromium(VI) esters at 350 mμ was followed by differential spectrophotometry using a blank solution of di-*t*-butyl chromate as a reference. A double beam instrument with thermostated cell compartment and automatic recorder was used. First order rate constants were calculated for the disappearance of chromium(VI) and are listed in Table I.

TABLE I

RATES OF OXIDATION OF *PARA* SUBSTITUTED PHENYL- *TERT*-BUTYL CARBINOLS

Para substituent	$k_1 \times 10^4$ min^{-1} at 25.85 ± 0.1°	$k_1 \times 10^4$ min^{-1} at 34.5 ± 0.2°
H	12.19	29.04
CH_3	9.53	23.37
CH_3O	5.17	17.97
F	10.98	27.74
Cl	11.30	27.41

The use of the sigma$^+$ values of Brown and Okamoto (5) for the modified Hammett relationship provided the best correlation between rate and electron releasing character of the *para* substituents. Figure 1 illustrates the correlation obtained at 25.85° where rho$^+$ had a value of +0.433. At 34.5° the value of rho$^+$ was +0.403. A plot of enthalpy of activation versus entropy of activation led to an estimate of 52° for the isokinetic temperature.

Unmodified Hammett plots indicated more scattering of points, with a definite positive slope. The correlation with sigma$^+$ values indicated that the *para* substituents participated with considerable mesomeric electron release in the transition state during the oxidative decomposition of the chromate ester. Retardation of the rate by electron releasing substituents on the ring is in accord with the accepted mechanism which includes the breaking of the carbinol C-H bond as the rate determining step. Westheimer and coworkers

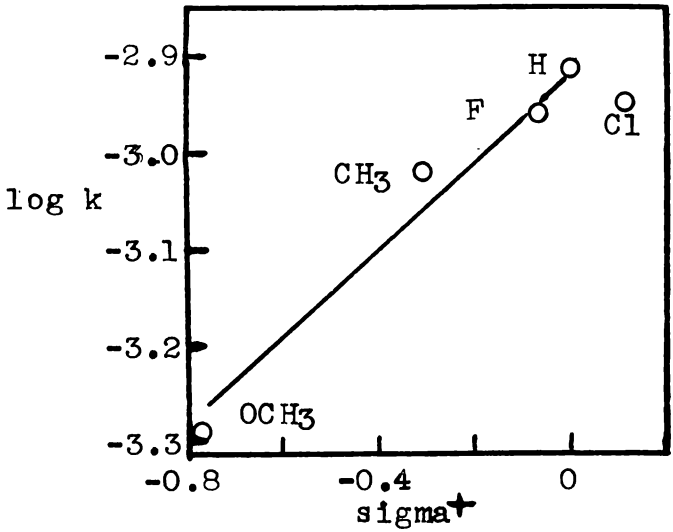

FIGURE 1—Modified Hammett plot.

(4) found a kinetic isotope effect of 6.6 at 25° for the oxidation of 2-deutero-2-propanol in 86% acetic acid. The rate of oxidation of 1-deutero-1-phenyl-2,2-dimethyl-1-propanol was measured in our laboratory. When compared with the rate of phenyl-*t*-butylcarbinol, the deutero isomer had a kinetic isotope effect of 3.30 at 25.85° and 3.09 at 34.5°.

ACKNOWLEDGMENTS

The authors thank Dr. R. L. Hillard of the American Cyanamid Company for the synthesis of the alcohols used in this study.

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VERTICAL MOMENTUM IN THE F_2 REGION OF THE IONOSPHERE

Roland H. Lamberson
Science Division
Minot State College, Minot, North Dakota
and
Willis L. Everett
Nuclear Engineering Department
University of New Mexico, Albuquerque, New Mexico

ABSTRACT

Momentum transfer equations for the constituents of the F_2 region of the ionosphere have been generated by taking velocity moments of the Boltzmann equation. The equations were reduced to manageable form by retaining only dominant terms. These simplified equations were used to study the coupling between the electron, ion, and neutral systems. The results show a strong coupling between the electron and the ion systems and between the ion and neutral systems.

DISCUSSION

The Boltzmann equation will be accepted as providing an adequate description of the behavior of each constituent of the ionosphere. Using the Boltzmann equation in the form

$$\frac{\partial f^\alpha}{\partial t} + v_i \frac{\partial f^\alpha}{\partial r_i} + a_i \frac{\partial f^\alpha}{\partial v_i} = \sum_\beta \left(\frac{\partial f^\alpha}{\partial t}\right)^\beta_{\text{collisions}} + \left(\frac{\partial f^\alpha}{\partial t}\right)_{\text{production}} + \left(\frac{\partial f^\alpha}{\partial t}\right)_{\text{loss}}$$

Equation 1

where α and β denate the type of constituent. The last two terms of equation (1) are expansions of the collision term which allow photo ionization and recombination. Taking the first velocity moment of this equation, Spitzer (1), the equation becomes

$$\rho^\alpha \left[\frac{\partial v_i^\alpha}{\partial t} + v_i^\alpha \frac{\partial v_i^\alpha}{\partial r_i}\right] + v_i^\alpha \left[\frac{\partial \rho^\alpha}{\partial t} + \frac{\partial}{\partial r_j}\left(\rho^\alpha v_j^\alpha\right)\right]$$

$$= n^\alpha q^\alpha E_i + \epsilon_{ijk} J^\alpha H_k - \frac{\partial \psi_{ij}^\alpha}{\partial r_j} + n^\alpha G_i^\alpha - 2\rho^\alpha \epsilon_{ijk} \omega_j v_k^\alpha$$

$$- \rho^\alpha \epsilon_{ijk} \omega_j \left(\epsilon_{kij} \omega_i r_j\right)_k + \sum_\beta \int_{\text{collisions}} m^\alpha v_i^\alpha \left(\frac{\partial f^\alpha}{\partial t}\right) d^3v$$

$$+ \int m^a v_i^a \left(\frac{\partial f}{\partial t}\right)^a_{\text{production}} d^3v + \int m^a v_i^a \left(\frac{\partial f}{\partial t}\right)^a_{\text{loss}} d^3v$$

Equation 2

The forces considered are those due to electric fields, magnetic fields, gravitation and the earth's rotation.

The last two terms of equation 2 are the momentum transfer in a unit volume due to photoionization and recombination respectively. The momentum of the ionizing photon will be divided between the positive ion and freed electron. If each is assumed to absorb one-half of the photon's momentum, then

$$\int m^a v_i^a \left(\frac{\partial f}{\partial t}\right)^a_{\text{prod.}} d^3v = Q\frac{h}{2\lambda}$$

Equation 3

This momentum is directed along a line parallel to the line of centers of the earth and the sun, so that the component normal to the earth's surface will be $-Q\frac{h}{2\lambda}\cos X$ on the day side of the earth and zero on the night side. The momentum change in a unit volume due to recombination is the recombination rate times the average momentum of the particles recombining. Since the probability of recombination is an inverse function of the relative velocity of the particles recombining, the average velocity of these particles will not necessarily be the same as the average for that type of particle. Thus

$$\int m^a v_i^a \left(\frac{\partial f}{\partial t}\right)^a_{\text{loss}} d^3v = -m^a v_1^a \alpha (n^e)^2$$

Equation 4

where v_1 is the average velocity of the particles recombining and $\alpha(n^e)^2$ is the recombination rate. Using the particle transport equation

$$\frac{\partial n^a}{\partial t} = Q^a - \alpha (n^e)^2 - \frac{\partial}{\partial r_i}(v_i^a n^a)$$

Equation 5

and denoting the collision term by P^α, equation (2) for electrons becomes using vector notation,

$$n^e e E_i + (\bar{J}^e \times \bar{H})_i = -K T^e \frac{dn^e}{dr_i} - n^e K \frac{dT^e}{dr_i} - \rho^e g_i$$

$$- P_i^e - Q\left[\frac{h}{2\lambda} f(x) + m^e v_i^e\right] + a (n^e)^2 m^e (v_i^e - v_{i_1}^e)$$

$$- 2 \rho^e (\bar{\omega} \times \bar{v}^e)_i - \rho^e (\bar{\omega} \times [\bar{\omega} \times \bar{r}])_i$$

Equation 6

for ions it is

$$n^i e E_i + (\bar{J}^i \times H)_i = K T^i \frac{dn^i}{dr_i} + n^i K \frac{dT^i}{dr_i} + \rho^i g_i$$

$$+ P_i^i + Q\left[\frac{h}{2\lambda} f(x) + m^i v_i^i\right] - a (n^e)^2 m^i (v_i^i - v_{i_1}^i)$$

$$+ 2 \rho^i (\bar{\omega} \times \bar{v})_i + \rho^i (\bar{\omega} \times [\bar{\omega} \times \bar{r}])_i$$

Equation 7

and for neutrals

$$K T^n \frac{dn^n}{dr_i} + K n^n \frac{dT^n}{dr_i} + \rho^n g_i + P_i^n + a(n^e)^2 (m^n v_i^n - m^i v_i^i - m^e v_i^e)$$

$$+ 2 \rho^n (\bar{\omega} \times \bar{v}^n) + \rho^n (\bar{\omega} \times [\bar{\omega} \times \bar{r}])_i = 0$$

Equation 8

It should be noted that $\alpha(n^e)^2$ is the rate of production of neutrals and Q is the rate of loss of neutrals.

Of particular interest in this study is the vertical component of these equations. This direction will be denoted by z. Assuming that the number density of electrons is equal to that of ions and that their kinetic temperatures are also nearly equal, all of the terms in the three equations may now be evaluated execept for those dealing with the electric field and the current density. The terms relating the momentum transfer resulting from the earth's rotation will not longer be considered since it has long been known that their effect on this region is insignificant. The evaluation of each of the terms may be seen in more detail in Lamberson (2).

From the graphs of the evaluated terms from the three momentum transfer equations, one may choose the terms which dominate the behavior of each of the constituent fluids.

Figure 1 indicates that the momentum transfer equation, for the electron fluid, may be approximated by only retaining the partial

pressure term containing the gradient of the number density and the electrodynamic terms. Thus,

$$- n^e e E_z - (\bar{J}^e \times \bar{H})_z - K T^e \frac{dn^e}{dz} = 0$$

Equation 9

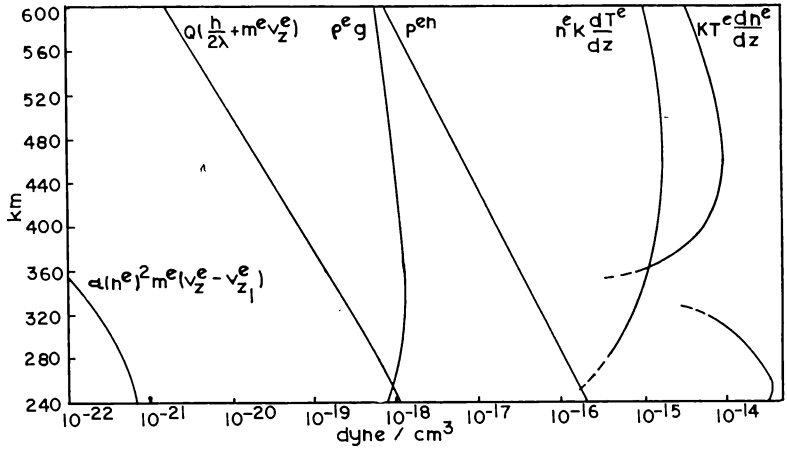

FIGURE 1—The momentum transfer terms for the electron fluid as a function of altitude.

Figure 2 shows that the momentum transfer equation for the ion fluid must also retain the gravitational force term and the ion-neutral collision term so that

$$n^i e E_z + (\bar{J}^i \times H)_z - K T^i \frac{dn^i}{dz} - \rho^i g_z - P^{in} = 0$$

Equation 10

The most influential terms in the conservation of momentum equation for neutrals are the gravitational term and the partial pressure term which contains the number density gradient. Thus,

$$K T^n \frac{dn^n}{dz} - \rho^n g_z = 0$$

Equation 11

Figure 3 indicates that the neutral fluid is not significantly influenced by its interactions with the two charged particle systems. Therefore, the motions of the two charged particle systems will not significantly effect the neutral system.

Figure 2 shows that the ion system is very closely coupled to the neutral system since the ion-neutral collisions are the most sig-

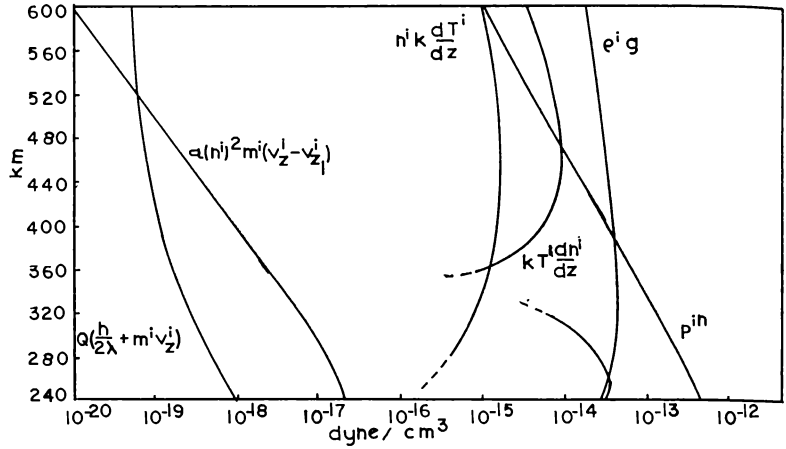

FIGURE 2—The momentum transfer terms for the ion fluid as a function of altitude.

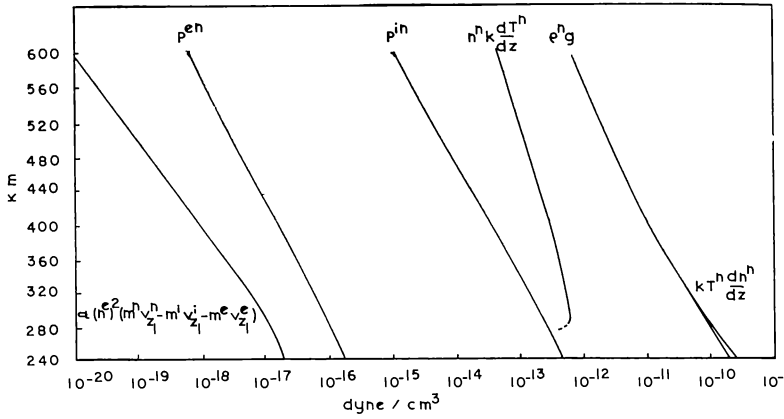

FIGURE 3—The momentum transfer terms for the neutral fluid as function of altitude.

nificant factor in the behavior of the ion fluid in the lower F_2 region and are significant throughout most of the F_2 region.

Figure 1 shows that the electron fluid is not directly coupled to the neutral fluid by collisions. However, it is strongly coupled to the ion system through the electric field term. This strong electric field coupling between the two charge particle systems causes an indirect coupling between the electron and neutral systems through the ion-neutral collision term.

Since there is a very great difference between the collision coupling of the ion and electron systems to the neutral system, the

electron will be able to diffuse much more freely. This raises the possibility of significant electric fields due to vertical charge separation.

In general, this work shows that the terms shown in equations 9, 10, and 11 are the most significant factors in the behavior of the F_2 region. It also shows that the electron and ion systems and the ion and neutral systems are closely coupled.

ACKNOWLEDGMENTS

We would like to thank the Natural Resources Research Institute at the University of Wyoming and its director, Dr. John C. Bellamy for sponsoring this research. We would also like to express our appreciation to the National Aeronautics and Space Administration for supporting this research through grant No. NsG-658.

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TECHNIQUE FOR ADAPTING T. C. CHROMOSOME CULTURE KITS FOR USE WITH BOVINE BLOOD[1]

George E. Staples and Myron F. Andrews

Department of Veterinary Science

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

The use of chromosome culture as a diagnostic tool in human medicine is assuming a place of increasing importance. Chromosome culture or cytogenetics has been employed in diagnosing Klinefelter's syndrome (4) and Turner's syndrome (3). These conditions are associated with maldevelopment of sexual characteristics in male and female respectively. The technique is valuable in differentiating cretinism from monoglism since the former responds to early therapy. Chromosome culture is also a valuable aid in genetic counseling of couples who produce an abnormal offspring (3). Other applications include blood dyscrasia diffentiation, dysproteinemia studies and in estimating radiation damage (3). Chromosomal aberrations are also found in some human abortions (2) and in some anemias (5).

The development and availability of chromosome culture kits

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[1]Published with the approval of the Director of the North Dakota Agricultural Experiment Station.

has greatly expanded the utility of this technique not only in human medicine and research but the animal fields as well. Pakes and Griesemer (6) have enumerated some animal applications for the technique including hermaphroditic studies in the feline, infertility relationships in the bovine male, neoplasia, bovine lymphosarcoma, and studies of the genetic effects of radiation exposure.

Another animal investigational area which might yield valuable information is that of somatic deformities known or suspected to be of genetic origin. It might be enlightening to learn to what degree chromosomal abberrations are produced by such deforming drugs as thalidomide, or the teratogenic plant substance reported by Binns *et al.* (1) to produce cyclopian and "monkey face" lambs. From a practical viepoint it would be valuable to investigate the possibility of detecting carriers of genetic deformities in normal appearing parents. If such proves possible it would be invaluable, particularly since a faulty animal's offspring can now easily number in the thousands through the use of artificial insemination.

The commercially available chromosome culture kit information leaflet states the kits have been successfully employed with bloods of man, horse, monkey, dog, rabbit, guinea pig, hamster, chinese hamster, mouse, rat, armadillo, frog, turtle, alligator and others; the bovine animal was excluded. Very little work has been done with chromosome culture in domestic livestock. Excluding the bovine animal from the simplicity of the commercial kit could discourage work with the bovine. However, with some minor changes in technique these kits can be employed in culture of chromosomes from bovine blood with a high percentage of successes. Procedural steps are numbered in the same order as the Difco information leaflet for comparison.

EXPERIMENTAL

Vial technique

1. (1) Ten ml of blood is withdrawn aseptically from the jugular vein of the bovine animal. Animals need not be fasted. Transfer blood immediately into the blood separation vial and let stand at 37° C for 3 to 4 hours. Suspend the vial in a loop of masking tape and centrifuge at 800 rpm (164 RCF) for 7 minutes. Following centrifugation, approximately ¼ to ⅜ inch of plasma-leukocyte suspension should be available above the red cell layer. It is not necessary to add the Bacto-Phytohemagglutinin M as suggested for the human procedure.
2. (2) Reconstitute a bottle of chromosome medium for each vial to be cultured. The chromosome reconstituting fluid should be warmed to 37° C prior to pouring it into the bottle of chromosome medium. Aseptic techniques should prevail throughout the cultural procedure.
3. (3) Inoculate each bottle of reconstituted chromosome medium with 2 to 2.5 ml of the plasma-leukocyte suspension. For

drawing off the inoculum a sterile disposable plastic syringe with 20-gauge 1-inch short-bevel needle is employed. The point of the needle is inserted to the line of demarcation between plasma and red cells so that mostly plasma (but some red cells) become the inoculum. Drawing plasma only from the top of the plasma layer may not yield metaphases.

Incubation

4. (4) Incubate the inoculated bottles at 37° C for 66 to 68 hours. Watch color change closely, particularly during the second and third days. It is necessary to loosen the lids on some vials, one-quarter turn for 4 to 8 hours daily during these two days, while other vials may require less than 1 hour of loosened lids out of a 24-hour period to permit the CO_2 to escape. It is beneficial to have the color a little on the pink to red side at the end of the day in order that the sample does not become too acidic (yellow) during the night. This is especially true of those samples which are most persistent in becoming or remaining too yellow.
5. (5) Add one vial of chromosome arresting fluid, tilt once or twice gently.
6. (6) Continue incubation at 37° C for 3 to 4 hours and resuspend with a Pasteur pipette and bulb; strict asepsis is no longer essential.
7. (7) Transfer the entire culture to a 1 ml graduated conical centrifuge tube and centirfuge 12 minutes at 1300 to 1500 rpm (432 to 575 RCF).
8. (8) Carefully pour off supernatant; centrifugation in step 7 may need to be adjusted slightly as to time and speed to give a good "button" in the cone of the tube which will not pour off with the supernatant.
9. (9) Procedures 9 through 29 as given on the Difco information sheet are then followed as given with the following exceptions:
   (a) Speed of centrifugation is increased to 1300 rpm (432 RCF) for 5 minutes following addition of the warm Hanks solution. Other centrifugations are carried out where indicated for 5 minutes at 1000 rpm (256 RCF). These steps have to do with concentrating the chromosome bearing cells, rupturing erythrocytes and swelling, fixing and staining the desirable cells so they will photograph under the microscope to advantage.
   (b) Procedure 21 is always repeated once and sometimes twice so the button of precipitate has a rather white appearance.
   (c) Staining time can be successfully reduced by using a higher ratio of Giemsa stain to distilled water. Filtered commerical Giemsa at a ratio of 8 ml to 100 ml of distilled water for 4 to 5 minutes gives good results.

Good metaphases were produced from more than 75 percent of the cultures where this technique was followed.

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A PRELIMINARY STUDY OF MICROBIAL *IN VITRO* INHIBITORY PROPERTIES OF CHICKEN PERITONEAL FLUID

Robert W. Barnes and Patric K. McIlwain

Department of Veterinary Science

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

SUMMARY

Chicken peritoneal fluid, when incorporated into media containing 5% sterile defibrinated sheep blood, exhibited an inhibitory activity against some microorganisms commonly associated with peritonitis. Sterile defibrinated chicken blood in similar media was noninhibitory to the same organisms.

It is the personal observation of the investigators that peritonitis is very infrequent in poultry. Assuming that a physical or chemobiological agent is responsible for the absence of peritoneal infections, one may conclude that further knowledge of this factor would be of value.

Two obvious physical varibles toward the infection are temperature and pH. Most of the organisms tested will grow at 41.1° C, and at the pH of the chicken peritoneal fluid, 7.1. It became apparent that if preliminary *in vitro* studies were conducted on peritoneal fluids these two varibles could be controlled.

MATERIALS AND METHODS

The peritoneal fluid was collected from fourteen apparently normal female Leghorn chickens by three methods. The first method involved the introduction of a sterile irritant, colloidal kaolin, into the peritoneal cavity of six of the birds. In the second method, approximately 20 ml of hypertonic saline in dialysis tubing were surgically placed into the peritoneal cavities of two chickens. After forty-eight hours, the birds were sacrificed and the fluid aseptically collected. The third method involved the introduction of 50 ml of sterile isotonic saline into the peritoneal cavity of each of six chickens. Two hours postinjection, the birds were sacrificed and the fluid collected. The peritoneal material obtained by each of the three methods was used individually as a 10% addition to blood agar plates (BAP) containing Difco blood agar base and 5% defibrinated sheep erythrocytes. Control plates with 10% sterile kaolin and 10% sterile isotonic saline added to BAP were used to determine if there was any inhibitory effect by these additions. BAP containing 5% defibrinated chicken blood also were used to ascertain whether the blood of poultry was involved in bacterial inhibition. The organisms used in the study were bacteriologically confirmed isolates obtained from the Diagnostic Laboratory, Department of Veterinary Science, North Dakota State University.

The organisms were streaked to the control sheep BAP, BAP with 10% kaolin, BAP with 10% physiological saline, control chicken BAP and BAP with the 10% peritoneal fluid addition. All plates innoculated with bacteria were held for 72 hours before being considered negative for growth, while the plates innoculated with fungi were held 5 days.

RESULTS AND CONCLUSION

The results obtained are shown in Table I. All of the microbes used in the trial grew well on the control BAP, BAP with 10% kaolin, BAP with 10% physiological saline and the chicken BAP. *Listeria monocytogenes, Mucor sp.,* and *Candida sp.* were the only organisms that were not inhibited by the addition of peritoneal fluid. No difference was noted in the inhibitory property of the peritoneal material regardless of means of collection. Fungi often are not affected by agents which are deleterious to bacteria, and neither *Mucor sp.* nor *Candida sp.* is generally considered the etiological agents of peritonitis. The growth of *L. monocytogenes* is of interest. Lucas et al., who have isolated *L. monocytogenes* from the largest number of birds conclude that they constitute the most important reservoir of infection. In the majority of these birds, there were no pathognomonic signs or lesions, so the investigators concluded that listeric infections of birds were either secondary or latent. Such survival *in vivo* correlates well with the refractory nature observed *in vitro.*

TABLE I

THE *IN VITRO* INHIBITION OF A NUMBER OF MICROBES BY CHICKEN PERITONEAL FLUID

	Control Sheep BAP	BAP with 10% Kaolin	BAP with 10% phys. saline	Control chicken BAP	BAP with 10% peritoneal fluid addition
Staphylococci, hemolytic	+	+	+	+	−
Staphylococci, non-hemolytic	+	+	+	+	−
Enterococci	+	+	+	+	±
Alpha Streptococci	+	+	+	+	−
Beta Streptococci	+	+	+	+	−
Gamma Streptococci	+	+	+	+	±
Bacillus subtilis	+	+	+	+	−
Listeria monocytogenes	+	+	+	+	+
Escherichia coli	+	+	+	+	±
Proteus mirabilis	+	+	+	+	±
Proteus vulgaris	+	+	+	+	−
Pseudomonas aeruginosa	+	+	+	+	−
Aerobacter aeruginosa	+	+	+	+	−
Nocardia sp.	+	+	+	+	±
Mucor sp.	+	+	+	+	+
Candida sp.	+	+	+	+	+

+ = Luxuriant growth
± = Sparse growth, definitely inhibited
− = No growth

The organisms most frequently associated with peritonitis were markedly suppressed by the peritoneal fluid addition, while the defibrinated chicken blood exhibited no inhibitory properties. Further studies involving the isolation and characterization of the agent(s) responsible are being attempted.

ACKNOWLEDGEMENT

This paper is published with the approval of the Director, North Dakota Agricultural Experiment Station and is supported by State funds from Project No. S-16-2.

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MICROBIOLOGIC INDICATORS OF POLLUTION IN THE RED RIVER OF THE NORTH

John W. Vennes and Janice M. Granum

Department of Microbiology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Microbiologic determinations on samples obtained from the Red River above and below Grand Forks (Bygland and Olso, Minnesota), in conjunction with a field survey of the International Joint Commission comprised of the North Dakota and Minnesota State Departments of Health and the Manitoba Department of Health, have indicated that considerable pollution of the Red River is present. Sampling occurred during September and November, 1965 and during January, February and March of 1966. These samples (a total of 32 at each station) were assayed for their total bacterial population, coliform and fecal coliform bacteria, and enterococci. Concomitant determinations for flow rate, pH, BOD, total and suspended solids, and other routine constituents were made.

The results of the above microbiologic determinations at Bygland and Olso were as follows: average 5-day BOD (mg/1) 3.7 and 3.5, average total bacteria (per 100 ml) 3×10^6 and 2.5×10^6, coliform bacteria (per 100 ml) 5×10^3 and 1.6×10^4, fecal coliform (per 100 ml) 1.5×10^3 and 4.2×10^3, enterococci (per 100 ml) 4.4×10^2 and 1×10^4.

When one considers the change in water temperature and the concentration effect of ice cover, it appears that the microbial population was reasonably stable throughout the testing period.

SEASONAL INCIDENCE OF *CLOSTRIDIUM BOTULINUM* TYPES

Myron W. Wentz, Richard A. Scott, Jr. and John W. Vennes

Department of Microbiology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

During the summer of 1965, a survey of mud and animal samples to determine the presence of *Clostridium botulinum* resulted in the identification of types A, C and F. The mud samples and animal intestinal contents were incubated anaerobically in brain heart infusion broth at 30°C for a period of 48 hours. Centrifuged samples of broth were injected into mice by the intraperitoneal route. Cultures exhibiting a heat-labile toxin, as determined by death in the mouse, were identified with suitable antitoxin.

Although *Cl. botulinum* types A and C had been previously isolated in this area, to our knowledge no isolation of *Cl. botulinum* type F has been made in the United States. It was of interest, therefore, to attempt actual isolation of this organism from the mud samples.

During the fall of 1965 attempts to isolate type F organisms were negative. In order to determine whether or not the organism was present in the mud sample or whether an inhibitory substance was present, the following experimental design was used. Mud samples, previously found to contain type F organisms, were incubated in brain heart infusion broth with a known toxin-producing *Cl. botulinum* type A. The results of these determinations suggested, since no toxin was produced, that an inhibitory substance must be present.

Further experimentation with mud and water samples found earlier to contain type F organisms, suggested the following: Both water and mud contain a heat labile, non-dialyzable substance, inhibiting to toxin production by *Cl. botulinum* type A. Studies on pH, temperature and medium constituents have tentatively suggested that a microorganism is responsible for the inhibition of toxin production by *Cl. botulinum* type A and that ambient temperature is the probable factor responsible for the occurrence of the inhibiting microorganism. The same inhibition may also be expected for toxin production by any of the types including the rare F.

THE EFFECT OF MENTAL ACTIVITY ON TEMPORARY AUDITORY FATIGUE IN THE HUMAN

James E. Fricke

Division of Special Education
Minot State College, Minot, North Dakota

ABSTRACT

Twenty subjects, both male and female, were exposed monaurally to Gaussian noise at intensity levels of 100, 110, and 120 dB, re. 0.0002 dyne/cm^2 for three minutes' duration. The auditory thresholds at 2000, 4000, 6000 and 8000 cps were determined for each subject and a comparison between pre- and post-fatigue thresholds constituted a measure of temporary auditory fatigue. Each subject participated under conditions of (1) listening to the noise in one ear and counting the "breaks" in the noise field during the three-minute test period; and (2) hearing the noise in one ear but also listening to a story being told in the opposite ear, with the knowledge that he would be tested on the passage at the end of the three-minute period.

The amount of auditory fatigue was shown to vary as a result of "focusing" in the opposite ear. Generally, the trend was for more auditory fatigue to result in the test ear when the subjects were concentrating on the message being presented in the other ear. This study combined with the results of previous work, tends to shed new light on the physiological role of the efferent auditory pathway and the results are discussed with this in mind.

COMPARATIVE STUDIES OF *IN VITRO* AND *IN VIVO* AMINO ACID TRANSPORT IN THE INTESTINE

M. L. McDaniel and F. A. Jacobs

Guy and Bertha Ireland Research Laboratory
Department of Biochemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Transport rates have been determined for L-leucine in the small intestine of the rat utilizing both the *in vitro* technique of Crane and Wilson (J Appl. Physiol. *12*:145, 1958) and a recirculated perfusion system in the intact animal. The perfusion system is designed for the continuous monitoring of a Carbon-14 perfusion mixture. The changes in radioactivity levels are measured by scintillation spectrometry and plotted directly. Studies using L-isoleucine as an inhibitor for the transport of L-leucine were carried out using both systems. Leucine has been studied over a concentration range up to 5.0 mM levels. The investigations were made in an attempt to correlate

results obtained from transport studies using the isolated *in vitro* segment with those obtained by perfusion of the intact animal. Isoleucine was found to inhibit leucine transport in both systems.

Supported in part by NIH research grant AM-02023-NTN.

DIELDRIN AND ALDRIN RESIDUES IN SUGAR BEETS AND THEIR SOILS

R. H. Smith, H. J. Klosterman, J. L. Dogger

Department of Agricultural Biochemistry

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

It has been shown that certain crops will absorb and translocate aldrin and dieldrin from the soil (1, 2). This absorption is controlled by such factors as the soil type, the pesticide, and the particular crop in question (3). Aldrin and its epoxide, dieldrin, are known to persist in the soil in measurable concentrations for extended periods of time. It has been further demonstrated that some plant varieties have a much greater absorptive capacity for aldrin and dieldrin than others. Carrots are especially noteworthy in this regard (3).

Recently analysis of dried sugar beet pulp from Red River Valley sugar beets grown in North Dakota has demonstrated aldrin and dieldrin residues. These beets had been treated at planting time with aldrin at the rate of one pound per acre. This was done as a control measure for the sugar beet root maggot, *Tetanops myopaeiformis.*

Since there is no tolerance set for any chlorinated pesticide on any product intended for use as a dairy cattle feed and since sugar beet pulp is marketed for this purpose, this residue is of great concern to the growers and processors of sugar beets as well as to the Food and Drug Administration. Therefore, this study was initiated to determine the level of aldrin and dieldrin in Red River Valley soils and the absorptive capacity of sugar beets for these materials. Also examined were the various fractions of the beet wherein the residues were concentrated.

METHODS

Sampling.—Samples consisting of ten beets each were selected at random in October, 1965, from fields treated with aldrin at the rate of one pound per acre in May, 1961, and in other fields treated with aldrin at the rate of one pound per acre in June, 1965. At the same time soil samples next to the beets were collected. These consisted of cores three inches in diameter and six inches long. Additional soil samples were also collected from fields treated with aldrin at the

rate of one pound per acre in June, 1962, but containing no sugar beets in 1965. Aldrin was mixed with the fertilizer and applied with the seed in the soil at a depth of 2-4 inches. The soil in this study was generally of the sandy loam type.

Analytical Methods.—The soil samples from each field were composited and frozen until analysis. Just prior to analysis 50 g samples were air-dried at room temperature and extracted continuously with n-hexane in a Soxhlet extractor for twelve hours. The extract was then concentrated to 10 ml using a Kuderna-Danish evaporator. Injections of 5 μl of this concentrate were made into the gas chromatograph for the determination of aldrin and dieldrin.

The beets from each field were washed, chopped, composited, weighed out in 200 g samples, and frozen until analysis. In addition, beets from fields treated in 1965 were peeled. A waxy outer layer (the periderm, fraction 1) approximately 0.2 mm thick was first removed with a razor blade. Next, a thicker layer (consisting of the secondary phloem, the vascular cambium, and the secondary xylem, fraction 2) approximately 0.8 mm in thickness was removed with a potato peeler. The peeled beet remained (fraction 3). Each individual fraction was composited from each lot of beets, frozen, and later analyzed separately to determine the extent of penetration into the beet by aldrin and dieldrin and their respective concentrations in each layer.

The whole beets and the beet fractions were extracted and partially cleaned up through solvent partition utilizing the Mills' procedure (4). The extract was further purified by running the petroleum ether solution over 9 g of Florisil activated according to the method of Beckman et al. (5). Aldrin and dieldrin were then eluted from the column with 250 ml of fifteen percent diethyl ether in petroleum ether. This eluate was concentrated to a suitable volume.

Injections of 5 μl were made into the chromatograph for the determination of aldrin and dieldrin. The residues from both soil and beet samples were confirmed by thin layer chromatography utilizing silica gel plates, 2 percent acetone in heptane as the developing solvent, and $AgNO_3$ as the chromogenic reagent.

A Barber-Colman Model 5000 gas chromatograph, equipped with an electron capture detector and a glass U-tube 6 ft x 3 mm I. D. packed with 10 wt percent Dok Corning Silicone fluid 200 (12,500 cstks) on Gas-Chrom Q was used. It was operated with a pulsed power supply and argon methane as the carrier gas at a flow rate of 100 ml per minute. The injector temperature was 230° C, the detector temperature 210° C, and the column temperature 200° C. The pulsed power supply was adjusted to give a pulse width of 0.5 microseconds and a pulse interval of 50 microseconds.

RESULTS

The various concentrations of aldrin and dieldrin in the whole sugar beets are given in Table I.

TABLE I

RECOVERIES OF ALDRIN AND DIELDRIN FROM SUGAR BEETS GROWN ON ALDRIN TREATED SOILS

Field	Treatment	Aldrin, P.P.M.	Dieldrin, P.P.M.	Total, P.P.M.
A.	1 lb/Acre 1961	<0.002	<0.002	
B.	1 lb/Acre 1961	<0.002	<0.002	.....
C.	1 lb/Acre 1965	0.026	0.096	0.122
D.	1 lb/Acre 1965	0.033	0.098	0.131

The residues found in the soils are given in Table II.

TABLE II

RECOVERIES OF ALDRIN AND DIELDRIN IN 1965 FROM SOILS TREATED WITH ALDRIN

Field	Treatment	Aldrin, P.P.M.	Dieldrin, P.P.M.	Total, P.P.M.
A.	1 lb/Acre 1961	0.010	0.040	0.050
B.	1 lb/Acre 1961	0.012	0.041	0.053
C.	1 lb/Acre 1965	0.82	0.10	0.92
D.	1 lb/Acre 1965	0.79	0.15	0.94
E.	1 lb/Acre 1962	0.030	0.095	0.125
F.	1 lb/Acre 1962	0.030	0.105	0.135

Due to degradation and dilution, the residue in the soil next to the beets in the 1961 treated fields is approximately 5 percent of the residue found next to the beets in the 1965 treated fields. This does not represent a loss of 95 percent of the pesticide, however, since the pesticide was applied only in the row with the beet and would be diluted when mixed with the untreated soil in between the rows. Analysis of samples of soil taken in between the rows of the 1965 treated fields showed residues of <0.002 P.P.M. aldrin and dieldrin.

It can also be seen from Table II that the sugar beets grown on the 1965 fields appear to contain residues of approximately 13 percent of those found in the soil. Thus, they exhibit a far smaller capacity for pesticide absorption than do carrots which have shown residues of 22-80 percent of those in the soil in which they were grown (3).

The various concentrations of aldrin and dieldrin in fractions 1, 2, and 3 of the beet (described earlier) are given in Table III.

Fraction 1, which represents approximately 2 percent of the weight of the beet, contains about 30-38 percent of the residue found in the entire beet. Fractions 1 and 2, which together represent approximately 4.5 percent of the weight of the beet, contain 45-50 percent of the total residue of the beet.

TABLE III

ALDRIN AND DIELDRIN RESIDUES IN VARIOUS FRACTIONS OF SUGAR BEETS GROWN ON FIELDS TREATED IN 1965 AT ONE POUND PER ACRE

Field	Fraction[a]	% of Total Weight	Aldrin, P.P.M.	Dieldrin, P.P.M.	% Dieldrin	% of Total Residue
A.	1	1.87	1.00	1.07	51.7	31.4
	2	2.53	0.16	0.47	74.6	13.0
	3	95.60	0.003	0.069	95.8	55.6
B.	1	2.11	1.19	1.21	50.4	38.8
	2	2.31	0.20	0.55	73.3	13.1
	3	95.58	0.003	0.063	95.4	48.1

[a]All figures determined on a fresh weight basis.

DISCUSSION

Small residues have been demonstrated in soils treated with aldrin at one pound per acre and analyzed three and four years after treatment. In addition, the uptake of aldrin by the 1965 treated sugar beets has been shown to be approximately 13 percent of the level found in the soil. In view of these facts, the further occurrence of significant aldrin and dieldrin residues in sugar beet pulp from sugar beets grown in the Red River valley is not anticipated. This assumes, however, that further treatment of these fields with aldrin and dieldrin is suspended and that a normal three or four year rotation plan on planting sugar beets is followed.

It can be seen from Table III that, as one progresses into the interior of the beet, the concentration of the total residue decreases. However, the percent of dieldrin in relation to aldrin increases during the same progression. It is not known at the present time whether this is due to selective absorption or increased metabolic conversion of the aldrin to dieldrin as it penetrates the beet.

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SOME RELATIONSHIPS BETWEEN ATMOSPHERIC GASES AND BIOCIDAL EFFECTIVENESS OF FUMIGANTS APPLIED TO WHEAT IN HERMETIC STORAGE

Ben Berck

Crop Protection Section

Canada Department of Agriculture Research Station, 25 Dafoe Road, Winnipeg 19, Manitoba, Canada

ABSTRACT

Investigation of the relationships between individual atmospheric gases and biocidal effectiveness of 9 fumigants was undertaken. Closed (hermetic) storage conditions were selected as a starting point. Changes in the CO_2-O_2-N_2 patterns of wheats of 12.5 - 22.5% moisture content stored hermetically in glass milk bottles at 22° C. (72° F.)

were monitored by gas chromatography. Effects on wheat quality and germination were also determined. Biocidal properties of the air of the stored wheats were assessed with 6 species of stored-products insects and 10 pure cultures of moulds.

Wheat of 15.0 - 22.5% moisture content developed a "silage" odor, in association with a high CO_2 and low O_2 content. It was found that the supernatant air of the bottled grain, *without addition of fumigants*, was insecticidal and fungistatic. After 2 weeks to 1 month storage under hermetic conditions, the test moulds showed marked loss of pigmentation, and significant inhibition of sporulation and growth. This was not due merely to the high CO_2 content of the air, as was ascertained in separate tests. Insecticidal and fungistatic behavior were correlated with presence of new peaks in the gas chromatograms. The peaks are not yet identified, nor have we determined whether the physiology of the test moulds was changed as a result of the treatment. The test moulds and those naturally present in or on the wheat gradually assumed apparently normal growth patterns after they were transferred from hermetic to room air. Nitrogen levels were distinctive and showed a marked drop in the 22.5% moisture wheat.

DETERMINATION OF ELECTRONIC PARTICLE COUNTER SETTINGS FOR THE ENUMERATION OF BOVINE, OVINE, AND PORCINE ERYTHROCYTES[1]

John W. Goertel and Rose M. Morgan

Department of Veterinary Science

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

INTRODUCTION

Erythrocytes of bovine, ovine, and porcine were sized utilizing a substage micrometer in an attempt to demonstrate that one aperture-threshold setting on the Electronic Particle Counter (EPC) could be utilized for the enumeration of the total erythrocyte count of these three species. Aperture-threshold curves were determined for the three species. The ideal curve was that which demonstrated the longest plateau following an initial drop at the noise level. The most efficacious setting was determined as being the middle of this plateau. An evaluation of the settings, as well as the instrument, was made by performing blood counts on numerous animals of each of the three species.

[1]Published with the approval of the Director of the North Dakota Agricultural Experiment Station.

METHODS

Blood—The blood was obtained by venous puncture into a B-D Vacutainer containing E.D.T.A. (Ethylenediaminetetraacetate) anticoagulant. The blood was inverted gently, but well, to avoid stasis. Blood samples were prepared for counting, utilizing the recommendations provided (1, 2). A dilution factor of 1:50,000 was utilized for bovine and porcine samples, whereas a dilution of 1:100,000 was utilized for the ovine samples. The total cell counts were performed within one hour of the drawing of the specimens to avoid any inaccuracies that have been previously described (3, 4). Simultaneous counts were performed on both the EPC and the counting chamber (hemocytometer) to assure the reproducibility of the settings.

Electronic Particle Counter—The instrument utilized in this investigation was a Coulter Particle Counter, Model A (5). The construction, principles, and instructions for operation of the EPC have been described by previous researchers (1, 4, 6, 7, 8, 9, 10, 11, 12).

RESULTS

Bovine erythrocytes ranged from 3.0-8.4 microns in diameter; porcine erythrocytes ranged from 3.0-10.0 microns in diameter; and ovine erythrocytes ranged from 3.2-8.6 microns in diameter.

From Figures 1, 2, and 3 it was demonstrated that the aperture-threshold curves utilizing an aperture current control settings of 4, 5, and 6 were undesirable, due to the sudden decrease of erythrocyte count and subsequent lack of a desirable plateau. The most desirable aperture-threshold curve for all three species was that utilizing an aperture current control setting of 7. This demonstrated the best

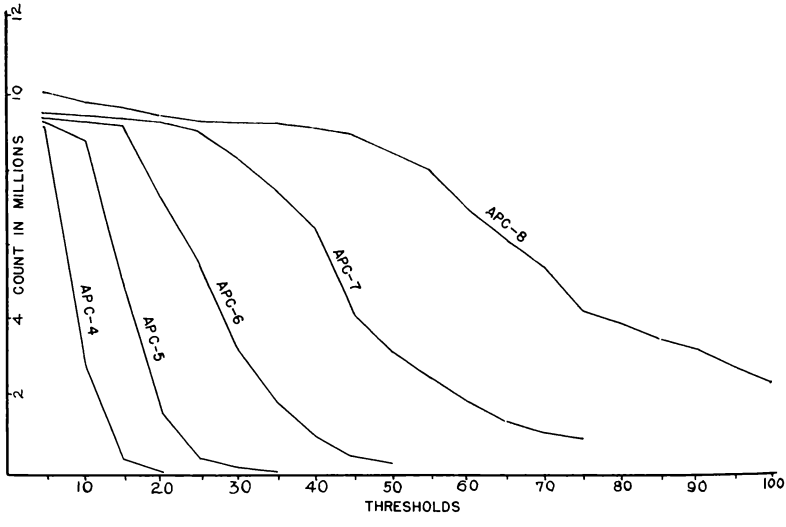

FIGURE 1—Bovine aperture-threshold curves.

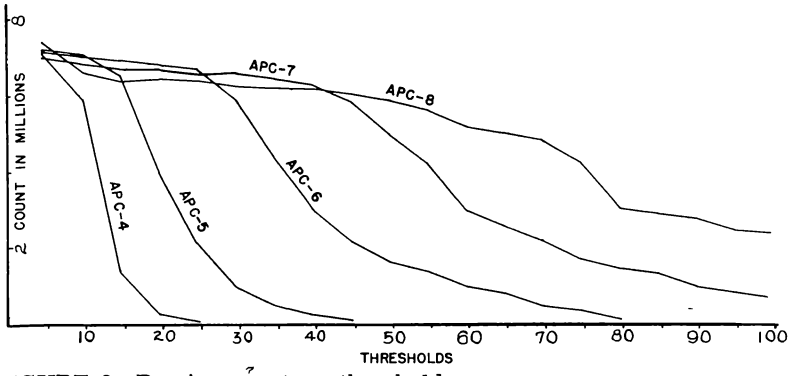

FIGURE 2—Porcine aperture-threshold curves.

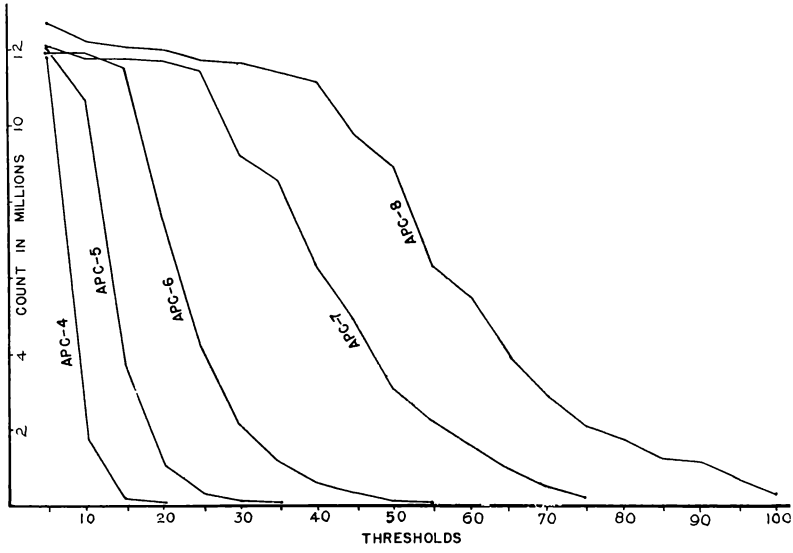

FIGURE 3—Ovine aperture-threshold curves.

plateau, giving a near-identical erythrocyte count for 3-5 successive threshold settings. The correct threshold setting was chosen as being the middle of this plateau, thus giving a threshold setting of 15.

Upon establishing the aperture threshold settings, an aperture current control setting of 7 and a threshold setting of 15, blood samples were counted by means of the EPC and the hemocytometer. To further evaluate the threshold setting of 15, threshold settings of 5, 10, and 20 were also utilized. Tables I, II, and III summarize the results found.

TABLE I

ENUMERATION OF BOVINE ERYTHROCYTES

COMPARISON OF HEMOCYTOMETER AND ELECTRONIC PARTICLE COUNTER

ANIMAL NUMBER	HEMOCYTOMETER 10^6	ELECTRONIC PARTICLE COUNTER THRESHOLDS 5	10	15	20
		CORRECTED COUNT (IN MILLIONS)			
B-6	9.21	9.62	9.40	9.32	9.32
" "	7.94	8.54	8.36	8.28	8.28
" "	8.48	8.04	7.98	7.86	7.86
B-1	8.14	8.44	7.86	7.86	7.88
" "	7.56	8.32	7.68	7.58	7.52
" "	8.31	8.02	7.98	7.62	7.54
B-2	9.72	9.30	9.12	9.18	9.08
" "	8.88	8.80	8.52	8.54	8.52
" "	8.43	8.42	8.28	8.20	8.16
B-3	9.67	9.24	8.96	8.78	8.78
" "	9.25	8.84	8.64	8.58	8.52
" "	8.84	8.96	8.66	8.66	8.64
B-4	10.62	10.10	10.04	9.96	9.96
" "	8.90	9.34	9.14	9.08	9.06
" "	8.20	9.14	9.00	8.88	8.84
B-308	8.39	9.98	8.40	8.24	8.18
" "	8.38	8.72	8.16	8.00	7.84
" "	8.16	8.72	8.14	8.10	8.00
B-309	8.62	9.12	8.84	8.78	8.76
" "	8.06	9.00	8.68	8.68	8.56
" "	9.94	10.06	9.82	9.76	9.56
B-310	7.69	7.74	7.20	7.04	6.92
" "	6.83	7.68	7.10	7.00	6.98
" "	9.28	9.94	9.24	9.10	9.00
1739	8.14	8.52	8.16	8.06	8.12
"	10.05	10.82	10.60	10.54	10.50
"	8.48	8.04	7.98	7.96	7.86
1742	10.43	9.48	9.38	9.24	9.18
"	10.78	9.40	9.22	9.02	8.92
"	9.30	9.22	9.00	8.98	8.96
1741	7.97	8.10	7.88	7.80	7.76
"	8.10	8.38	8.02	8.02	7.98
"	10.20	9.18	8.84	8.80	8.68
1740	10.20	10.92	10.60	10.58	10.50
"	12.36	11.90	11.70	11.64	11.52
"	11.63	11.94	11.68	11.66	11.52

TABLE II

ENUMERATION OF PORCINE ERYTHROCYTES

COMPARISON OF HEMOCYTOMETER AND ELECTRONIC PARTICLE COUNTER

ANIMAL NUMBER	HEMOCYTOMETER	ELECTRONIC PARTICLE COUNTER THRESHOLDS			
	10^6	5	10	15	20
		CORRECTED COUNT (IN MILLIONS)			
P-4	8.63	9.44	8.24	7.96	7.78
" "	8.61	9.54	8.56	8.40	8.24
" "	8.87	9.66	8.72	8.72	8.30
P-3	8.85	9.20	8.16	8.16	7.76
" "	8.79	9.92	9.14	8.88	8.88
" "	8.67	9.64	8.98	8.86	8.76
P-1	8.96	9.74	8.80	8.48	8.40
" "	8.73	9.52	8.42	8.20	8.12
" "	8.87	9.76	8.90	8.78	8.58
P-2	9.47	10.14	9.50	9.34	8.98
" "	9.97	10.76	10.06	9.78	9.56
" "	9.86	10.56	9.96	9.78	9.76
P-106	8.87	8.42	8.24	7.84	7.16
" "	8.96	8.50	8.38	8.14	7.96
" "	8.55	8.80	8.48	8.24	7.96
P-107	7.27	7.76	7.34	7.12	7.08
" "	7.92	8.40	8.16	7.76	7.18
" "	8.56	8.48	8.24	8.04	7.90

DISCUSSION

By increasing the threshold settings of the EPC, smaller cells are excluded or screened out due to the inability of the pulses produced by such cells to reach the pulse - height level established by the individual threshold settings. The desired pulse - height level is that one which will exclude the counting of small undesirable debris, but yet will count small desired cells. Thus, in the counting procedure the threshold control is set at a sufficiently low setting in order to count the smallest cells.

From this description of the threshold control settings and the results found by the substage micrometer sizing of the bovine, ovine, and porcine erythrocytes one can readily see that the same aperture-threshold setting could be utilized for the enumeration of erythrocytes for all three species.

TABLE III

ENUMERATION OF OVINE ERYTHROCYTES

COMPARISON OF HEMOCYTOMETER AND ELECTRONIC PARTICLE COUNTER

ANIMAL NUMBER	HEMOCYTOMETER	ELECTRONIC PARTICLE COUNTER THRESHOLDS			
	10^6	5	10	15	20
		CORRECTED COUNT (IN MILLIONS)			
OV-201	11.92	12.66	12.30	12.30	12.24
" "	10.34	10.42	10.10	10.10	10.02
" "	11.29	12.34	11.52	11.46	11.26
OV-202	9.70	10.06	9.80	9.82	9.78
" "	10.71	11.28	11.10	10.94	10.86
" "	7.10	7.48	7.24	7.24	7.10
OV-203	10.97	10.96	10.76	10.68	10.30
" "	10.71	11.01	10.82	10.88	10.54
" "	11.34	12.68	12.22	12.10	12.02
OV-204	11.50	11.82	11.70	11.70	11.32
" "	11.63	12.18	11.86	11.82	11.74
" "	10.97	11.56	11.46	11.48	11.18
OV-205	9.43	9.78	9.50	9.42	9.42
" "	9.60	9.91	9.78	9.70	9.46
" "	10.03	10.65	10.38	10.22	9.92
OV-206	10.60	10.96	10.50	10.48	10.30
" "	12.28	11.92	11.76	11.76	11.38
" "	11.63	12.58	12.22	12.18	12.02
OV-207	12.85	13.08	12.90	12.88	12.20
" "	13.42	14.28	14.06	14.06	13.66
" "	12.95	14.02	13.66	13.52	13.22
OV-208	11.41	11.42	11.20	11.12	10.98
" "	12.06	13.08	13.00	13.00	12.82
" "	11.86	12.66	12.22	12.14	11.90

To further evaluate the instrument and the chosen settings, an aperature current control setting of 7 and a threshold setting of 15, replicate counts were made on a number of animals of various age groups of all three species. In conjunction with the EPC the chamber count (hemocytometer) was also utilized. However, the error in count that has been associated with the chamber count (hemocytometer) made its use as a reliable standard undesirable (4, 8, 12).

The results on Tables I, II, and III further substantiate the validity of 15 as the correct threshold setting. The counts received at the threshold settings of 10, 15, and 20 are in close proximity, whereas the erythrocyte count enumerated at a setting of 5 deviated from the 10, 15, and 20 settings. Over an extended scale, there is a sudden drop at a threshold setting of 5, whereas 10, 15, and 20 produce a relatively flat plateau. Again, the threshold setting of 15 would be the middle of this plateau. When comparing the hemocytometer count and the EPC, one finds that the EPC threshold setting of 15 is not always the most desirable setting. However, as mentioned previously, the hemocytometer produces a high error of reproducibility, thus making it a poor instrument for comparative purposes.

SUMMARY

Using the Electronic Particle Counter, threshold-aperture curves were determined for the three species: bovine, ovine, and porcine. The ideal curve was that which presented the best plateau, giving approximately the same count for three-four successive threshold settings and then gradually dropping due to the exclusion of smaller cells. The settings to be utilized for the enumeration of erythrocytes for the three species were determined as being the middle of this plateau. As an evaluation of the instrument and of the predetermined settings, triplicate counts of each of several animals of the various species were determined on the Electronic Particle Counter and were correlated to those same blood samples enumerated by the hemocytometer method. An aperture of 7 and a threshold of 15 were shown to be the most efficacious settings for all thre species.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Miss Charlotte Graves.

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THE PARTIAL THROMBOPLASTIN TIME TEST (PTT) EMPLOYING *PLATELET FACTOR REAGENT WITH CELITE* AS OBSERVED IN SUIS

Rose M. Morgan and John W. Goertel

Department of Veterinary Science

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

INTRODUCTION

For diagnosis of possible thromboplastic factor deficiencies in suis the partial thromboplastin time (PTT) employing *Platelet Factor Reagent with Celite* has been shown to be reliable, simple and sensitive, as well as versatile. Few studies thus far have shown the significance of the PTT in bleeding tendency units for suis.

MATERIALS AND METHODS

Blood Samples.—Approximately 4.5 ml of blood was obtained from each healthy and apparently hematologically normal individual animal by means of a cardiac puncture into a B-D vacutainer tube containing 0.5 ml of 0.1 M sodium oxalate obtained from Becton, Dickinson and Company, Rutherford, New Jersey. Special care was taken in drawing the blood to avoid stasis. The specimens were care-

fully mixed by inversion of the tubes. The blood-sodium oxalate mixtures were then centrifuged at 1,500 to 2,000 r.p.m. for 15 minutes. To avoid hemolysis all plasmas were separated from the cells within 30 minutes after the actual drawing of the specimens. The specimens were placed at a refrigerator temperature of 4° to 6°C until used. All specimens were obtained from non-fasting animals.

Procedure.—Lyophilized *Platelin Plus Activator* was reconstituted with 2.5 ml of distilled water with a known pH of 7.2. (The pH of this water was determined just prior to the reconstitution since it is recommended that the pH of the water be above 6.0).

The $CaCl_2$ solution was placed in the 37°C constant *Thermolyne Dri-Bath* for 10 to 15 minutes. The *Platelin Plus Activator* was also placed in the 37°C constant *Dri-Bath* for 10 to 15 minutes. Repeated tests have shown that a 10 to 15 minute incubation period is required for minimal clotting time (1), rather than 0 to 6 minutes as previously described (1, 3, 4, 5, 6).

The test was performed employing the following methods:

Step 1.—One tenth ml of *Platelin Plus Activator* and 0.1 ml of 0.025 M $CaCl_2$ are placed in 10 x 75 mm test tubes.

Step 2.—After the 15-minute incubation period, 0.1 ml of plasma is rapidly blown into the *Platelin Plus Activitor* - $CaCl_2$ mixture and the timer started simultaneously. Ionic calcium is added to the plasma to initiate the reaction and *Platelin Plus Activator* reagent is added to assure optimum platelet activity.

Sept 3a. Loop Method.—A small nichrome wire loop is inserted into the tube, moving it across the bottom of the tube in a sweeping motion at approximately 2 times per second. At the first appearance of fibrin strands the timer is stopped and the length of time recorded.

Step 3b. Tilt Method.—The tube is tilted at intervals of 2 seconds until the first appearance of a fibrin clot. The timer is then stopped and the length of time recorded.

Control determination on all plasmas were made employing *Diagnostic Plasma.* The control time for this plasma was 39.0 and 39.5 seconds, respectively, employing the loop method, and 40.0 ind 39.5 seconds, respectively, employing the tilt method.

RESULTS

PTT studied in suis of three age groups have shown ranges of 22.5 to 38.0 seconds with a mean of 28.24 seconds for piglets 7 days of age; 28.0 to 38.0 seconds with a mean of 31.76 seconds for those 14 days of age; and 25.0 to 31.5 seconds with a mean of 29.36 seconds for those 35 days of age. The seven day old group recorded the shortest PTT, 22.5 seconds, and the longest PTT, 38.0 seconds, (Tables I, II, and III).

DISCUSSION

Previous studies have shown the PTT to be reliable in all factor deficiencies with the exception of Factor VII, a very rare isolated factor which has shown itself to be necessary only in the extrinsic

clotting system. All other clotting factors of the intrinsic clotting system are normally found in the plasma and may be detected by means of the PTT.

Only recently has it been shown that previous details of procedure (such as the need for a refrigerated centrifuge and siliconized glassware) are not essential for reproducibility or sensitivity of this particular test (2).

TABLE I
COMPARATIVE PARTIAL THROMBOPLASTIN TIME TEST RESULTS IN 7 DAY OLD SUIS UTILIZING LOOP AND TILT METHODS

Animal number	Loop method (seconds)	Tilt method (seconds)	Average of both methods
7	25.0	25.0	25.37
	26.0	25.5	
4	22.5	23.0	22.87
	23.0	23.0	
6	31.0	30.5	30.62
	30.0	31.0	
2	27.0	26.5	26.50
	26.0	26.5	
8	31.0	32.0	31.00
	30.0	31.0	
5	26.0	26.0	26.00
	26.0	26.0	
1	38.0	38.0	37.75
	37.0	38.0	
3	25.5	26.0	25.87
	26.0	26.0	
		mean	28.24

In comparing a standard procedure with a more simplified one (such as the study by Goulian and Beck (2)) the two procedures were shown to be indistinguishable.

The determinations were performed using both the tilt and the nichrome loop methods. The latter is preferred since the end point is more easily visualized and does not require the removal of the tube from the 37°C Dri-Bath. The nichrome loop method greatly decreases any chance of induced errors due to rapid temperature changes.

ACKNOWLEDGEMENTS

This paper is published with the approval of the Director, North Dakota Experiment Station, Fargo, North Dakota.

The authors wish to acknowledge the assistance of the following individuals:

Myron F. Andrews, D.V.M., Chairman, Department of Veterinary

Science, North Dakota State University, Fargo, North Dakota; Mrs. Dorothy I. Good, M.S., M.T., (ASCP), Chief Technologist, Laboratory - The Fargo Clinic, and Supervisor, School of Medical Technology,

TABLE II

COMPARATIVE PARTIAL THROMBOPLASTIN TIME TEST RESULTS IN 14 DAY OLD SUIS UTILIZING LOOP AND TILT METHODS

Animal number	Loop method (seconds)	Tilt method (seconds)	Average of both methods
2	33.0	33.0	32.25
	33.0	30.0	
3	29.5	30.0	30.12
	30.0	31.0	
5	31.0	32.0	31.50
	31.0	32.0	
6	31.0	31.0	31.00
	30.5	31.5	
8	28.0	29.0	28.37
	28.5	28.0	
4	37.5	36.0	37.37
	38.0	38.0	
		mean	31.76

TABLE III

COMPARATIVE PARTIAL THROMBOPLASTIN TIME TEST RESULTS IN 35 DAY OLD SUIS UTILIZING LOOP AND TILT METHODS

Animal number	Loop method (seconds)	Tilt method (seconds)	Average of both methods
3	26.0	25.5	25.36
	25.0	25.0	
16	30.0	30.0	30.12
	30.0	30.5	
1	30.0	30.0	29.87
	29.5	30.0	
5	29.5	30.0	29.87
	30.0	30.0	
10	30.0	30.0	30.00
	29.5	30.5	
4	31.5	31.0	31.00
	30.0	31.5	
		mean	29.36

St. Luke's Hospital, Fargo, North Dakota; Allan Anderson; Marsha Costello; Mrs. Nora Edhlund; Jeannie Juen; Charlotte Graves; Donald Graves; Annette Kisser; and Kay Smith.

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ICE-THRUST BEDROCK IN NORTHWEST CAVALIER COUNTY, NORTH DAKOTA[1]

John P. Bluemle

North Dakota Geological Survey, Grand Forks, North Dakota

INTRODUCTION

During the summer of 1964, geologists of the North Dakota Geological Survey visited excavations under construction for Minuteman Missile installations in eastern North Dakota. An unusually well-exposed ice-thrust bedrock feature near Hannah in northwest Cavalier County was studied at one of these sites. This paper is a discussion of the geology at and near the site, and the stratigraphy, structural relationships, and possible methods of formation of the thrust feature. The particular missile site where the thrust was exposed will hereafter be referred to as the "Hannah Site."

GENERAL GEOLOGY IN THE IMMEDIATE AREA

Most of Cavalier County is underlain by the Cretaceous Pierre Shale which crops out in several places; some Niobrara Shale crops out along the Pembina Escarpment at the east edge of the county. Bedrock elevations are slightly higher east of the Hannah Site but west of there they drop markedly. The area around the Hannah Site is underlain by ground moraine till that averages between 10 and 30 feet thick. The till is slightly thinner east of the Hannah Site but it is much thicker a few miles to the west.

Observations of the cuts made for missile site installations revealed numerous exposures of two or more tills separated by out-

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wash, boulder pavements and buried soil profiles. The presence of paleosols on the lower tills at many of the missile sites indicates a period of time during which glacier ice was absent over the area. The lower tills were deposited during glaciations earlier than the one responsible for the deposition of the surface drift, but their ages are not yet known.

The lower tills are distinctly different from the upper tills at most of the missile sites studied. Generally, they are compact, hard, jointed and highly limonite-stained. The surface tills seldom have any of these characteristics. Where lower-type till occurs near the surface, the more typical surface drift is missing—either it was not deposited or it has been eroded away. The first of these possibilities is more likely because the area is only slightly eroded.

At the Hannah Site, two tills are separated by a block of overthrust shale. Both tills are very shaly clay loams with only a few granite and limestone pebbles. The lower till is much more dense, cohesive and uniform with a very few granitic and limestone pebbles; it is essentially ground-up shale. However, the presence of a few of these pebbles proves it is glacial drift and not bedrock.

DESCRIPTION OF THE HANNAH THRUST FEATURE

No topographic expression is associated with the subsurface ice-thrust feature at the Hannah Site. Ridges (washboard moraines) are abundant in nearby areas but because exposures are not present on them, it is impossible to verify whether they are the surface expression of similar ice-thrust features. Ice-thrust features described by Kupsch (1962) in an area of Saskatchewan to the northwest of Cavalier County have associated surface ridges.

Figure 1 illustrates the structural and stratigraphic relationships exposed by the excavation. The nearly vertical cut was about 30 feet deep. Beneath the topsoil and a 1- to 2-foot-thick surface till is a discontinuous pavement of igneous and metamorphic boulders and cobbles. Directly beneath the pavement, except at the westernmost end of the excavation, is shale that has been thrust over the lower till. At the westernmost end of the cut, the cobble pavement is directly underlain by the dense, sandy, shaly and pebbly till.

Three thrust-fault planes were exposed on the wall of the cut. The lowermost fault plane, A (Figure 1), is on the lower till surface. Its trace, which can be seen in only two dimensions, has an apparent dip of about 40° to the east. Highly contorted, ground-up, overturned and dragfolded shale lies on the till in many places. The thrust-plane trace is very sharp (Figure 2) as is the second fault plane, B (Figure 1), which is about six feet above the first. The shale above the fault trace is essentially undisturbed. Movement of the second thrust was along bedding planes. Because this fault trace was exposed on intersecting walls of the cut, it was possible to determine that the dip of the plane is 35° to the north and the strike

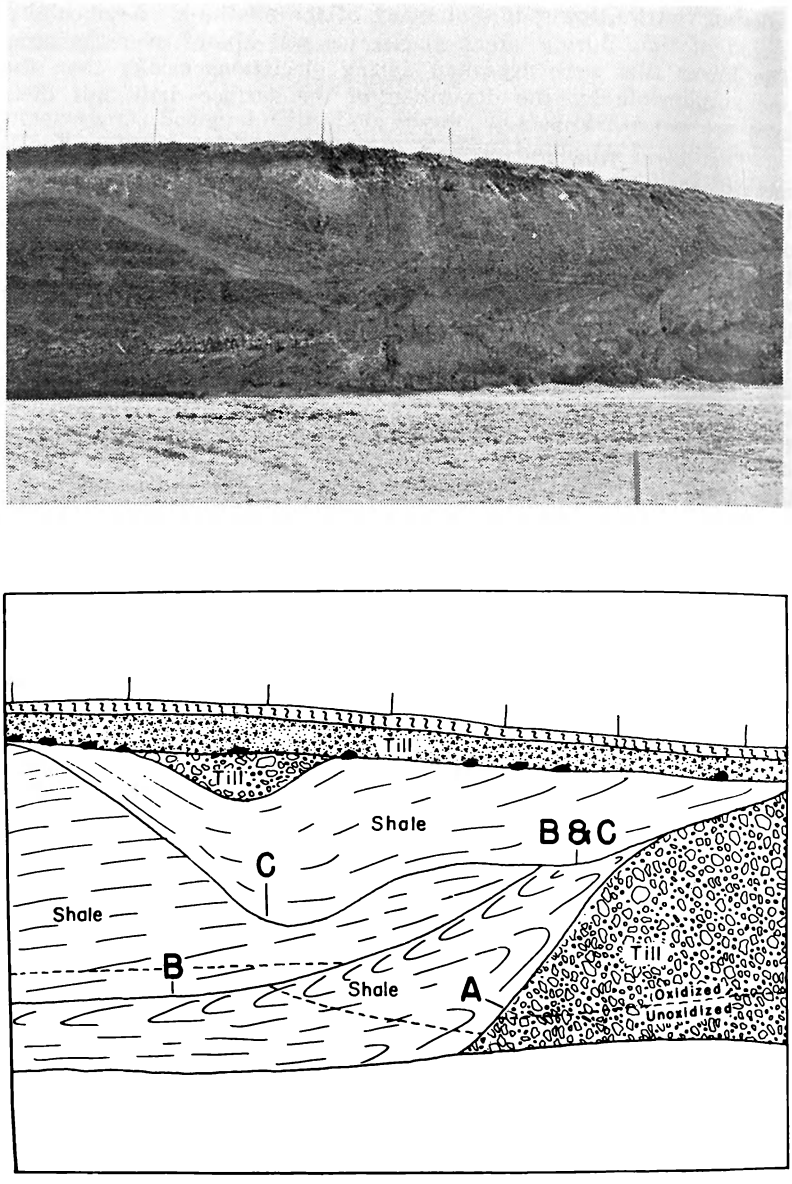

FIGURE 1—Photo of thrust with diagram showing the relationships. Thrust planes are lettered to correspond with the text. Excavation is about 30 feet high.

is 30° east of north, so movement must have been primarily from the northeast. The uppermost thrust plane, C (Figure 1), merges on the west with the thrust plane below it (B) and rises to about 25 feet above thrust plane B on the southeast. Generally, the shale on either side of the uppermost thrust plane has not been contorted. Movement along this thrust plane was probably mainly along bedding planes and from the north. The thrust plane dips northward at about 16°.

POSSIBLE METHODS OF FORMATION OF THE THRUST FEATURE

The thrust faults at the Hannah Site have spoon-shaped, concave-upward fault planes. According to Brinkmann (1953), different types of thrust structures can form depending on their position relative to the ice front. He says that near and ahead of the terminus, steeply dipping imbricate thrust blocks are pushed into shape and farther back behind the terminus, flat-lying folds result from frictional drag of the ice on the surface of the ground. The lowest thrust, A, is the most steeply dipping and the uppermost one dips the least. Perhaps, as the ice approached the area, the steep thrust (A) was pushed into shape and as the ice moved over, the less steeply-dipping thrusts (B and C) formed. The changes in direction of the thrusting may have resulted from changes in the ice flow direction as it thickened and became less controlled by the underlying topography.

Kupsch (1962, p. 593) stated that ice-thrust ridges may form in an area of thrusting in the marginal zone of a glacier where the upper part of the bedrock down to the lower limit of permafrost is incorporated in the glacier. The effective base of the glacier therefore would not be the top of the bedrock but the lower limit of permafrost on top of the water-bearing, unfrozen shales beneath the ground ice. Kupsch maintained (p. 596) that the presence of ground ice is an essential condition for the formation of ice-thrust ridges because, in their unfrozen state, sands and silts would not maintain their bedding during the thrusting. I think this is an unnecessary restriction because unlithified and unfrozen sediments do deform while maintaining recognizable bedding. I have seen intricately deformed silts associated with recent landslides at several locations in the Montana Rocky Mountains. The fault traces at some of these slides were very sharp, the materials a few inches on either side of the traces essentially undisturbed even though thousands of tons of materials had slid on the fault planes. Most of these landslides resulted when water-lubricated (and unfrozen) materials slid into too-steep highway cuts. Similarly, excavations at Oahe Dam in South Dakota revealed numerous large-scale slump thrust faults with unmodified slices of Pierre Shale between individual fault planes. Individual faults were hundreds of feet long. These faults were caused by slumping of the bluff during river downcutting, certainly in an

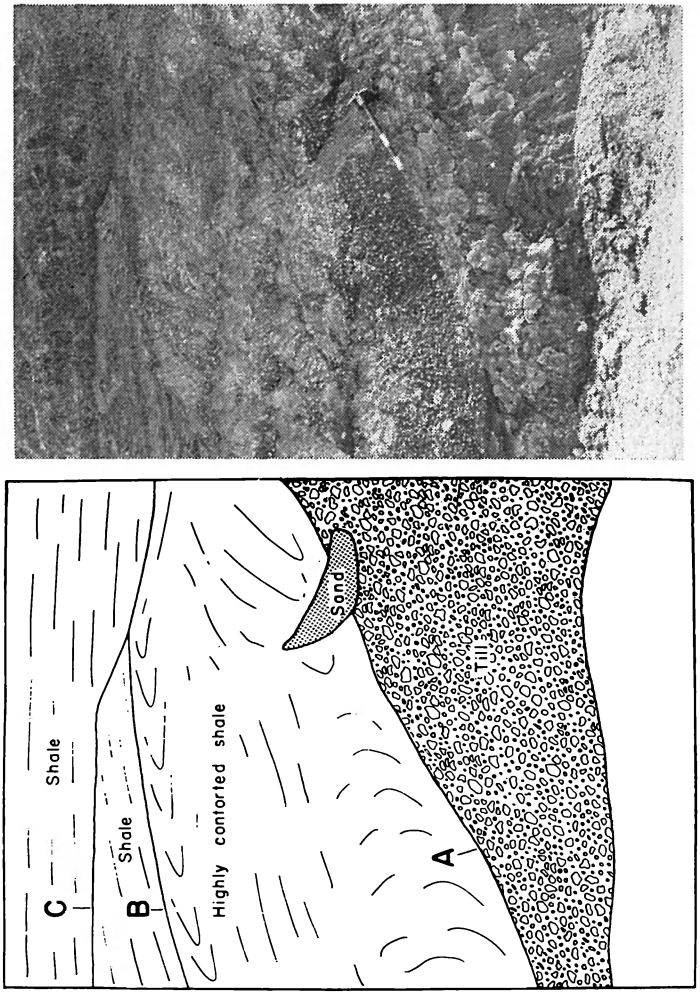

FIGURE 2—Close-up view of part of Figure 1 showing all three fault traces and the conditions of the materials above and below each trace. Shovel is about two feet long.

unfrozen condition. On the Missouri Coteau of central North Dakota I have seen similar slump faults and folds in collapse stagnation outwash. These, too, were probably unfrozen when they slumped into their present positions. During the present study, highly contorted sands and silts were observed in many exposures in eastern North Dakota. These contorted sediments have maintained their bedding planes in spite of the squeezing and thrusting they have undergone.

Engineering tests at the Hannah site show that the shales at the site have compressive strengths that vary from as little as 37 psi at 10 feet depth to as much as 378 psi at 80 feet depth. Therefore, taking the confining pressures at those depths into account, 37 to 378 pounds of stress per square inch are required to cause failure (crushing or shearing in any direction) of the shales. A glacier 650 feet thick exerts a static pressure of about 250 lbs/sq. in. (these figures are for clean ice; if the ice is debris-laden, the static pressures increase markedly). The ice was probably not this thick when it first advanced over the Hannah area but it must have been much thicker there when its margin was far to the south. The compressive strengths of the shale were undoubtedly exceeded by several times so it would not be at all surprising if all the shale had been completely crushed and all bedding had been obliterated. This did happen at many sites where as much as 100 feet of till composed almost entirely of crushed shale lies on undisturbed bedrock.

The compressive strengths given above for the Pierre Shale at the Hannah Site are certainly much greater than the strength of the shale along bedding or jointing planes. They are probably sufficient to allow the shale to fault and contort without being crushed or otherwise altered provided that the static glacier pressure does not exceed them (the compressive strengths) greatly. Such conditions might be obtained when ice first moved over the area.

Because the strengths of the shale along bedding and jointing planes are so much less than the static strengths of the shale, freezing is not a necessary condition to account for the undisturbed bedding above fault plane B and on either side of the uppermost thrust plane (C). However, where the main component of force exerted by the ice did not coincide with any bedding or jointing plane, the compressive strengths probably were exceeded and crushing such as that between fault planes A and B resulted.

Osmotic equilibrium may have played an important role in reducing the frictional resistance to sliding. As pointed out by Hubbert and Rubey (1959), anomalously high pore-water pressures may facilitate overthrust faulting. The high pore-water pressures spoken of by Hubbert and Rubey are caused in part by the considerable thicknesses of overlying rocks, a condition not met in the near-surface thrusting with which we are concerned. However, Hanshaw and Zen (1965) theorize that anomalously high pore-water pressures

can result from osmotic processes. The Pierre Shale, because it has layers of bentonitic clay that could act as semi-permeable membranes to circulating ground water, might be particularly susceptible to high pore pressures at certain depths due to osmotic processes. The bentonitic clays in the shale are very continuous laterally and, if abundant fresh water was supplied by the nearby melting glacier ice, a relatively significant difference in the chemical potential of H_2O across the membrane might develop. The fresh water, supplied from above, would tend to migrate downward through the bentonite membranes toward high salt regions of relatively lower H_2O concentration. This would raise the H_2O concentration and pressure in those beds beneath the bentonite membrane causing the overlying beds to "float" on a layer of high-pressure water. If the dissolved components in a body of circulating ground water were filtered out by one or more beds rich in clay minerals such as bentonite, then under equilibrium conditions the pressure on the influx side might be anomalously high.

Because glaciers supply large amounts of fresh water, it seems likely that when they move over areas underlain by bentonitic shale such as the Pierre, that osmotic equilibrium might be a major factor in loosening material and forcing it upward ahead of and into the moving ice body. This should be especially true in areas such as North Dakota where the ground was probably not frozen to great depths during the glaciation. Such hydraulic raising of the bedrock may have played an important role in helping the glacier pick up its load of debris.

ACKNOWLEDGMENTS

I wish to thank Col. H. D. Weston, Corps of Engineers Area Engineer at the Grand Forks Air Force Base who obtained the security clearance necessary for publishing this paper.

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THE DAHLEN ESKER OF GRAND FORKS AND WALSH COUNTIES, NORTH DAKOTA[1]

Jack Kume[2]

North Dakota Geological Survey, Grand Forks, North Dakota

INTRODUCTION

A spectacular ridge of glacial origin was studied by the North Dakota Geological Survey during a Grand Forks County geologic and ground-water investigation in 1964 and 1965. The ridge, interpreted as an esker, is about three miles northeast of Dahlen, North Dakota and is here named the Dahlen esker. An esker is a sinuous, narrow ridge of rudely stratified gravelly or sandy sediments deposited by a stream in contact with glacial ice. Eskers also contain unsorted debris of all grain sizes called till, deposited directly from the adjacent ice.

The Dahlen esker has attracted local interest from both the layman and the geologist because of three reasons: first, the landform has a rather anomalous appearance, that of a conspicuous, high-relief sinuous ridge adjacent to an area of relatively low-relief topography; second, its excellent preservation, as it is virtually unmodified by man —many eskers are destroyed in the process of mining for gravel; and third, it is easily accessible as well as noticeable, for it is next to North Dakota highway 32. The purpose of this report is to provide a description of the Dahlen asker and to briefly discuss its origin and geologic setting.

ESKER LOCATION AND DESCRIPTION

The esker is in secs. 5 and 6, T. 154 N., R. 56 W. in northwestern Grand Forks County, and in secs. 31, 32, and 33, T. 155 N., R. 56 W., and in secs. 25 and 36, T. 155 N., R. 57 W. in south-central Walsh County, North Dakota.

Physiographically, the esker lies upon the Pembina escarpment in the Drift Plains district. This escarpment, an eastward-facing relatively steep slope, forms the boundary between the Drift Plains and the Agassiz Lake Plain districts in northeastern North Dakota. In the Dahlen vicinity the escarpment is a conspicuous rise in land surface of about 300 feet in seven miles.

Field observations of the esker were conducted in seven areas or sites on the ridge (Figure 1). The sites consist of exposures along section line trails, ridge crests, and roadcuts. Two of the sites contain five small gravel pits in a third of a mile ridge segment. Excellent exposures of the esker sediments occur within the gravel pits. The observed characteristics of the Dahlen esker are summarized in Table I.

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[2]Present address: 1383 Iowa Ave. S.E., Huron, South Dakota 57350.

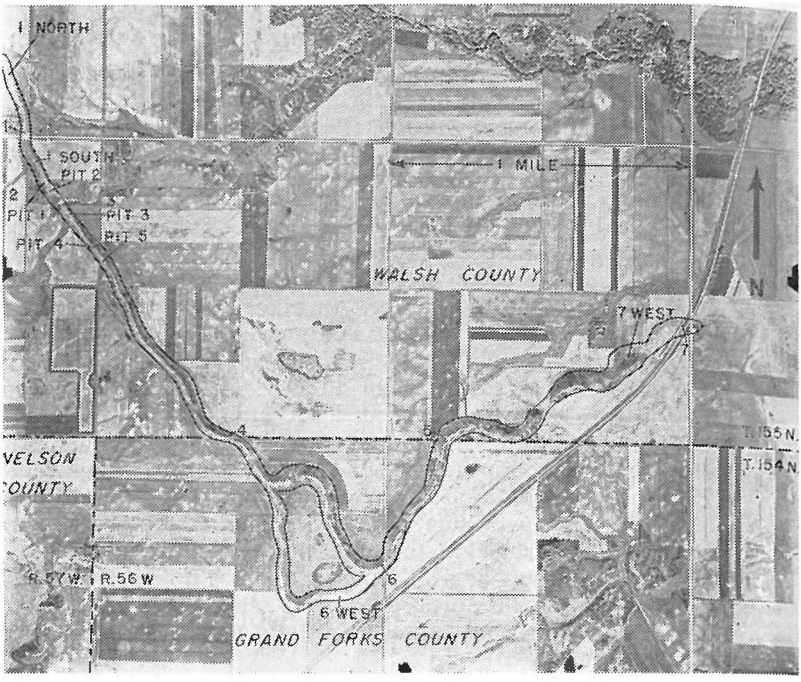

FIGURE 1—Aerial photo of the Dahlen esker in Grand Forks and Walsh Counties.

As seen on the aerial photo in Figure 1, the Dahlen esker is V-shaped in plan view with the apex to the south and branches to the northwest and northeast. The apex has an adjoining subdued ridge segment, sort of a double apex, which is looped around a kettle, an undrained depression. The length of the esker is about four miles, and it is about 400 feet wide. The entire esker is sinuous, but the northwest branch is straighter as compared to the strongly curved apex and the northeast branch.

Elevations of the northeast branch range from 1250 feet above sea level near the base of the ridge to 1330 feet at the ridge crest. In cross-section the Dahlen esker is steep-sided and asymmetrical; the slope is steeper and higher on the north of the ridge. The ridge height at the apex is 53 feet on the north slope and 39 feet on the south slope. About a half a mile northeast of the apex the height of the ridge is 63 feet on the north side and 29 feet on the south side. The maximum relief of the ridge is 80 feet.

The ridge generally has an accordant crest level, but the crest appears irregular because of numerous minor gaps (Figure 2). These gaps, accentuated and modified by present ephemeral stream erosion,

TABLE I

OBSERVED CHARACTERISTICS OF THE DAHLEN ESKER

SITE NO.	EXPOSURE					COMPOSITION					SORTING		BEDDING		RELIEF	
	RIDGE CREST	TRAILCUT	ROADCUT	GRAVEL PIT NO.	CUT SIZE (Feet)	SAND	GRAVEL	TILL	SILT	BOULDERS	POOR	GOOD	HORIZONTAL	CROSS-BEDDED	NORTH SIDE (Feet)	SOUTH SIDE (Feet)
1			X		7	X	X			X	X				44	24
1 NORTH	X															
1 SOUTH	X							X		X						
2 (PIT 1)				1	7	X	X	X	X	X	X	X	X			
2 (PIT 2)				2	15	X	X	X			X					
3 (PIT 3)				3	7	X	X				X					
3 (PIT 4)				4	12	X	X	X		X	X		X			
3 (PIT 5)				5A	16	X	X	X		X	X	X	X		39	29
4		X			6	X	X			X	X		X		45	
5		X					X			X	X				63	29
6		X			14	X	X	X	X	X	X				53	39
6 WEST	X						X	X		X	X					
7			X		34	X	X	X	X	X	X	X	X	X	34	
7 WEST	X									X					80	

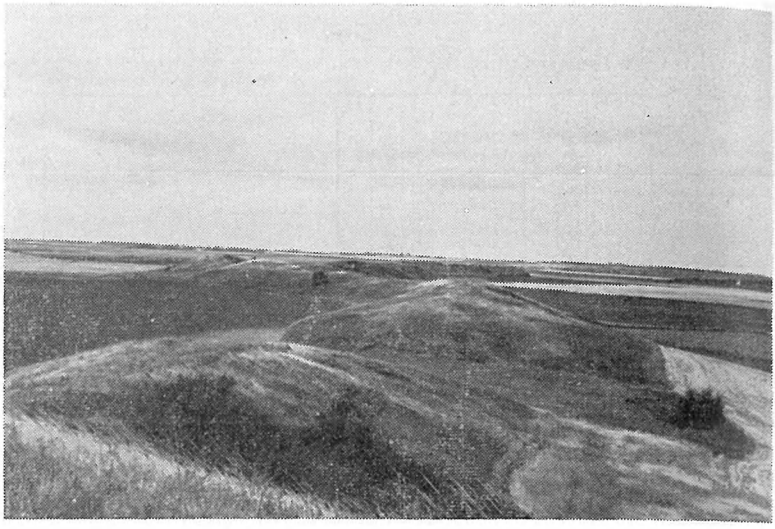

FIGURE 2—Northwest branch of the Dahlen esker. View toward the northwest and site 4.

probably were formed during the deposition of the esker. The gaps could have formed by sediment collapse as the underlying glacial ice melted. The accordant crest level is interrupted by a small hill near the terminus of the northeast branch. This hill has the form of a kame, a moundlike accumulation of outwash.

The ridge consists of two main types of sediments: (1) outwash, the meltwater stream deposits of bedded and non-bedded sand, gravel, and silt; and (2) till and boulders deposited directly by glacial ice.

The sands of the ridge are generally brown (oxidized), range in size from very coarse to fine-grained, and are usually associated with gravel. They are poorly sorted where the bedding is not apparent, and poorly to well-sorted where the bedding is developed. The shale content of the sand in some areas is very high, and where the shale particles are abundant the sand is dark gray. Horizontally-bedded sand was observed in five exposures, and cross-bedded sand was observed in one exposure.

The till of the esker is a stony loam because of an abundance of pebbles in a loamy sediment. The till is very light olive-gray to buff, oxidized, calcareous, unsorted, and unstratified. It is a heterogeneous material, the particle size of which ranges from boulders to clay. Scattered free boulders generally are abundant on the crest of the ridge. The boulders are of various sizes; boulders four feet in width were commonly observed and one measured seven feet across.

Till and boulders generally occur beneath, adjacent to, and upon

the outwash. However, in some areas outwash is exposed at the crest of the ridge without any overlying till or boulders. A mixture of sand, gravel, till, and boulders was observed in several of the gravel pit exposures. The mixing probably was caused by sediment collapse and slump as the adjacent ice melted.

ESKER ORIGIN AND GEOLOGIC SETTING

Approximately 12,000 radiocarbon years B. P. (before present) the Wisconsin glacier, the main ice mass, was mostly in Canada, but an ice lobe projected into the broad Lake Agassiz basin of North Dakota and Minnesota. During the waning stages of this lobe and while the ice margin was on the Pembina escarpment in the Dahlen vicinity, the Dahlen esker was deposited.

The Dahlen esker was deposited by a meltwater stream in an ice-walled channel, most likely a tunnel, near the base of a stagnant zone of the ice lobe. The stream flow probably was from east to west, toward the margin of the ice lobe. The flow direction, although suggested mostly by the position of the ice lobe, may be indicated by a kame near the terminus of the northeast branch. Kames differ from eskers by forming in a surface opening in the ice rather than in a tunnel or ice-walled channel. The possibility exists that the water entered an opening in the ice and then flowed through an ice-walled channel or tunnel. The meltwater stream deposited outwash in the surface opening and within its stream course now marked by the esker ridge.

Subsequent ablation of the adjacent ice resulted in the deposition of ablation till and boulders upon the stream sediments. Sediment collapse and slump also occurred as the adjacent ice, especially the underlying ice, ablated. Contemporaneously with the deposition of the esker, a low-relief till plain called ground moraine, was deposited in the area adjoining the esker.

Following the deposition of the Dahlen esker, ablation continued until the margin of the ice lobe had retreated five miles east. The glacier, because of an increase in activity, attained a stable position or stillstand. During this stillstand and along this active ice margin a till ridge, the Edinburg end moraine, was deposited. Meltwater flowed along the ice margin in a channel formed between the end moraine on the east and the Pembina escarpment on the west. Within this channel in the Dahlen vicinity an outwash plain was deposited, and to the south where the channel extended into an inlet of glacial Lake Agassiz, a delta-lake plain was deposited.

CONCLUSIONS

It is evident, as a result of the geologic mapping of Grand Forks County, that the ridge in the Dahlen vicinity is glacial in origin and can be designated an esker because of its landform and composition. This esker was deposited by a melt-water stream within the marginal zone of an ice lobe of the Wisconsin glacier.

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DIET AND ESTIMATED ENERGY ASSIMILATION OF THREE COLORADO LIZARDS

Donald R. Johnson

Department of Biology

Minot State College, Minot, North Dakota

ABSTRACT

Ants and beetles were the primary foods of 60 eastern fence lizards and 25 desert spiny lizards during the spring and summer months in southwestern Colorado. Iepidopterous larvae and grasshoppers comprised most of the food of 49 western whiptails. Although there was some seasonal change in the diet of all species there was little qualitative difference in diet between sexes or age groups. Sand grains and small pebbles found in stomachs may act as abrasives which macerate hard exoskeletons. Female fence and desert spiny lizards had larger amounts of food in their stomachs during the spring months than did the males, probably to meet the metabolic requirements of egg-laying. Assuming that these species fill the stomach twice daily, that stomach capacity is twice that of the mean weight of the stomach contents, an assimilation efficiency of ⅔, and an energy value of 5363 cal/g, the energy assimilated by an adult eastern fence lizard (15 g), western whiptail (22 g), and desert spiny lizard (30 g) is estimated as 0.83, 1.57, and 2.17 kcal/day (55, 71, and 72 cal/g/day), respectively.

HIGH TEMPERATURE TOLERANCES OF FIVE SPECIES OF ANURAN AMPHIBIANS[1]

Russell A. Dean and William D. Schmid
Department of Biology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

High temperature tolerances of five species of anuran amphibians, *Bufo hemiophrys* Cope, *Pseudacris nigrita* Le Conte, *Rana pipiens* Schreber, *R. septentrionalis* Baird and *R. sylvatica* Le Conte, were measured according to the method described by Schmid (1965, Ecology, 46:559). Specimens of *Bufo hemiophrys* and *Pseudacris nigrita* were collected in the vicinity of Grand Forks, North Dakota, while specimens of the remaining species were collected in the vicinity of the University of Minnesota Biological Station, Lake Itasca, Minnesota.

The order of high temperature tolerance (highest to lowest) was *Bufo hemiophrys, Rana pipiens, R. septentrionalis, Pseudacris nigrita* and *R. sylvatica.* The interspecific variations in tolerance to high temperature of these anurans was correlated with the known ecologies and geographic distributions. Comparisons were made among different methods of statistical analysis of data of response curves obtained in this study.

[1]The field work of this study was supported in part by a National Science Foundation Research and Training Fellowship obtained through the Biological Station of the University of Minnesota, Lake Itasca, Minnesota.

THE FEEDING RATE OF *DIAPTOMUS LEPTOPUS*[1]

J. E. Schindler[2] *and G. W. Comita*
Deparment of Zoology
North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota

The purpose of this paper is to present some measurements of the feeding rate of *Diaptomus leptopus* made at seven temperatures. A Coulter Counter was used for the determination of the cell concentrations before and after the feeding period.

Among the many investigators that have attempted to measure

[1]Supported by NSF Grant 16415 and the North Dakota Institute for Regional Studies. The help of Leroy D. Zinsli, Paula J. Kubik and D. Ted Eastlund is gratefully acknowledged.
[2]Undergraduate research participant (NSF).

feeding rates of copepods, the following have produced results that can be campared to our data. Gauld (1951) worked with five species of calanoids, feeding them *Chlamydomonas* Marshall and Orr (1955) measured the feeding rates of *Calanus finmarchicus* using ^{14}C; Corner (1961) and Cowey and Corner (1963) worked with *Calanus helgolandicus* and Conover (1956) measured the filtering rate of *Acartia clausi* and *A. tonsa* using *Skeletonema costatum*. Many other measurements have been made on *Daphnia*. Ryther (1954) has studied feeding rates of *Daphnia magna*. Rigler (1961) determined the feeding rate of *Daphnia magna* by measuring the animals ^{32}P activity after ingestion of labelled yeast, and McMahon and Rigler (1963) observed directly the feeding habits of *D. magna*. In addition to the above mentioned, Reeves (1963) studied the feeding rate of a branchipod, *Artemia*.

These investigators used three different methods for obtaining the rate of food ingestion: radio tracers (^{14}C, ^{32}P), direct observation and the counting cell. The method we used is most nearly like the counting cell method, making use of the Coulter Counter for demining cell concentrations.

MATERIALS AND METHODS

An initial culture of *Chlamydomonas* having approximately 50,000 cells per ml was obtained from a stock suspension. Since the Coulter Counter requires that the particles to be counted must be suspended in an electrolyte, the concentration of this initial suspension was determined by taking 25 ml of algal culture and adding 25 ml of 1% KNO_3 as the electrolyte. This suspension was placed in the counter and a cell count was made. The experiment was then set up using the algal concentration determined from this initial suspension.

Five flasks were set up as controls without copepods, and eight flasks with five animals per flask were used for the experimental determination. Each flask was filled with 50 ml of algal suspension into which the animals were introduced. These copepods were conditioned by suspending them in millipore filtered (5 μ) water for approximately 24 hours and the flasks were kept in the dark during this time. After the feeding period, 25 ml of the algal suspension were withdrawn and diluted to 50 ml by adding an additional 25 ml of 1% KNO_3 and the cell concentration was determined in the counter. The counts were corrected for coincidence using the data supplied by the Coulter Company.

Our analysis is based on the reasonably well established premise that diaptomids are filter-feeding animals, and that when suspended in an experimental vessel where the physical and chemical conditions are kept constant, the concentration of food particles in the presence of a steadily grazing copepod should decline exponentially, and at any given time it is given by the expression,

$$C_t = C_o e^{-kt} \qquad (1)$$

where C_t is the concentration after time t, and C_o the initial concentration.

Further, if v is the volume of water per animal, then vk is the volume of water swept free by one animal in unit time, so that the filtering rate, F, is given by,

$$F = vk \qquad (2)$$

This expression can be evaluated from (1) in the form

$$F = \frac{v(\log_{10}C_o - \log_{10}C_t)}{t \log_{10}e} \qquad (3)$$

After each set of experiments, the feeding rate was computed using the following simpler form of this equation:

$$F = \frac{v(\ln C_o - \ln C_t)}{t} \qquad (4)$$

RESULTS AND DISCUSSION

Seventy sets of observations (Table I) were analyzed for a possible regression relationship between rate of feeding and temperature. The first analysis made was to test the hypothesis that the variances are homogeneous. This hypothesis was rejected at the 5% level but accepted at the 0.5% level. The computed $\chi^2 = 17.03$, ($\chi^2_{.05} = 13.59$, $\chi^2_{.005} = 18.55$, d.f.6).

The equation derived for the feeding rate, F, was: $F = 0.080\ T^{\circ} + 0.071$, $r = 0.57$, ($r_{.05} = 0.302$ df 70), and $R^2 = 32\%$ ($F = 32.5$, $F_{.005} = 5.8$).

When an analysis of variance to test deviation from linearity was performed on the data (Table II), an F of 1.833 was obtained ($F_{.05} = 2.40$, df: 3, 63) indicating that the data do not deviate significantly from linearity at the 5% level. However, when the means of the feeding rates at the different temperatures are plotted (Figure 1), a line fitted by sight curves slightly suggesting a possible logarithmic relationship. As a consequence, a log transformation was made and the resulting regression relationship was examined. The equation obtained was $\text{Log } F = 0.068\ T^{\circ} - .985$, $r = 0.54$ ($r_{.05} = 0.302$, df: 70) and $R^2 = 29\%$ ($F = 27.4$, $F_{.005} = 5.8$). Comparing the F values obtained from the linear ($F = 32.5$) and log transformation ($F = 27.4$) equations it is evident that the regression equation obtained from the unmodified data is a better expression, and this reinforces the test which indicated that the data do not deviate significantly from linearity. These results are in agreement with those obtained by Conover (1956) whose data showed that the grazing rate for both

TABLE I
FEEDING RATES OF *DIAPTOMUS LEPTOPUS* (F)
(ml filtered/female/day)

	5°C	10°C	15°C	20°C	23°C	25°C	26.2°C
1)	0.482	0.069	0.721	2.283	2.054	2.540	3.465
2)	0.882	1.074	0.827	2.085	1.542	3.557	1.987
3)	0.189	1.539	0.317	2.485	2.444	1.326	2.349
4)	0.978	0.063	0.771	1.435	2.973	0.935	2.144
5)	0.477	1.828	1.491	2.133	2.270	0.493	1.943
6)	1.182		0.244	2.002	2.008		2.123
7)	0.937		0.970	2.197	3.777		1.828
8)			1.224	1.452	2.673		1.277
9)			1.167		2.379		2.651
10)			0.913		1.238		3.682
11)			1.866		0.986		2.901
12)			0.860		1.726		1.485
13)					1.250		3.342
14)					0.891		3.914
15)					0.385		
16)					1.622		
17)					1.622		
18)					0.575		
19)					0.188		
$\bar{y}$	.733	.915	.947	2.009	1.716	1.770	2.507
s^2	.125	.673	.205	.143	.852	1,578	.697

TABLE II
TEST FOR LINEARITY OF REGRESSION
(Ho: regression in the population is linear)

	S.S.	d.f.	M.S.	F	$F_{.05}$
Treatment	25.934	6	4.322		
Linear Reg	20.488	1	20.488		
Dev. from linearity	5.446	5	1.089	*1.833*	2.368 d.f. 5, 60
Error	37.416	63	.594		
Total	63.045	69	.916		

Conclusion: Accept Ho: Regression in the population is linear.

Acartia clausi and *A. tonsa* were essentially linear with respect to temperature and that this linear relation between temperature and grazing rate was characteristic of results he obtained throughout the fall of the year in which he made his study.

The F value was computed by dividing the regression sums of squares by the sample variance. The high value obtained indicates that the slope of the regression equation was significantly different from zero at the 5% level ($F = 32.5$; $F_{.05} = 4.0$ d.f. 1, 70).

A wide range of feeding rates have been reported, from less than 1 to 71.03 ml/animal/day. Corner (1961) stated that there is a considerable variation between different estimates of the grazing rate found with one particular species of diatom (e.g., *Ditylum brightwellii*) or flagellate (e.g., *Chlamydomonas*); and further pointed out that in recent work by Conover *et al.* (1959) these values can very greatly depending on the method of estimation. For example, a grazing rate determined by the technique of counting cells can be as high as 224.7 ml/animal/day, whereas that found using ^{32}P-labelled diatoms can be as low as 11.5.

The feeding rates obtained also vary considerably. Coefficients of variation computed from the mean values obtained at each temperature ranged from a minimum of 17.5% at 20°C to a maximum of 80.1% at 10°C (mean C.V. = 48%). The reasons for this very high

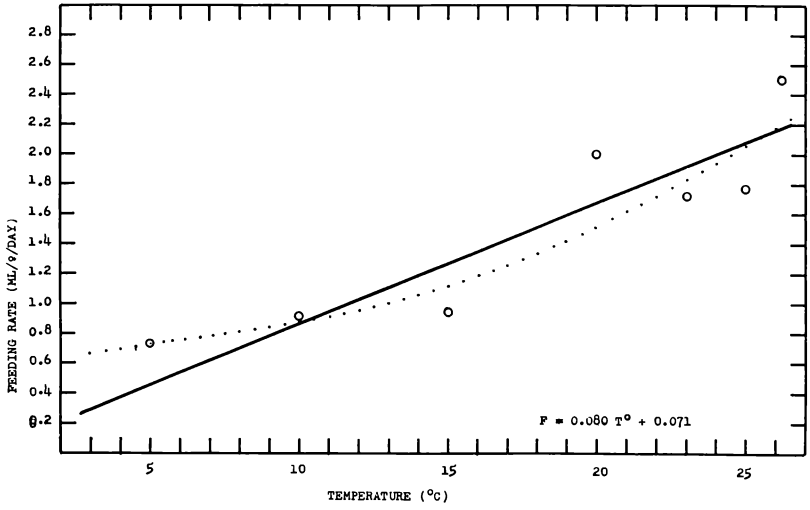

FIGURE 1—The relation between temperature and feeding rate for *Diaptomus leptopus*. The points plotted are the means of the data listed in Table I. The dots represent a line sighted to the data but the straight solid line is the computed regression line. F is the feeding rate in milliliters cleared per female per day.

amount of variability are probably very complex. Copepods in natural conditions certainly experience quite drastic temperature changes of the order of magnitude used in our experiments. The extent to which they are affected by handling and confinement was not determined. This should be done. An analysis should also be made of other influencing factors such as type and density of food which they were fed. The age of the organisms is another obviously important influencing factor. Without culturing the animals this latter factor will be very difficult to determine.

A final analysis was made by computing Q_{10}'s on the feeding rates derived from the regression equation. These were as follows: 5°-15°: 2.7, 10°-20°: 1.9 and 15°-25°: 1.6.

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LIFE HISTORY STUDIES ON *MASTOPHORUS NUMIDICA* (SEURAT, 1914) READ AND MILLEMANN, 1953 (NEMATODA:SPIRURIDAE) OCCURRING IN THE DEER MOUSE, *PEROMYSCUS* MANICULATUS[1]

William G. Dyer

Department of Biology

Minot State College, Minot. North Dakota

ABSTRACT

In an effort to determine the intermediate host of *M. numidica,* eggs containing first-stage larvae were fed to adult crickets, *Acheta domestica,* adult grasshoppers, *Melanoplus femur-rubrum,* and adult beetles, *Eleodes obsoleta.* Cysts containing infective third-stage larvae were subsequently obtained from the hemocoels of all three species. The first molt usually occurs in cysts, 7 to 9 days after exposure, with subsequent development of second-stage larvae continuing inside. The second molt occurs 19 to 22 days after exposure. A single control specimen of *M. femur-rubrum* and four control specimens of *Eleodes obsoleta* containing systs in the hemocoels, indicated natural infections.

Successful infection of *M. numidica* was established in the stomachs of deer mice fed cysts containing third-stage larvae as well as crickets and grasshoppers, 38 days after exposure of the insects. The larvae remain in the stomach of the deer mice, where they develop to maturity without migration in the tissue of the host.

Larvae undergo the third molt 8 to 11 days after exposure. The fourth and final molt occurs 15 to 17 days after experimental infection. At 35 to 42 days, the adults are mature and ova appear in the feces of the host.

[1]This investigation was supported by a training grant (5 T1 A1 94-05) from the Institute of Allergy and Infectious Disease of the National Institute of Health, U.S. Public Health Service.

THE INFLUENCE OF SELECTED GRAMINEAE ON THE BEHAVIOR OF THREE CEREAL-INFESTING APHIDS

Nadine K. Williams and J. T. Schulz

Department of Entomology

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

ABSTRACT

Studies on the influence of twelve selected Gramineae on the behavior of *Macrosiphum avenae* (Fab.), the English grain aphid, *Rhopalosiphum padi* (Linn.), the oat-bird cherry aphid, and *Schiza-*

phis graminum (Rond.), the greenbug, were carried out under greenhouse conditions.

The influence of each host on the length of life of each aphid species was determined. Hosts were then categorized as excellent, good, fair or poor.

Grasses rated as poor hosts had an inhibitory effect on the behavior of the aphids. Canadian brome, green foxtail, timothy, quackgrass and reed canary grass had an inhibitory effect on the behavior of all three aphid species. Russian wildrye, orchard grass, crested wheatgrass, Kentucky bluegrass, and sudan grass had an inhibitory on *M. avenae,* and yellow foxtail had an inhibitory affect on *S. graminum.*

Grasses which were given a fair rating inhibited the behavior of the aphids to the extent that they were not able to live over two weeks. Russian wildrye, sudan grass and yellow foxtail had this effect on *R. padi.*

Grasses given a good or excellent rating did not inhibit the behavior of the aphids. Meadow fescue did not inhibit the behavior of the three aphid species. Orchard grass, crested wheatgrass and Kentucky bluegrass did not inhibit the behavior of *R. padi* and *S. graminum.* Russian wildrye and sudan grass did not inhibit the behavior of *S. graminum,* and yellow foxtail did not inhibit the behavior of *M. avenae.*

The grasses which had an inhibitory effect upon the length of life of the aphids also had an inhibitory effect upon the rate of oviposition.

FOOD HABITS OF THE WESTERN BURROWING OWL (*SPEOTYTO CUNICULARIA HYPUGAEA*) IN SOUTHWESTERN NORTH DAKOTA

Ted R. James and Robert W. Seabloom

Department of Biology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

A study of the natural history and food habits of the western burrowing owl (*Speotyto cunicularia hypgaea*) was made in southwestern North Dakota in 1964. The analysis of the contents of 42 owl pellets collected at eight burrow sites in Slope County indicated that the diet of the western burrowing owl consists primarily of grasshoppers and various other insects. The remains of five species of small rodents and one fringillid bird were also identified.

THE PREVALENCE OF ALVEOLAR ECHINOCOCCOSIS, *ECHINOCOCCUS MULTILOCULARIS* LEUCKART, 1863, IN NORTH DAKOTA[1]

Paul D. Leiby

Department of Biology

Minot State College, Minot, North Dakota

Echinococcus multilocularis Leukart, 1863, is an important cestode zoonosis of man. Human infection, acquired from infected carnivores, is due to parasitism by the larval stage. *E. multilocularis* was first reported from the North American continent by Rausch (1956) who found adult cestodes in red foxes (*Vulpes vulpes*) and arctic foxes (*Alopex lagopus*) in Alaska. Because of the wide occurrence of *E. multilocularis* in Alaska, Rausch believed that this cestode would be introduced into high-boreal regions of southern Canada and the United States. Six years later Choquette et al. (1962) found *E. multilocularis* in arctic foxes at Eskimo Point on the western coast of the Hudson Bay, Northwest Territories, the first reported occurrence of this cestode on the Canadian mainland. Recently Leiby and Olsen (1964) found adult cestodes in red foxes in Ward County, North Dakota. These were indistinguishable from those found in Alaska and were identified as *E. multilocularis* on a morphological basis. Kagan et al. (1965) fed eggs from *E. multilocularis* recovered from foxes in North Dakota to cotton rats (*Sigmodon hispidus*) and infection of his host was obtained. This constituted biological verification of the occurrence of this species in the conterminous United States. Leiby (1965) found field mice (*Microtus pennsylvanicus* and *Peromyscus maniculatus*) in North Dakota naturally infected with the alveolar larval stage of *E. multilocularis*. Thus, the sylvatic cycle of this cestode (fox to rodent to fox) was shown to be established in North Dakota.

During 1965-66, 57 *V. vulpes* from North Dakota were examined for adult cestodes. Those found in 27 (47.37%) were confirmed as *E. multilocularis*. The infected foxes came from seven counties in north central and central portions of the State. Out of 12 *Mephitis mephitis* examined from this locality during early summer when young foxes were becoming infected, one juvenile skunk was found harboring immature *E. multilocularis*. Due to the worms being immature it cannot be stated whether or not striped skunks are suitable definitive hosts for this parasite. *E. multilocularis* was not observed in 5 *Procyon lotor*, 5 *Canis latrans*, 18 dogs and 3 cats examined from the enzootic area.

A total of 832 rodents were examined for the alveolar larvae of this cestode. These animals were collected throughout North Da-

[1]This investigation was supported by Grant AI-06633 from NIH.

kota, adjoining portions of Minnesota and South Dakota, and the Black Hills of Wyoming. The livers examined showed numerous cystic lesions. Many were diagnosed as bacterial infections, coccidiosis and cysticercosis. However, cysts found in 32 (4.74%) of the 705 rodents examined from North Dakota were ascertained as the alveolar larvae of *E. multilocularis*. Of this total larvae were encountered in the liver of 6 (4.13%) of 145 *M. pennsylvanicus*, 23 (4.93%) of 467 *P. maniculatus* and 1 (7.14%) of 14 feral house mice. Larvae were not observed in 8 *Onychomys leucogster*, 13 *Zapus hudsonius*, 34 *Citellus richardsoni*, 21 *C. tridecemlineatus* and 3 *Sorex cinereus*. Also, larvae of this cestode were not found in 127 rodents (deer mice, microtines and ground squirrels) collected in Minnesota, South Dakota and Wyoming.

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BEHAVIORAL RESPONSES AT VARIOUS THERMAL GRADIENTS IN SPECIES OF *ONYCHOMYS*

Bill A. Texera

Department of Biology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The effect of a one-hour exposure to various environmental temperatures was studied in two species of desert rodents, *Onychomys leucogaster* and *O. torridus*. Postural attitudes, and core and skin temperatures were noted at specific air temperatures from 2.0 to 38.0 C in 22 *O. leucogaster* and 13 *O. torridus*. Each species assumed four basic stances between 10.0 and 38.0 C. These attitudes were similar in each species; but the temperature at which they were elicited, and the range over which they were maintained, varied below 10.0 C. *O. leucogster* sacrificed the insulative advantage of its posture that minimized exposed surface area by its active behavior. Body

temperatures in *O. leucogster* reached lethal temperatures from 36.0 to 38.0 C. At these air temperatures, *O. torridus* held significantly lower body temperatures. In general, the thermal gradients from body-to-surface and surface-to-air temperatures were similar in both species at common environmental temperatures. The interrelationship between behavioral responses was discussed in relation to internal and external thermal conditions. The behavioral responses, core and surface temperatures, and thermal gradients were graphed over the environmental temperature range from 2.0 to 38.0 C.

THE DETECTABILITY OF A COMPLEX AUDITORY SIGNAL AS A FUNCTION OF THE RELATIVE PHASE OF THE COMPONENTS

James E. Fricke

Division of Special Education
Minot State College, Minot, North Dakota

ABSTRACT

Controversy has raged for almost a century as to whether or not the human ear can distinguish changes in the relative phase of the components of a complex signal. Recent studies have demonstrated that phase relationships are a significant auditory experience and that these changes are realized subjectively as changes in pitch, quality, and intensity. Moreover, it has been shown that changes in phase, which result in alteration of the peak characteristics of the time envelope, serve as the possible basis of these subjective differences.

The present study was designed to investigate the detectability of a complex signal as a function of the relative phase of the components of that complex waveform. The signal used in the present study was composed of a fundamental and its second harmonic. The combination of the two components was altered through six relative-phase relationships: 0°, 30°, 60°, 90°, 120°, and 150°. A statistical analysis of the data revealed significant differences with regard to the detectability of the complex signal as a function of the relative-phase of the components. The results indicated that at the 120° relative-phase relationship detectability was maximum.

PRIMARY PRODUCTIVITY MEASUREMENTS AT DEVILS LAKE, NORTH DAKOTA

Richard Armstrong[1], *David W. Anderson*

Department of Biology

and

Edward Callender

Department of Geology

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

Devils Lake, a closed lake, is located in northeastern North Dakota (48° N lat, 99° W long; Figure 1). The lake is part of a chain of waterways situated in the Devils Lake drainage basin which has an approximate area of 10,000 sq km (3,900 sq miles). During the late 1800's, Devils Lake had an area of approximately 207 sq km (80 sq miles), but it has been steadily declining and presently occupies an area of only 48 sq km (18.5 sq miles).

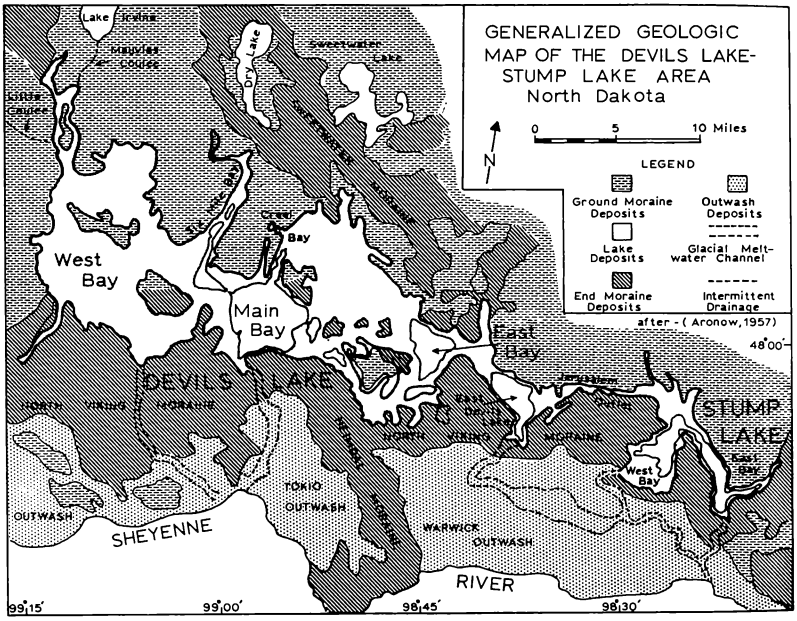

FIGURE 1—Generalized geologic map of the Devils Lake-Stump Lake area, North Dakota (after Aronow, 1957).

[1]Present address: Institute of Ecology, University of California, Davis, California.

The mean annual temperature of the Devils Lake basin ranges from 2° to 6° C (Swenson and Colby, 1955) with extremes varying from above 36° C to below -40° C. The average annual precipitation at the city of Devils Lake is 44.5 cm (Swenson and Colby, 1955) with most of the precipitation occuring during the summer months. The prevailing wind is from the northwest during all months of the year and has an average annual velocity of 10 mph.

The surficial deposits in the Devils Lake area are primarily glacial and post-glacial in origin (Figure 1). The glacial drift varies in thickness from a meter to nearly 120 m and is composed primarily of limestone and shale. Glacial Lake Minnewaukan, predecessor of the present Devils Lake-Stump Lake complex, originated as a moraine-dammed proglacial lake behind the North Viking morainal complex. The lake declined rapidly as the volume of meltwater subsided and eventually split into several isolated lakes and bays of which Devils Lake is the largest.

Devils Lake has a simple morphometry (Figure 2). The lake basin is shaped like a pie plate with a present mean depth of 2.6 m. During the ice-free months between May and November, strong winds thoroughly mix the lake waters and they remain well oxygenated. Any thermal stratification is temporary and easily destroyed by the slightest wind.

Summer water temperatures reach 26°-28° C while winter temperatures under the ice approach 0° C. Transparency is low and a common Secchi disc reading during the ice-free months is 0.4 - 0.6 m. The lake is sealed off by ice during the winter and early spring months and reducing conditions develop.

The lake has a total dissolved salt content of 14 parts per thousand (about 40% that of sea water), but beneath the ice in March the salinity may reach 18 parts per thousand due to concentration by freezing. Devils Lake water differs from normal sea water in that it has more sulfate and much less chloride. The organic nitrogen and phosphorus contents of the lake water are very high, reflecting the influx of sewage and agricultural pollutants into the lake.

In spite of apparently high concentrations of available nutrients, the biota of the lake is not especially rich in terms of biomass and is distinctly poor in terms of biological diversity. The meager phytoplankton populations are dominated by diatoms. Two species, one a diaptomid, the other a moinid, dominate the zooplankton.

The vertebrates are almost solely represented by one species of salamander, *Ambystoma tigrinum*. The bottom fauna of the lake is not well know but preliminary samples revealed only meager populations of a few dipterous larvae.

There is but one rooted plant in Devils Lake, *Ruppia maritima*. Except in Creel Bay it plays a very minor role in the bioeconomy of the lake. Extensive beds of *Enteromorpha prolifera*, a filamentous

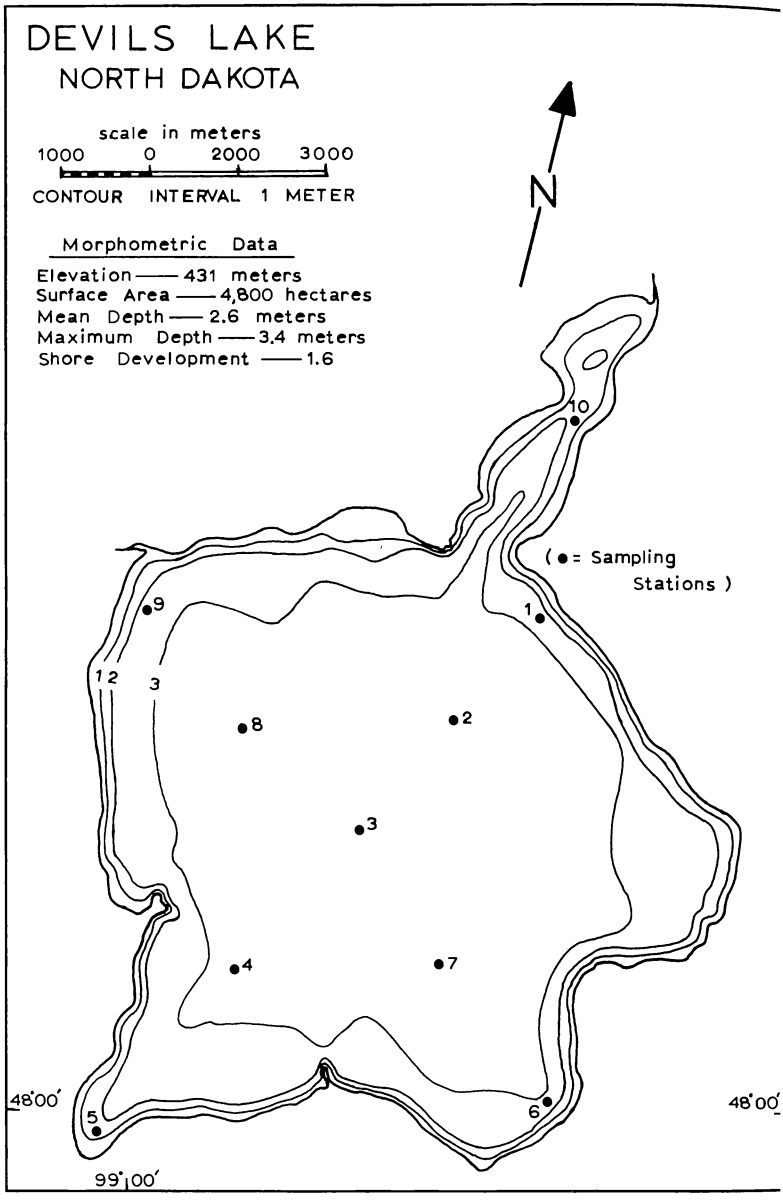

FIGURE 2—Morphometric map of Devils Lake, North Dakota.

alga which grows attached to submerged objects are also found in the littoral zone.

The surface sediments consist of two major types: (1) a black, reducing, foul-smelling, silty clay that occurs throughout 75% of the bottom area, and (2) a light grayish brown near-shore sand and calcareous mud in the shallower zones that are commonly heavily populated by attached algae. Cobbles and boulders are commonly found in these shallow areas.

Previous investigations of Devils Lake have dealt with glacial geology and limnology. Aronow (1957) mapped the beach deposits and major strand lines around the lake and concluded that it had fluctuated greatly during post-glacial time. Young (1924) made a detailed limnological study of Devils Lake from 1911 through 1923, and Metcalf (1931) briefly studied it during a limnological survey of North Dakota lakes. Swenson and Colby (1955) studied the chemistry of Devils Lake (as well as several other lakes in the Devils Lake drainage basin). The present authors are conducting an intensive investigation of the natural history of Devils Lake including studies of the glacial geology, biogeochemistry of nutrients, sedimentation, paleolimnology, plankton dynamics, and primary productivity.

A study of the primary productivity of a lake, the rate at which inorganic carbon is reduced to organic forms, provides the focal point for the interpretation of other processes taking place in the lake. The rate of carbon fixation at the level of the primary producers is influenced by the morphometry of the lake and the geology and hydrology of the drainage basin. It is the best available assessment of the actual fertility of the lake (Goldman and Wetzel, 1963) and provides the best estimate of the rate at which matter moves through biogeochemical cycles. Both the qualitative and quantitative aspects of sedimentation are influenced by primary productivity, high rates being recorded in the sediments by increased content of high energy materials (organic matter as well as reduced forms of elements other than carbon). The population dynamics within the biota of the lake are, in fact, the dynamics of the dispersal of energy originally concentrated by photosynthesis.

METHODS

In the study of Devils Lake, standing crops of larger littoral species (*Ruppia maritima, Enteromorpha prolifera,* and *Cladophora sp.*) were measured by harvesting a swath 18 cm (7 inches) wide extending from the shoreline outward to the depth at which no more plants were found. Efficiency of this method within any single swath was estimated at 80%. A total of 15 swaths, evenly spaced around the main lake, were laid out and harvested on a single day, August 9, 1965. Continuous observations of a control area during the study period indicated that littoral vegetation built up slowly to a peak on this date and declined very rapidly afterwards. Harvested plants were air dried for four months in a warm room and then weighed.

Instantaneous rates of production by littoral species were estimated by enclosing known amounts of littoral algae with known volumes of lake water in transparent polyethylene bags. The changes in inorganic carbon concentration in these bags were compared with changes in similar bags incubated in a dark box. Inorganic carbon concentrations were measured by potentiometric titration of carbonate and bicarbonate ions with 0.03 N H_2SO_4 (American Public Health Association et al., 1960). Each sterile polyethylene bag had a rubber hose affixed to the bottom which allowed samples to be withdrawn anaerobically. The bags were not suitable for studying fluctuations in dissolved oxygen as polyethylene is quite permeable to gasses. Diffusion of CO_2 through the bag was not considered to be a problem because of the high carbonate alkalinity of Devils Lake. A correction factor was applied to compensate for the appreciable precipitation of $CaCO_3$ in the light bags.

Primary productivity of planktonic algae was measured by the oxygen light-and-dark-bottle method. Glass-stoppered 250 ml autoclaved bottles were used. Bottles were filled anaerobically from an unlined, three-liter Kemmerer water sampler. Three bottles were filled from each Kemmerer sample, one bottle to measure the original concentration of dissolved oxygen, one light bottle and one dark bottle. Two such sets were assembled for each depth at which productivity was to be measured. Oxygen was determined by the azide modification of the Winkler method (American Public Health Association, et al., 1960). Bottles were suspended at half-meter intervals from the surface down to 3 m. Dark bottles were wrapped with black adhesive tape to exclude light and with aluminum foil to limit heat absorption.

The bottles were incubated between noon and sunset. The amount of oxygen produced during an entire day was taken to be twice the amount produced during the incubation period. Amount of carbon fixed was calculated from the gross production of oxygen (light bottle oxygen concentration minus dark bottle concentration) by assuming a photosynthetic quotient (PQ) of 1.2 (Strickland and Parsons, 1960).

A single station at the center of the lake was used throughout the study with measurements of production being made approxmately biweekly. At selected times primary production was measured at other locations on the lake to defect any variations that might exist.

Each time the productivity was measured, phytoplankton populations in the lake were estimated. Methods and results are described elsewhere (Anderson and Armstrong, 1966).

Transparency of the water was estimated with a standard Secchi disc and light attenuation was measured with a light meter constructed by the authors (Anderson and Armstrong, in manuscript). Water temperatures were measured with a portable thermistor unit. Samples of the sediments at the mud-water interface were obtained

by a siphon sampler described elsewhere (Armstrong, Callender, Anderson, in manuscript).

Phosphorus was measured by the stannous chloride method with benzene-isobutanol extraction (American Public Health Association

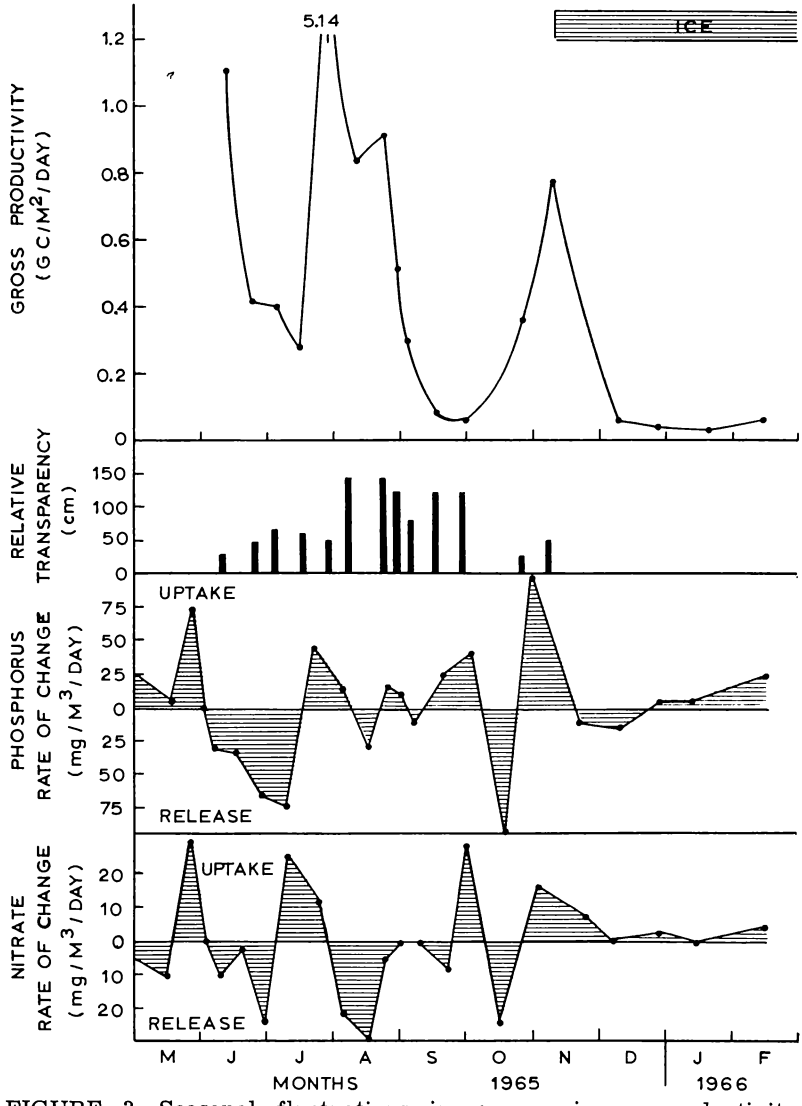

FIGURE 3—Seasonal fluctuations in gross primary productivity, Secchi disk transparency, rate of change of nitrate and total phosphorus.

et al., 1960). Iron was measured with orthophenanthroline after hydrolysis in 2 N HCl (American Public Health Association et al., 1960). Silica was measured by the molybdosilicate method (American Public Health Association et al., 1960). Nitrate was measured by Jenkins and Medsker's (1964) improved brucine method. Nitrite, ammonia, and organic nitrogen were measured by standard methods (American Public Health Association et al., 1960).

RESULTS

Primary productivity measurements, Secchi disc transparencies and the rate of change of nitrate and total phosphorus are shown in Figure 3. Fluctuations in productivity occurred throughout the study. These fluctuations reflect not only changes in the photosynthetic potential of the phytoplankton but certainly also variations in light intensity.

Appreciable fluctuations in total phophorus content of the water occurred during the study period. In general, these fluctuations coincided with changes in the intensity of photosynthesis. During periods of high productivity phosphorus concentrations decreased (shown as uptake on the differential cure, Figure 3) and during periods of low photosynthetic activity increased (release in Figure 3). In general, a similar relationship existed between fluctuations in nitrate concentration and photosynthetic rate but there were more pronounced discrepancies than was the case with phosphorus.

Seasonal fluctuations in the concentrations of nitrate, nitrite, ammonia, total phosphorus, silica, carbonate and bicarbonate are shown in Figure 4.

Throughout the entire study period the dominant phytoplankter was a diminutive diatom (*Cyclotella?*) having a diameter of only five microns. This ultraplankter accounted for at least 99% of the phytoplankton cell surface area. Numbers of cells per liter ranged from 20×10^6 to 150×10^6. There was no discernible relationship between phytoplankton population size and primary productivity (Anderson and Armstrong, 1966).

Rapid extinction of light in the turbid waters of Devils Lake was evident from the photometer values. Absorbance of light (-log % transmission) at different depths for three representative dates is given in Figure 5. Also shown in Figure 5 are comparisons of Secchi disc transparencies and gross productivity at different depths for the same dates. The euphotic zone seldom extends beyond two meters due to the high turbidity.

Highest productivity measured was on July 28, 4.14 g C/m^2/day. This measurement was made on the third day of a four day winds with maximum daily wind velocities above 20 mph. During this unusually calm period a well-defined chemical and thermal stratification developed (Figure 6). We attribute the sharp increase in productivity to the release of one or more essential micronutrients

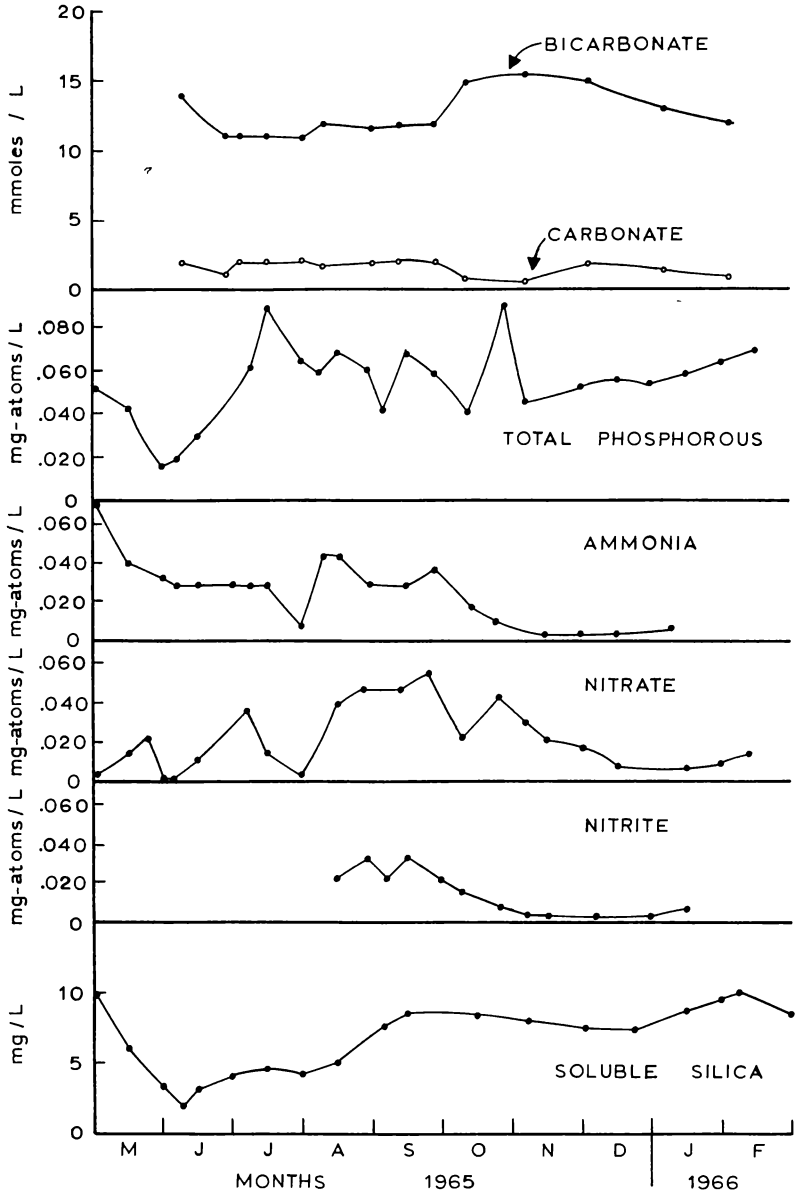

FIGURE 4—Seasonal fluctuations in the concentrations of nitrate, nitrite, ammonia, total phosphorus, silica, carbonate and bicarbonate.

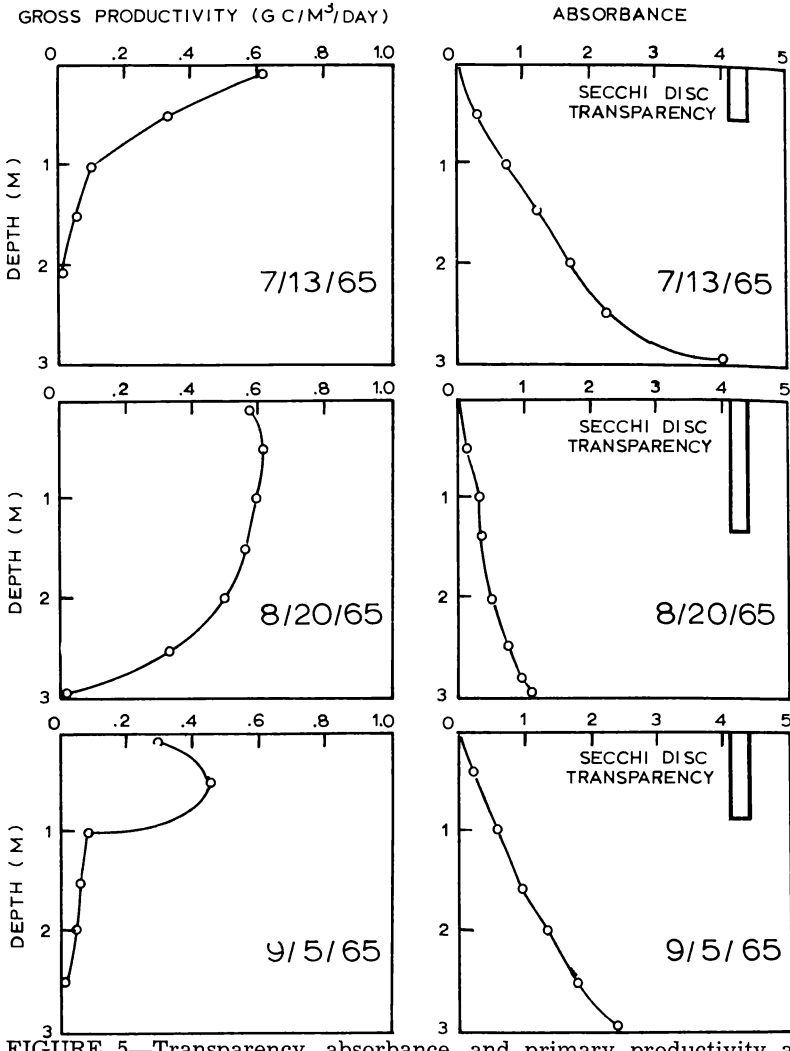

FIGURE 5—Transparency, absorbance, and primary productivity at different depths on three respective date.

from the normally oxidized but temporarily reduced mud surface. Total iron content of unfiltered water increased nearly threefold during the calm period. The increases in concentration of iron in the water coincided with decreases in the percentage of iron in the sediments at the mudwater interface throughout the study (Figure 7) and is discussed in detail elsewhere (Armstrong, Callender, and Anderson, in manuscript).

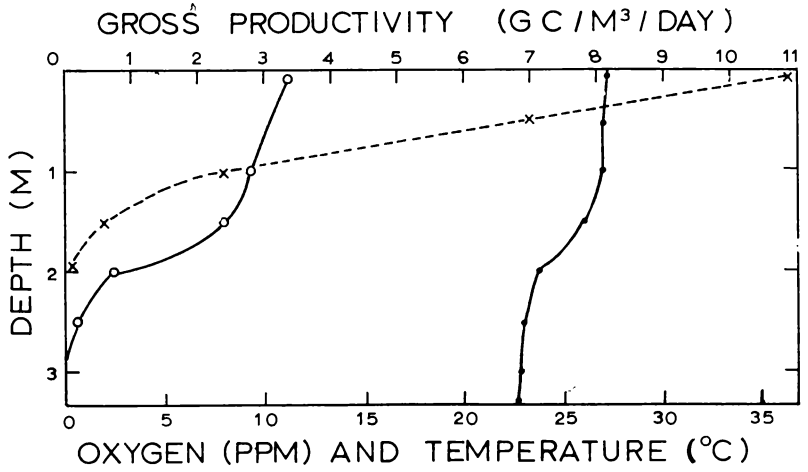

FIGURE 6—Gross productivity (Cx), oxygen concentration (Co), and temperature (C.) at the center of the lake on July 28, 1965.

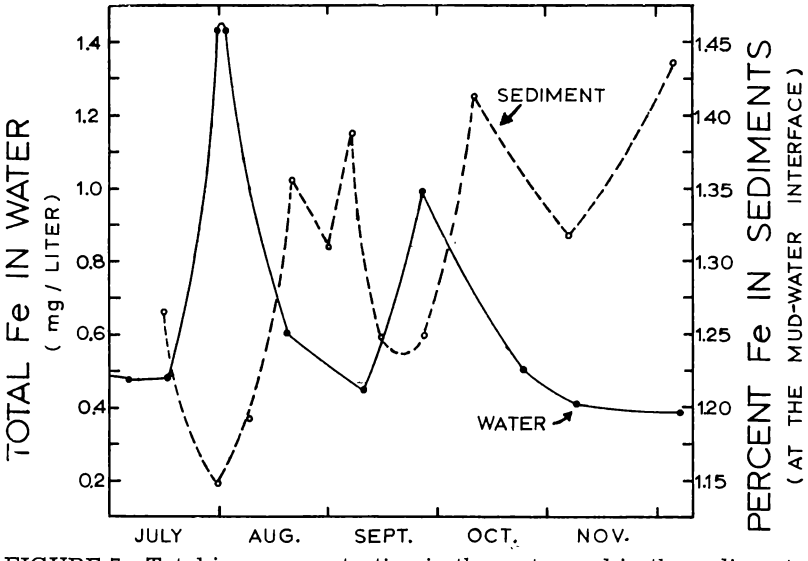

FIGURE 7—Total iron concentration in the water and in the sediments at the mud-water inter face.

Productivity of *Enteromorpha prolifera* was estimated from the plastic bag experiments. On July 28, the day of highest phytoplankton productivity, photosynthesis by *E. prolifera* accounted for the gross incorporation of 0.94 g C/m² in the littoral areas where it

was abundant. This figure is close to the average daily gross production of *E. prolifera*, 1.06 g C/m²/day, suggesting that the rocky littoral area was not influenced greatly by the reducing conditions which developed in the deeper zones.

Air temperature on July 28 reached 102° F (38.9° C) while the peak water temperature (at the shore) was 81° F (27.2° C). Noontime dissolved oxygen concentration at station 1 was 39.2 mg/1 (509% supersaturated). The sound of oxygen issuing from the water was quite perceptible. By nightfall dead copepods (*Diaptomus sicilis*) were on the shore in windrows ankle deep. Large masses of water, especially in Creel Bay, were deeply rust colored, presumably because of ferric hydroxide precipitation.

Photosynthesis by the phytoplankton produced 67.3 x 10^5 kg organic carbon during the study period. Production by the larger attached algae of the littoral zone is estimated for the same period as 1.86 x 10^5 kg organic carbon. The sum of these figures underestimates the total production of the lake by not taking into account the abundant growth of epilithic diatoms (principally *Navicula* spp.) on rocks and boulders in shallow water. Also missing is an estimate of the production of *Ruppia maritima*. This plant is not an important producer in the main lake but it is locally abundant in Creel Bay and near station 5.

This estimate of production must be considered minimal because it is based on rate of carbon assimilation between noon and sunset which may be appreciably less than half the total daily production (Doty and Oguri, 1957).

Net production of *Enteromorpha prolifera* from May 25 to August 9 was calculated from standing crop data. Total net production for the seventy-five day period was .49 x 10^5 kg. The area available to *E. prolifera* was 1.0 x 10^6 m² so the net production (i.e. net gain in dry weight) during the period of most rapid growth was 49 g/m² or 0.26 g C/m²/day (assuming carbon to be 40% of dry weight). By November 7, the entire crop of *E. prolifera* had distintegrated.

Respiration of the planktonic community during the study period was calculated by averaging the dark bottle respiration on each sampling date, extrapolating the result to 24 hours and summing for the entire volume of the lake. Because of the shallowness of Devils Lake and the unremittent circulation of its waters this method of calculating community respiration is considered to be as valid as the calculation of production to which it is compared. Total community respiration during the study period was 64.2 x 10^5 kg C. This figure is 95% of the planktonic production indicating that most of the annual production is utilized while the lake is still free of ice.

DISCUSSION

Mean gross phytoplankton productivity during the 175-day study period was 0.80 g C/M²/day. Photosynthesis under the ice is negligible

(no photosynthesis could be detected by the O_2 method on 12-29-65 or 4-8-66; both days were cloudless). There are no data for the first 40 days of the 1965 growing season (5-1 to 6-10). If the average photosynthetic rate for these forty days is assumed to be 0.80 g C/M^2/day, the total annual gross production of Devils Lake can be calculated as 1720 kg C/ha. Although the intensity of production by attached forms in the littoral areas exceeds that of the phytoplankton, the contribution of attached forms to the total annual production of the entire lake is probably not great. A minimum estimate of this contribution based on the plastic bag experiments is 2.8%.

Measurements of primary production have been made on a large number of lakes and annual production varies widely with the type of lake (Table I).

TABLE 1

COMPARISON OF THE ANNUAL MEAN PRIMARY PRODUCTIVITY OF SEVERAL LAKES TO VALUES FOR DEVILS LAKE, NORTH DAKOTA (AFTER WETZEL, 1964).

Lake	Annual Mean (mg C/m^3/day)
Devils Lake, North Dakota	470
Borax Lake, California	250
Clear Lake, California	438
Castle Lake, California	98
Esrom So, Denmark	370
Fureso, Denmark	750
Frederiksborg Slotsso, Denmark	1,030
Sollerod So, Denmark	1,430
Store Gribso, Denmark	230
Grange Langso, Denmark	248
Kattehale Mose, Denmark	80

The four-day calm period in July and attendant events illustrated the important relationship existing between the planktonic community and the sediments. This relationship appears more dramatic perhaps in a deeper lake in which a regular seasonal cycle of thermal and chemical stratification often imposes a seasonal cycle on plant production (Mortimer, 1942). It would have been easy to overlook the importance of mud-water exchange in the continually circulating Devils Lake had not the unusual meterological conditions developed.

An important finding of the present study is the circumstantial evidence that ultraplankters are responsible for virtually all of the productivity in Devils Lake. The total annual production of 1720 kg C/ha in spite of a relatively short growing season qualifies Devils Lake as a highly productive or eutrophic lake. Goldman and Wetzel (1963) observed that ultraplankters or nannoplankters are more important in less productive waters while larger phytoplankers are the

principle producers in eutrophic situations. Apparently this general observation does not fit Devils Lake. The same authors (Goldman and Wetzel, 1963) also observed that the relative importance of smaller phytoplankters increased in situations where light was an important limiting factor; "especially in waters with heavy ice cover, the smallest size division of phytoplankton, or ultraplankton, can completely dominate the primary producers". They cite an increased efficiency of utilization of light and present experimental evidence that the relative contribution of smaller phytoplankters (less than 5-10 microns) to the productivity was greatest during winter and spring when turbidity of their study area was highest. Smaller phytoplankters sink more slowly than large ones (Munk and Riley, 1952) and thus small size is likely to be a distinct advantage in a lake having a shallow euphotic zone. It seems unlikely, however, that sinking rate would be an important consideration at Devils Lake because of the nearly constant circulation.

ACKNOWLEDGMENTS

This research was supported by a North Dakota Water Resources Institute grant (WI-2-100-1). The authors were supported by NDEA predoctoral fellowships.

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LOCAL DISTRIBUTION OF MUSSELS, TURTLE RIVER, NORTH DAKOTA

Alan M. Cvancara, Robert G. Heetderks and Floyd J. Iljana

Department of Geology

University of North Dakota, Grand Forks, North Dakota
Sylvan Christian School[1], Grand Rapids, Michigan
Mira Loma High School[2], Sacramento, California

INTRODUCTION

The purpose of this study is to analyze certain factors which might affect the local distribution of mussels in a small river. During the summer of 1965, two sites were studied in detail on the Turtle River, a tributary of the Red River near Grand Forks in eastern North Dakota. These sites were selected because of their abundance of mussels. Cvancara and Harrison (1966) reported on the distribution and ecology of mussels throughout the Turtle River. The writers wish to thank the following persons for their aid in this study. Mr. George Pike and other members of the Surface Water Branch of the U. S. Geological Survey at Grand Forks aided in water velocity measurements. Professor F. D. Holland, Jr., of the geology department at the University of North Dakota, read and criticized the manuscript. Professor Vera Facey, of the biology department at the University of North Dakota, checked the identifications of the aquatic plants.

[1]R. G. H.
[2]F. J. I.

LOCATION OF SITES

The two sites, each about a 200-foot linear segment, are on the middle part of the Turtle River about nine river miles apart (Figure 2, index map):

Site 1.—Turtle River State Park, NE¼ sec. 36, T. 152 N., R. 54 W., about 1½ miles north of Arvilla (or about 20 miles west of Grand Forks), Grand Forks County, North Dakota. The downstream edge of the mapped area (Figure 1) on the right bank is 224 feet upstream from the most northerly bridge in the park.

Site 2.—SW¼ sec. 11, T. 152 N., R. 53 W., about 2½ miles southwest of Mekinock (or about 16 miles west-northwest of Grand Forks), Grand Forks County, North Dakota. The upstream edge of the mapped area (Figure 2) on the left bank is 55 feet downstream from the bridge on the section line common to sections 11 and 14.

Sites 1 and 2 are at stations 11 and 13, respectively, of Cvancara and Harrison (1966, p. 134-135).

METHODS

Each site was mapped with steel tape and Brunton compass, and divided by a reference grid with 10-foot quadrats. Mussels were located visually with a Turtox Fishscope, an aluminum alloy cylinder, 24 inches long and six inches in diameter with a glass plate. Each mussel was identified in the field, and its position and orientation were plotted on the map. Four cross-profiles were constructed at each site. Velocity readings and bottom samples were taken along each cross-profile. Water velocity was measured in fps with a Price Pygmy current meter. Bottom samples were analyzed for particle size by a combination of wet sieving and decantation. From these analyses the areas of sediment type were delineated, using the percentage limits of Shepard (1954).

Turbidity was measured with a Hach Chemical Company Model DR-EL photoelectric colorimeter. Water was analyzed for dissolved oxygen and total alkalinity by titration, following the manual of the American Public Health Association and others (1960). Dissolved oxygen was determined by the Alsterberg (sodium azide) modification of the Winkler method, titrating with 0.024N sodium thiosulfate solution and a starch indicator. Total alkalinity was measured by titrating with 0.02N sulfuric acid using phenolphthalein and methyl red indicators.

RESULTS

Site 1

Mussel distribution and orientation.— The following species and their respective numbers of individuals were noted and plotted:

Lampsilis siliquoidea (Barnes)	130
Anodonta grandis Say	46
Lasmigona complanata (Barnes)	24
Anodontoides ferussacianus (Lea)	18
Total	218

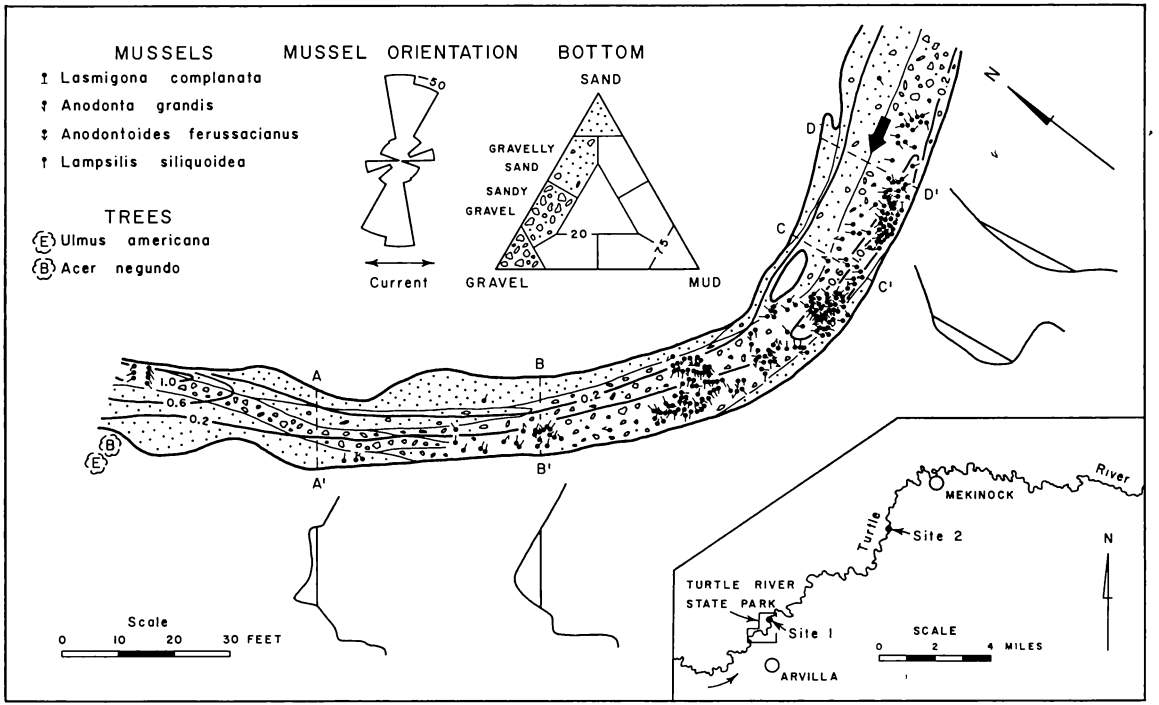

FIGURE 1—Map of site 1 on the Turtle River showing mussels, bottom sediment, channel configuration, velocity, and trees along banks. Mussels are indicated by dark circles, from each of which is extended a line indicating direction of siphons. Rose diagram showing mussel orientation is based on plot of 218 individuals. Stream velocity, shown by heavy isopleths at 0.6 of the depth, is in fps. Vertical exaggeration of cross profiles is approximately X1.7. Index map is shown on Figure 2.

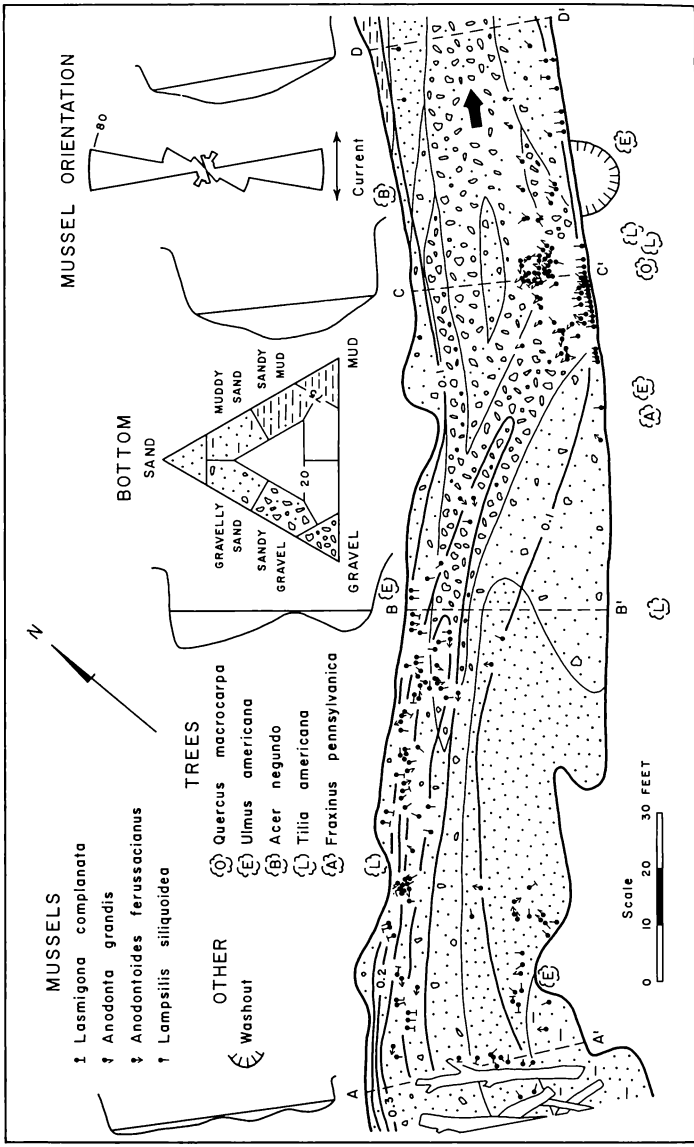

FIGURE 2—Map of site 2 on the Turtle River showing mussels, bottom sediment, channel configuration, velocity, and trees along banks. Mussels are indicated by dark circles, from each of which is extended a line indicating direction of siphons. Rose diagram showing mussel orientation is based on plot of 243 individuals. Stream velocity shown by heavy isopleths at 0.2 ft. off the bottom, is in fps. Vertical exaggeration of cross-profiles is approximately X1.7. Index map shows location of sites 1 and 2.

Most individuals of *Lampsilis siliquoidea* were apparently mature and about 80 percent of these were females as determined by shell shape.

All mussels were plotted on a rose diagram relating the orientation of their siphons to current direction. Most of the mussels were oriented predominantly perpendicular to the direction of stream flow (Figure 1) and the siphons of most mussels were directed toward the middle of the river (from either bank), as might be expected.

Stream velocity.—Stream flow was very low at this site and was difficult to measure near the bottom (readings were taken at one or two points along each of the profiles). Consequently, the isopleths of velocity shown in Figure 1 were based upon readings taken at 0.6 of the depth. Although water velocity was low, a strong suggestion is that concentrations of mussels are directly related to areas of relatively higher velocity. Most mussels occurred near the banks along the apparent thalweg. This phenomenon is in apparent contrast to the idea of Matteson (1955, p. 142) who doubted water current has any direct effect on mussels except the mechanical effect of "pressure."

Bottom.—The configuration of the bottom and water depth (few inches to four feet) is indicated by four cross-profiles. Four to seven bottom samples were taken along each cross-profile, and a map showing sediment types (Figure 1) was constructed from this information. Bottom sediment varied from gravel to sand to sandy mud. Coarser sediment occurred where stream velocity was higher, as might be expected. Most mussels occurred on a bottom varying from pebble gravel to gravelly sand.

Chemical and other factors.—Dissolved oxygen, total alkalinity, and turbidity were determined for site 1 (Table I) and all showed

TABLE I
DISSOLVED OXYGEN, TOTAL ALKALINITY, AND TURBIDITY AT TWO SITES ON THE TURTLE RIVER*

Site	Date and Time	Dissolved Oxygen (PPM)	Total Alkalinity (PPM)	Turbidity (PPM)
1	7/10/65 (1600)	6.9	.....	.....
1	7/11/65 (0400)	4.8	230	12
2	7/11/65 (0500)	4.9	228	16
1	7/26/65 (1600)	.....	231	
2	7/26/65 (1700)	5.6	237	.....

*All values are averages of analyses of samples from four depths.

no apparent variation with depth. Daily variation in dissolved oxygen is indicated by a value of 6.9 ppm at 1600 hours on 10 July and 4.8 ppm 12 hours later (0400). Negative results with phenolphthalein indicate all alkalinity was present as the bicarbonate ion. Total alkalinity values, measured 15 days apart, showed no change. Turbidity, measured only once, was low.

Banks were well shaded by bushes and trees, and the latter are shown in Figure 1. Aquatic plants, which were scarce and occurred mainly along the banks, are listed in Table II.

Site 2

Mussel distribution and orientation.—The following species and their respective numbers of individuals were noted and plotted:

Lampsilis siliquoidea (Barnes)	206
Anodonta grandis Say	29
Anodontoides ferussacianus (Lea)	5
Lasmigona complanata (Barnes)	3
Total	243

TABLE II
AQUATIC PLANTS AT TWO SITES ON THE TURTLE RIVER

Aquatic Plants	Site 1	Site 2
Equisetaceae		
Equisetum arvense Linnaeus	X	X
Sparganiaceae		
Sparganium eurycarpum Engelmann	X	X
Potamogetonaceae		
Potamogeton sp.	X	
Alismaceae		
Alisma subcordatum Rafinesque	X	
Gramineae		
Leersia oryzoides (Linnaeus)	X	X
Cyperaceae		
Carex aquatilis Wahlenberg	X	X
Carex sp.		X
Ranunculaceae		
Ranunculus pennsylvanicus Linnaeus		X
Scrophulariaceae		
Limnosella aquatica Linnaeus	X	
Veronica americana (Rafinesque)	X	X
V. anagallis-aquatica Linnaeus	X	X
Compositae		
Bidens cernua Linnaeus	X	X
B. frondosa Linnaeus	X	X

Lampsilis siliquoidea and *Anodonta grandis* were the most common mussels as was also true for site 1. Fewer specimens of the other two species were present here than at site 1. Most individuals of *Lampsilis siliquoidea* were young as indicated by their small size.

A plot of all mussels on a rose diagram relating the orientation of their siphons to current direction shows similar results as for site 1. Most mussels were approximately perpendicular to the direction of stream flow (Figure 2).

Stream velocity.—Stream flow was higher here than at site 1

and consequently it was possible to measure velocity near the bottom; readings were taken at one to three points along each of the profiles. The isopleths of velocity shown in Figure 2 were based on measurements made at 0.2 ft. off the bottom; this approximates the level of the siphons of many of the mussels. Here, as at site 1, most mussels were concentrated where velocity was higher (Figure 2), and followed the apparent thalweg. Perhaps higher velocity, as well as an orientation approximately perpendicular to the current, offers better conditions for a mussel to take food, soluble mineral matter, and oxygen from the water.

Bottom.—Bottom configuration and water depth (few inches to four feet) is indicated by four cross-profiles. Four bottom samples were taken along each of the profiles to prepare a map of the distribution of sediment types (Figure 2). Bottom sediment varied from pebble gravel to sand and, as at site 1, coarser sediment occurred where velocity was greater. Most mussels occurred on a bottom of sandy pebble gravel, and, in fact, were nearly restricted to this type of sediment (Figure 2).

Chemical and other factors.—Measurements of dissolved oxygen, total alkalinity, and turbidity at site 2 were similar to those at site 1 (Table I). All alkalinity existed as the bicarbonate ion.

Banks were essentially open at this site with only two trees. Aquatic plants, which were scarce and occurred mainly along the banks, are listed in Table II.

SUMMARY

Detailed mapping of two sites on the Turtle River near Grand Forks, North Dakota suggests that stream velocity is the main environmental factor affecting local mussel distribution on a small river bed. Analysis of more than 200 individuals at each site indicates that mussels occur in greater concentrations where stream velocity is higher. It is also apparent that most mussels are oriented with their siphons approximately normal to the current. Presumably this position and orientation offers the best conditions for a mussel to extract food, soluble mineral matter, and oxygen from the water.

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DISTRIBUTION AND ECOLOGY OF MUSSELS IN THE RED RIVER VALLEY, GRAND FORKS TO DRAYTON[1]

Alan M. Cvancara
Department of Geology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

During the summer of 1965, 49 stations were sampled on the Red River and its seven tributaries between Grand Forks and Drayton: the Turtle, Forest and Park rivers in North Dakota, and the Red Lake, Snake, Middle and Tamarac rivers in Minnesota. In addition to collecting mussels and other mollusks, data were gathered on the following: pH, dissolved oxygen, free carbon dioxide, nitrate, nitrite, carbonate and total alkalinity, calcium and total hardness, chloride and iron content, water velocity, water temperature, bottom sediment, aquatic vegetation, and physical characteristics of the river.

Thirteen species of mussels, most from the Red and Red Lake rivers, were collected: *Fusconaia flava* (Rafinesque), *Amblema costata* Rafinesque, *Quadrula quadrula* Rafinesque, *Lasmigona compressa* (Lea), *L. costata* Rafinesque (not collected alive), *L. complanata* (Barnes), *Anodonta grandis* Say, *Anodontoides ferussacianus* (Lea), *Strophitus rugosus* (Swainson) (not collected alive), *Proptera alata* (Say), *Ligumia recta latissima* (Rafinesque), *Lampsilis siliquoidea* (Barnes), and *L. ventricosa* (Barnes). Two species, *Quadrula quadrula* and *Anodontoides ferussacianus,* are apparently new for the Red Lake River and one species, *Lasmigona compressa,* is new for the Forest River. More species of mussels occur in the Red River than in any of its tributaries, with the exception of the Red Lake River. Live species in the tributaries range from one in the Park River (*Anodontoides ferussacianus*) to five in the Forest River (*Lasmigona compressa, L. complanata, Anodonta grandis, Anodontoides ferussacianus,* and *Lampsilis siliquoidea*). Other tributaries have two to four of the species found in the Forest River. The Park River in North Dakota and the Tamarac, Middle, and Snake rivers in Minnesota are particularly poor in mussels.

About 530 chemical analyses were made in the field at 46 of the 49 stations, including 15 stations on the Red River and three to five stations on each of the tributaries. The North Dakota tributaries all show an increase in chloride content downstream, and high values (up to 2200 ppm) correlate with a downstream decrease or absence of mussels in two of the tributaries. Other factors, in addition to

[1]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.

chloride content, seem to limit the distribution of mussels in the third tributary. The Minnesota tributaries generally have low chloride values, and the Red River has relatively low and nearly constant values of chloride content.

The absence or decrease of mussels in parts of the studied area may be attributed to at least three possible causes: high chloride content, pollution, and long periods of no flow. High chloride values affect mussels in the lower reaches of the Turtle and Forest rivers. The apparent lack of mussels at two stations in Grand Forks is attributed to industrial or human sewage. The scarcity or lack of mussels in the lower reaches of the Tamarac, Middle, and Snake rivers may be the result of occasional long periods of no flow.

A PRELIMINARY STUDY OF SOME CALCIUM MINERAL EQUILIBRIA IN THE WATER-SEDIMENT SYSTEM OF DEVILS LAKE, NORTH DAKOTA

Edward Callender and Richard Armstrong

Departments of Geology[1] and Biology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Equilibria calculations using calculated activity coefficients for Devils Lake water (salinity 12.5 parts per thousand) and taking into account inorganic complexing of specific ions indicate that Devils Lake is supersaturated with respect to calcite, dolomite, and apatite at all temperatures. X-ray analysis showed the presence of these minerals in sediment samples from the water-sediment interface.

The analytical calcium, magnesium, carbonate, and phosphorus contents of these sediment samples show a definite variation throughout the sampling period from July, 1965 to February, 1966. Displacements in the X-ray peaks of calcite (i.e., a bimodal calcite peak) in the sediment from the water-sediment interface indicate substitution of smaller ions for calcium. Chemical analyses of carbonate fractions from several samples indicate that both iron and magnesium may be substituting for calcium in the calcite structure. This disordered calcite appeared to be the only carbonate mineral which varied significantly during most of the sampling period and is believed to be the only carbonate mineral whose presence is controlled solely by physicochemical and biochemical processes within the lake. This conclusion is strengthened by the apparent absence of disordered calcite in several glacial till samples adjacent to Devils Lake which were analyzed by X-ray diffraction. Also, the amount of disordered calcite in sediment grab samples and samples

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from several depths in a one-meter core is much less than that in the interface samples. The relative amount of apatite in the water-sediment samples also varied significantly during the sampling period. However, this mineral was not detected on X-ray diffractograms of glacial till samples, sediment grab samples, and core samples.

Large fluctuations in the chemistry and mineralogy of sediment from the water-sediment interface appear to reflect significant shifts in certain carbonate and phosphate mineral equilibria. These shifts are a result of physicochemical and biochemical processes which appear to be associated largely with primary productivity. A much more detailed study of the water and sediment chemistry, and the sediment mineralogy should clarify the nature and magnitude of these processes and their effect upon sediment mineralogy. Such a detailed study should also provide useful information concerning apparently rapid diagenetic processes affecting disordered calcite and apatite found in water-sediment interface samples from Devils Lake.

ZOOPLANKTON-PHYTOPLANKTON RELATIONSHIPS IN DEVILS LAKE, NORTH DAKOTA

David W. Anderson and Richard Armstrong[1]

Department of Biology

University of North Dakota. Grand Forks, North Dakota

INTRODUCTION

This paper describes quantitatively and qualitatively the seasonal variations in composition of the plankton of Devils Lake, North Dakota between June and January, 1965-66. Results presented here are compared with primary productivity measurements made during the same period (Armstrong, Anderson, Callender, 1966). An attempt has been made to trace the movement of energy through the various components of the lake-sediment system.

The first published record of work done on Devils Lake was a study by Pope (1909) which dealt with the physical and biological conditions affecting fish acclimation. Two years later, Brannon (1911) described the factors influencing the flora of the lake. Subsequently, Moberg (1918) studied the variation in the horizontal distribution of plankton within the lake. Young (1924) made a detailed limnological study of Devils Lake from 1911 through 1923, and Metcalf (1931) briefly studied the lake during a limnological survey of North Dakota lakes. Swenson and Colby (1955) studied the chemistry of Devils Lake and several other lakes in the Devils Lake basin.

[1]Present address: Institute of Ecology, University of California, Davis, Calif.

METHODS

Phytoplankton samples were collected every two weeks from each of ten stations during the ice-free months and monthly from stations 1, 2, 3 and 10 during the period of ice cover (Figure 1). Samples were collected with a three-liter Kemmerer water bottle at the surface, middle (1.5 m) and bottom (3.0 m). Water samples collected from each depth were drained into a large plastic bucket, mixed and a one-liter subsample was withdrawn for filtration. Time elapsed from collection to filtration was never greater than two hours. Each subsample was remixed, and a 200 ml aliquot withdrawn and

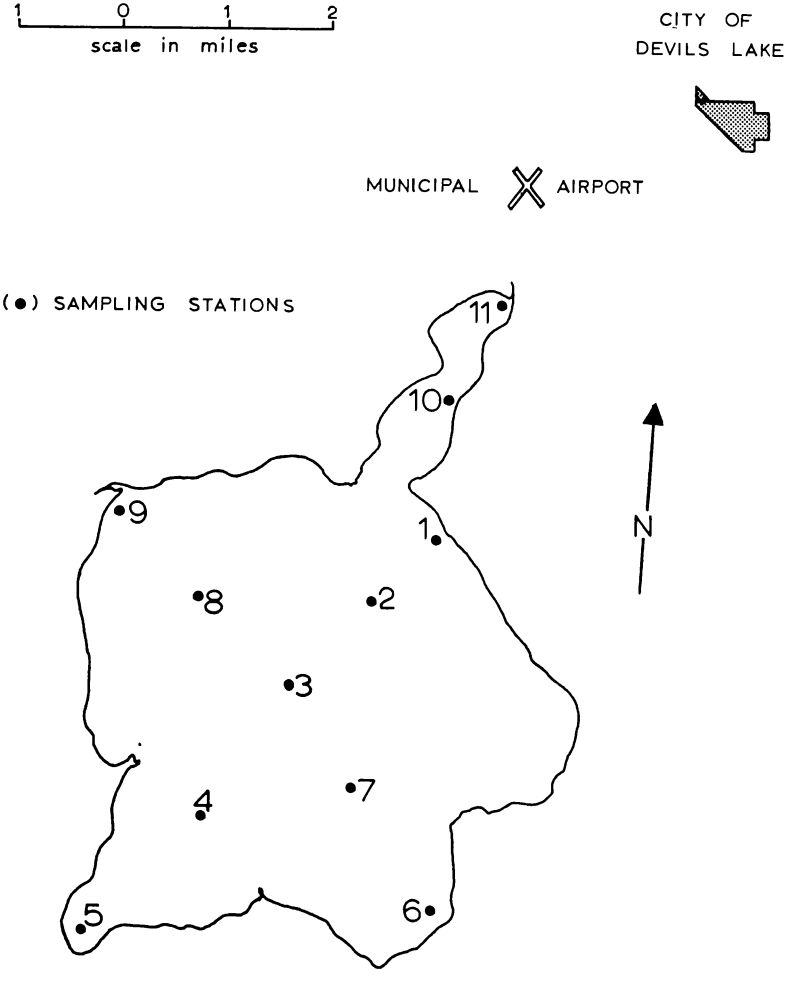

FIGURE 1—Location of sampling stations.

vacuum filtered through a RA Millipore filter (pore size, 1.2 microns). The filter was placed upon a large microscope slide and covered with six drops of immersion oil; it was then put in a dark slide cabinet to dry. After 24 hours at room temperature, the oil replaced water in the interstices of the filter making it transparent.

Two enumerations of the phytoplankton cells were made with a binocular microscope; the first at 450 X, the second at 2000 X magnification. Strip counts and identifications were made at 450 X magnification by focusing on the perimeter of the effective filtering area and traversing the filter at its widest diameter. Three such traverses were made for each filter and an average drawn from the total number determined from these three traverses. The portion of the total effective filtering area represented by each traverse was determined and the number of cells distributed over the entire effective filtering area was calculated. A calibrated ocular grid was used for the second count at a magnification of 2000 X. With the grid it was possible to enumerate the smaller phytoplankton cells not seen at 450 X. Twenty-five grids were counted and an average drawn. The area of one grid was determined and used to calculate the total number of cells distributed over the filtering area.

Zooplankton samples were collected utilizing the same techniques and stations as the phytoplankton collections. The nine liters of water which were taken at each station were filtered through a 250 micron screen that retained the large crustaceans but allowed the smaller rotifers and phytoplankton to pass through. Crustaceans collected from each station were rinsed off the screen into a small jar and preserved for counting and identification. Quantitative estimates of the microscopic rotifers were made by the method utilized for phytoplankton.

Phytoplankton cells or colonies were measured with a Whipple micrometer and their surface areas and cell volumes were computed using appropriate formulae. Cell volumes were converted to weight of carbon by assuming each cubic centimeter of volume contained 0.125 grams of carbon (Lewin, 1962). Values for standing crop of reduced C/m^2 were calculated by multiplying the concentration of C/m^3 by the mean depth of the lake.

Numbers of zooplankton/m^3 were converted to mg C/m^2 by weighing known numbers of dried specimens and assuming a carbon content of 45% of dry weight. The areal density of carbon in the sediments at the mud-water interface was measured directly by methods described elsewhere (Armstrong, Callender, Anderson, in preparation). Dissolved organic carbon was estimated from Kjeldahl determinatins of dissolved organic nitrogen using a C:N ratio of 8.07. Primary productivity and community respiration was measured by the oxygen light and dark bottle method. Details of the productivity and resipiration measurements are presented elsewhere (Armstrong, Anderson, Callender, 1966).

RESULTS

Figures 2 and 3 show the seasonal variations in the most important phytoplanktonic genera. *Cymatopleura, Gyrosigma, Chlorococcum, Polycystis, Selenastrum,* and *Centritractus* were found during the study but are not included in these figures. In spite of apparently high concentrations of available nutrients, the lake was not especially rich in terms of biomass and was distinctly poor in

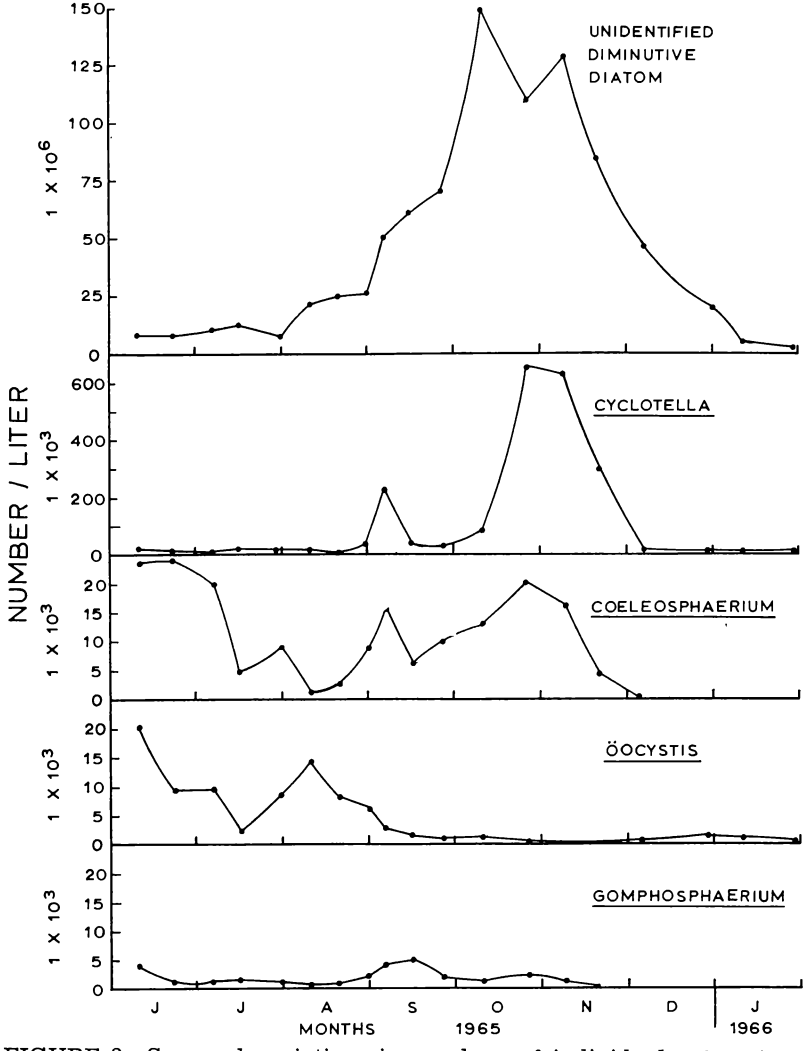

FIGURE 2—Seasonal variations in numbers of individuals of various genera of phytoplankters.

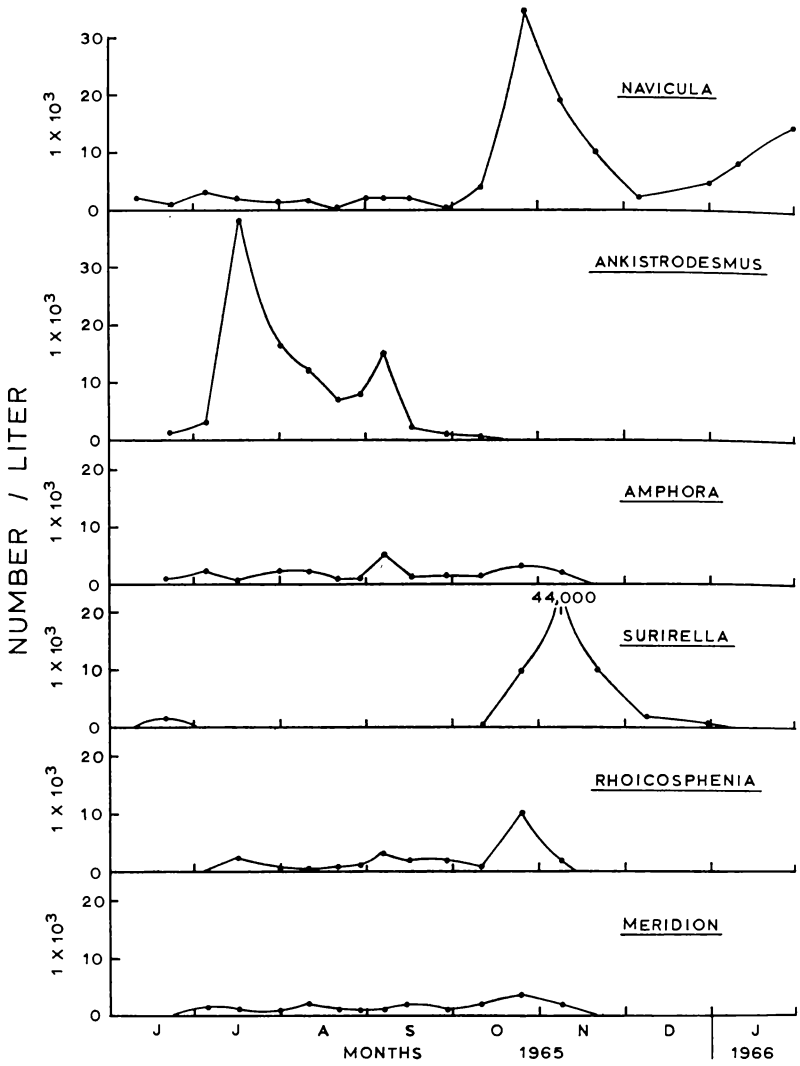

FIGURE 3—Seasonal variations in numbers of individuals of various genera of phytoplankters.

terms of biological diversity. The rather meager planktonic flora was dominated by a single diminutive diatom (*Cyclotella*, Figure 2). The importance of this ultraplankter was not diminished by converting cell numbers to cell volumes (100 u^3/cell) or cell surface area (120 u^2/cell) as suggested by Paasche (1960). It was determined

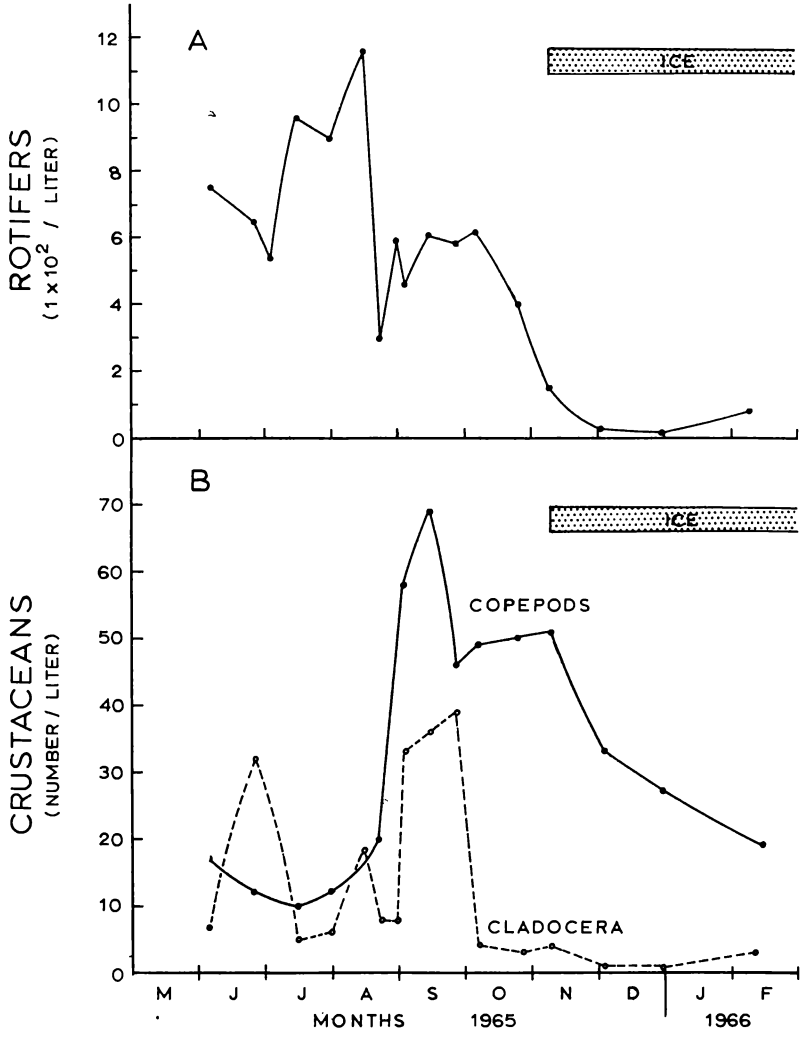

FIGURE 4—Seasonal variations in numbers of crustaceans and rotifers.

that this diminutive species consistently accounted for at least 95% of the phytoplankton biomass and consequently all calculations of the standing crop of phytoplankton carbon were based solely on this species.

Fluctuations in the zooplankton standing crop are shown in Figure 4. Rotifers were not identified to species as they appeared

distorted on the millipore filter preparations. There were three distinct periods of growth by the cladoceran, *Moina hutchinsoni* Brehm while the copepod, *Diaptomus sicilis* Forbes had only a single broad peak in the late summer and fall months. There were changes in the composition of both crustacean populations during the period of study as the relative contribution of immature forms to the total counts

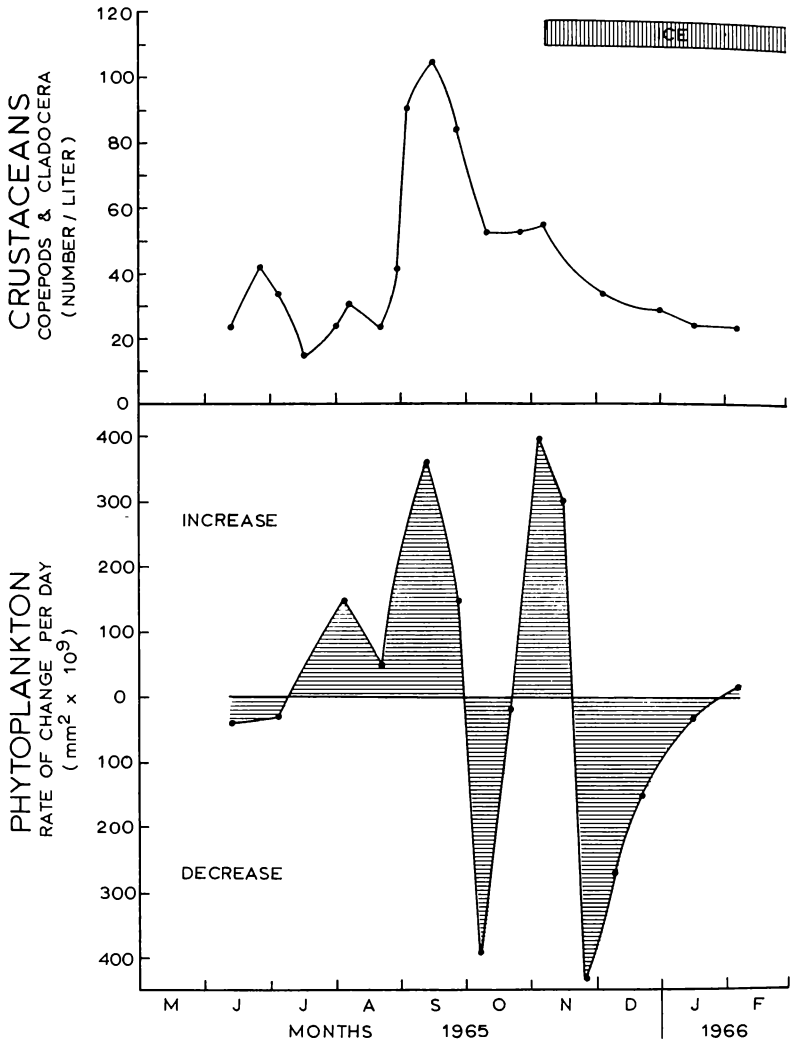

FIGURE 5—Rate of change of phytoplankton cell surface area and total crustacean populations.

fluctuated. Unfortunately these fluctuations were not studied quantitatively.

Figure 5 compares the rate of change of phytoplankton cell surface area (1.2 x cell volume) with crustacean populations (total individual copepods plus cladocerans per liter). Seasonal variation in standing crop of crustacean carbon was likewise computed in order to assess the effect of fluctuation amounts of carbon per individual. This curve had the same shape as the population curve and is not shown. Figure 5 indicates that growth of phytoplankton occurred when crustacean numbers were low but phytoplankton growth was accompanied by an increase in crustacean populations. As crustaceans reached their peak population density the phytoplankton population declined and the crustaceans followed. With reduced grazing pressure, the phytoplankters again increased in number until the lake froze over.

Table I shows the areal density of carbon in various components of the lake system at different times of the year. Table II presents the increments in these values during each sampling interval and contains, for comparison, data on the primary production and aerobic community respiration. It is apparent from Table I that the lake

TABLE I

AREAL DENSITY OF ORGANIC CARBON IN VARIOUS COMPONENTS OF THE LAKE SYSTEM.

Sampling Date	Sediments at the Mud-Water-Interface (mg C/m^2)	Soluble Organic Matter (mg C/m^2)	Particulate Organic Matter (mg C/m^2)	Phytoplankton (mg C/m^2)	Zooplankton (mg C/m^2)
6/9	68,000	114,100	21,400	330	1830
6/23	66,000	108,500	20,300	330	4220
7/4	63,000	116,500	16,100	370	4290
7/15	61,000	118,100	8,600	415	3750
7/31	62,600	115,900	12,000	330	3210
8/9	60,000	115,500	10,600	825	2750
8/21	68,000	116,100	12,400	950	2310
8/28	65,800	119,000	12,600	1,075	3450
9/5	65,500	128,300	13,500	2,065	6770
9/14	66,600	123,900	14,950	2,480	8150
9/26	63,600	121,800	17,700	2,890	7660
10/10	63,000	114,300	21,600	6,196	4260
10/24	60,400	122,000	30,600	4,445	4420
11/7	57,700	120,900	25,500	5,270	4700
12/5	64,400	117,900	14,350	1,860	2080
12/28	54,200	124,000	7,750	745	1560
2/8	48,400	85,600	4,600	330	1750

TABLE II
INCREMENTS IN AREAL DENSITY OF ORGANIC CARBON IN VARIOUS COMPONENTS OF THE LAKE SYSTEM DURING EACH SAMPLING INTERVAL.

Sampling Interval	Total Load of Organic Carbon (mg C/m^2)	Total Gross Photosynthesis (mg C/m^2)	Total Community Aerobic Respiration (mg C/m^2)	Phytoplankton (mg C/m^2)	Zooplankton (mg C/m^2)
6/9-6/23	- 8,700	10,150	23,400	0	+2390
6/23-7/4	+ 800	4,400	4,200	+ 40	+ 70
7/4-7/15	- 7,900	3,300	6,500	+ 45	- 540
7/15-7/31	+ 2,800	40,000	21,000	- 85	- 540
7/31-8/9	- 4,400	36,000	17,400	+ 495	- 460
8/9-8/21	+10,400	8,800	6,000	+ 125	- 440
8/21-8/28	+ 900	5,760	6,400	+ 125	+1140
8/28-9/5	+ 9,900	4,050	8,100	+ 990	+3320
9/5-9/14	-1,850	1,600	4,960	+ 415	+1380
9/14-9/26	- 2,350	840	3,000	+ 410	- 490
9/26-10/10	- 4,200	2,000	4,000	+3305	-3400
10/10-10/24	+14,100	3,800	11, 900	-1750	+ 160
10/24-11/7	- 8,900	8,000	8,400	- 825	+ 280
11/7-12/5	- 7,550	12,300	11,140	-3410	-2620
12/5-12/28	-10,600	0	36,700	-1115	- 520
12/28-2/8	-47,350	0		- 415	+ 190
6/9-2/8	-64,900	141,000	173,100	—	—

contained a large store of organic carbon in soluble form and a further large reserve in the sediments at the mud-water interface. Total particulate carbon in the water, while less by a power of ten than the soluble organic carbon, still greatly exceeded the organic carbon definitely assignable to any of the three dominant species.

A more coherent picture emerges when the results for the entire study period are examined. There were net losses of organic carbon from the sediments at the mud-water interface, from solution, and from the particulate component. The total net loss during the study period was 64,900 mg C/m^2. Total gross productivity during this time was 141,000 mg C/m^2 while total aerobic community respiration was 173,000 mg C/m^2 for a net deficit of 32,100 mg C/m^2. Respiration between 12-5-65 and 2-8-66 was calculated from the observed depletion of oxygen during this period, proper allowances being made for the reduction in volume due to ice formation. Approximately half of the organic carbon removed from the system can be attributed to aerobic respiration. This estimate of total aerobic metabolism is certainly low because it does not take into account aerobic respiration occurring at the mud-water interface. The estimate of total community respiration is also low because it does not include anaerobic respiration.

DISCUSSION

Even though the photosynthetic rate was rather high, the lake suffered a net loss of reduced carbon. This loss must certainly represent materials which had been stored in the sediments during the previous growing season. Approximately forty days of growth had passed before the study commenced and some of the missing carbon may have been reduced during this period. The average rate of production during the ice-free months was 800 mg C/m²/day. An average of twice this rate would be required in the total absence of respiration to make up the observed loss. With average respiration (760 mg C/m²/day) the required productivity would be unnaturally high (2360 mg C/m²/day). Therefore, the most acceptable conclusion would be that during 1965 the production of the lake was not high enough to support the catabolic processes taking place within it.

The attempt to trace energy transfers during short intervals was unsuccessful. The most carefully observed organisms never contained more than 5% of the total load of reduced carbon and usually contained much less. Because the store of soluble organic carbon was large compared to the amount being produced, small analytical and sampling errors in its determination possibly obscured the results. The same consideration applies to the determinations of carbon in the sediments at the mud-water interface and in the particulate fraction.

An interesting finding of the study was that the standing crop of herbivores (i.e. in dry weight of carbon) generally exceeded and often greatly exceeded the standing crop of phytoplankton. This situation undoubtedly places a strong grazing pressure on the phytoplankton and keeps the crustaceans in constant danger of exhausting their food supply. The virtual absence of secondary consumers in Devils Lake is a likely cause of the unstable zooplankton-phytoplankton ratio.

SUMMARY

1. Data are presented describing phytoplankton-zooplankton relationships at Devils Lake, North Dakota for a 10-month period in 1965-66. Energy present as standing crop in both trophic levels at different times of the year is compared with energy captured by photosynthesis.
2. The energy budget for the entire period showed a net loss of potential energy from the system even though total annual gross production was rather high (1720 kg C/ha).
3. Zooplankton grazing was the most important factor controlling the size of phytoplankton standing crop.

ACKNOWLEDGEMENTS

This work was supported in part by the North Dakota Water Resources Research Institute, with funds provided by the Department of Interior, Office of Water Resources Research, under P. L. 88-379.

The authors were supported by NDEA predoctoral fellowships. Dr. Rufus Thompson, University of Kansas, identified the phytoplankton.

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THE REVERSIBILITY OF HYDRATION OF POLYVINYL METHYL ETHER MALEIC ANHYDRIDE

Anatol M. Hnojewyj[1] *and Wasyl S. Hnojewyj*

College of Chemistry and Physics

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

ABSTRACT

Adsorption and desorption isotherms of H_2O vapor on polyvinyl methyl ether maleic anhydride and acid are determined gravimetrically by means of equilibration in a high vacuum system at 17° and 27°C. The isothemic data presented indicate a similar process at both temperatures: an adsorption of H_2O vapor up to 3 mg/100, (3%) is reached in the range of relative pressure equal to 0.62 and does not cause hydration of the anhydride into acid.

[1]Research Laboratory, Toni Co., St. Paul, Minnesota

Increase of equilibrium pressure of H_2O, however, initiates the chemical reaction resulting in the formation of an acid by consuming one molecule of H_2O per unit of polyvinyl methyl ether maleic anhydride. Further increase of equilibrium pressure caused the additional adsorption of three molecules of H_2O which were physically bonded as shown by calculation of differential heats of sorption. These water molecules were contained in the monolayer. The reversibility of hydration-dehydration at different conditions of equilibration is presented and discussed on the basis of isothermic data.

THE CONCENTRATION DEPENDENCE OF THE SPECIFIC ROTATION OF *TRIS* (ETHYLENEDIAMINE) COBALT(III) NITRATE IN AQUEOUS SOLUTION

J. A. Juhala and J. G. Brushmiller

Department of Chemistry

The University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Recent published data (1) indicate that the specific rotation of the complex ion (+)-*tris*(ethylenediamine)cobalt(III), $[Co(en)_3]^{+3}$, changes when the optically inactive anion associated with the optically active cation is changed. In the above mentioned report no attempt was made to determine the concentration dependence of the specific rotation nor was the effect of changes in the ionic strength of the medium on the specific rotation evaluated, although either of these effects might explain the unusual and unexpected results of the experiments. This paper reports on the results of an investigation into the solution behavior of the specific rotation of the (+)-$[Co(en)_3](NO_3)_3$.

The concentration dependence of the specific rotation was investigated over a wide range of concentrations and the effect of the ionic strength of the medium on the specific rotation was also measured. The data are examined using the specific rotation computed on both a volume and a weight basis and the results compared. The experiments show that at complex concentrations between 0.0008 M and 0.01 M, the specific rotation is independent of complex concentration. Furthermore, when the ionic strength of the medium is adjusted between $\mu = 0.01$ and $\mu = 5.0$ with $NaNO_3$, the specific rotation remains independent of ionic strength. However, different results are obtained when the ionic strength is adjusted with NaI. Between $\mu = 0.01$ and 0.5, the specific rotation retains its concentration independent nature. Above $\mu = 0.5$, significant changes are observed in the specific rotation which are a function of the ionic strength of the medium. The results above $\mu = 0.5$ in solutions containing NaI are explicable in terms of ion pair formation of the

type $[Co(en)_3]I^{+2}$, an explanation not original with the present authors. It appears that at high I^- to complex ratios, these ion pairs can be formed and their specific rotation is significantly different from those of the solvated cation. On the basis of these experiments it appears that the specific rotation of the (+)-$[Co(en)_3]^{+3}$ cation is independent of the nature of the optically inactive anion associated with it provided that the experiments are carried out on samples which are sufficiently dilute to preclude the formation of the ion pair.

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pH-DEPENDENT CITRATE INHIBITION OF RAT LIVER MICROSOMAL INORGANIC PYROPHOSPHATE-GLUCOSE PHOSPHOTRANSFERASE AND GLUCOSE 6-PHOSPHATASE[1]

David G. Lygre and Robert C. Nordlie

Department of Biochemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Rat liver microsomal glucose 6-phosphatase has been shown to possess inorganic pyrophasphate (PP_i)-glucose phosphotransferase activity (1). Citrate inhibition of these two activities has been studied with regard to 1) effect of pH and 2) inhibitor specificity. Inhibition was seen only below pH 7.0. Lineweaver-Burk plots show that citrate inhibition is competitive with respect to phosphate substrates and non-competitive with respect to glucose. Minimal K_m values for glucose 6-phosphate and PP_i were 0.56 mM at pH 6.5 and 1.1 mM at pH 6.1, respectively. K_i (citrate) values agreed closely for two activities over the entire pH range studied (pH 4.65-6.5), substantiating the common identity of both enzymic activities.

K_{PP_i} values are about ⅕ those reported earlier with citrate as buffer, strengthening the possibility of a metabolically important role for the phosphotransferase activity. Citrate inhibition of this activity may constitute a feedback mechanism for control of hepatic glucose utilization This mechanism may be especially important in diabetes, where decreased liver citrate levels could allow an elevation in the rate of glucose phosphorylation. Indeed, in diabetes it is seen that the phosphotransferase activity doubles (2), whereas

[1]Guy and Bertha Ireland Research Laboratory, School of Medicine. This work was supported in part by grants from the Hill Family Foundation and Research Grant AM 07141-01 from the National Institutes of Health.

the other major hepatic glucose phosphorylating system, glucokinase, nearly disappears (3). An activity-pH profile comparing phosphotransferase activity with that of the newly evaluated K_{PP_i} values with glucokinase on a "per g wet liver" basis shows the latter predominates above pH 7, while the former far exceeds glucokinase below pH 7. The difference is even more pronounced in diabetes. Intracellular pH values for liver are found predominantly between pH 6 and 7.1 (4).

Plots of pK_i or pK_m *vs.* pH consist of line segments with -1, 0, and +1 slopes as predicted by the rules formulated by Dixon (5). Inflection points on these plots suggest the involvement of the imidazolium group of histidine as part of the active site. However, further work is necessary to definitely implicate histidine as playing a role in enzyme-substrate complexing.

Of various citrate analogs and other chelating agents tested, only oxalate approached citrate in effectiveness as inhibitor. Since nonspecific chelation of metal ions can be ruled out by the ineffectiveness of other chelating agents with approximately the same affinities for metal ions as citrate, it appears likely that inhibition may be attributed to protein-bound metal at the active enzymic site, as has been seen to be the case in several other enzymic reactions.

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EVIDENCE FOR THE SEPARATE IDENTITY OF α-KETOISOVALERIC AND α-KETOISOCAPROIC ACID OXIDATIVE DECARBOXYLASES

D. J. Danner and J. L. Connelly

Department of Biochemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Evidence has been gained in support of the separate identity of alpha-ketoisovaleric (KIvD) and alpha-ketoisocaproic (KIcD) acid decarboxylases. Earlier reports adhere to the concept that these substrates are decarboxylated by a single enzyme complex (1). This idea arose from the fact that high levels of these compounds along with alpha-keto-beta-methylvaleric acid are found in the serum and urine of patients with maple syrup urine disease (2).

The enzyme source used in these studies was rat liver mito-

chondria, prepared by the method of Lardy and Wellman (3). Assays were conducted according to the method previously described by Gubler (4) wherein reduction of $K_3Fe(CN)_6$ is followed continuously with time at 420 mμ using a Cary 15 recording spectrophotometer.

Attempts to solubilize KIvD from the mitochondria were unsuccessful. Therefore, differentiation studies on mitochondria were undertaken *in situ*. The activities of alpha-ketoglutaric and pyruvic acid decarboxylases were used for comparison in these experiments since these enzymes have been shown to exhibit absolute specificity in purified preparations (5).

Stability studies using heat, repeated freeze and thaw, storage at various temperatures and hydrogen ion concentrations produced responses which were found to be characteristically different for the four decarboxylases. An activation was seen as a response to the various treatments in the case of all the enzymes. The degree and time of occurrence was the differentiating factor. The inevitable inactivation which followed, likewise varied with treatment for any one decarboxylase. This property was used to advantage in kinetic studies.

Kinetic studies added support for the idea of separate enzymes. Included in these studies were the determination of apparent Km values using Lineweaver-Burk (6) plots and pH optima of the four enzyme activities. The method described by Dixon and Webb (7) was used to test directly the activity of one enzyme on one or two substrates in the same system. If one enzyme is acting on two substrates the observed rate of reaction should equal the calculated rate. (Values used for Vmax were determined according to the Michaelis-Menton equation (8). Experimental runs were conducted using a variety of substrate combinations and concentrations all being below Vmax concentrations. In no case was any agreement found between the calculated and observed rates.

The interference of membrane permeability was ruled out on the following basis: mitrochondria heated to 35° for ten minutes to inactivate pyruvic acid decarboxylase activity were used to examine the ability of pyruvate to inhibit intact KIvD and KIcD. Similarly, treatment of mitochondria to pH 8 for three minutes to destroy KIcD enabled the examination of alpha-ketoisocaproic acid to inhibit KIvD. In both cases inhibition was observed, showing that the substrate can approach the active enzymes by penetration of the membrane.

The composit of these studies provides strong evidence for the existence of separate enzyme complexes acting to decarboxylate the various keto acids investigated.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF α-KETO*ISO*CAPROIC DECARBOXYLASE FROM BEEF LIVER

Joe A. Bowden and Jerald L. Connelly

Department of Biochemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Investigations using inorganic phosphate (P_i) extracts of ground beef liver have been used to demonstrate the separate identity of α-keto*iso*caproic (KIcD) and α-keto*iso*valeric (KIvD) decarboxylases. This finding is contrary to the current concepts that both acids are decarboxylated by a single enzyme complex (1). The importance of this observation lies in the fact that these acids along with α-keto-β-methylvaleric acid, are markedly elevated in the serum and urine of patients afflicted with maple syrup urine disease.

Beef liver ground, homogenized and subjected to subcellular fractionation demonstrated that KIcD and KIvD activities can be separated with the KIvD activity sedimenting in the mitochondrial fraction. Purification of KIcD was extended as follows: the preparation was centrifuged at 80,000 x g for 45 minutes, decanted and mixed with 0.1 volume of 2% protamine sulfate and centrifuged. The supernatant was brought to 55% saturation with $(NH_4)_2SO_4$ and the precipitate dissolved in 0.03 M P_i (pH 7.4) and cooled to -5° C. One and a quarter volumes of 95% ethanol (-40° C) was added with rapid stirring, the precipitate was extracted with 0.03 M P_i (pH 7.4). This green solution, which contained about 25% of the original activity, represented about a 70 fold purification. The activities were assayed continuously using a modification of the $K_3Fe(CN)_6$ method of Gubler (2) at 420 mμ, on a Cary 15 recording spectrophotometer.

Stability studies were carried out using the various partially purified fractions. KIcD (very labile) is most stable between pH 6.9 and 7.4 and below 20° C. The enzyme is unstable to dilution and is

activated by the addition of $(NH_4)_2SO_4$ and other salts to the assay mixture. Reduced glutathione protects the $(NH_4)_2SO_4$ fraction against denaturation at 10^{-3} molar concentrations but not the ethanol extract. An apparent K_m of 7×10^{-3} and apparent V_m of 6.24×10^{-5} moles/min/mg protein/3.5 ml were determined. Using the ethanol extract fraction a profile of pH *versus* activity revealed two optima in phosphate buffer; one at pH 5.6 and the other at pH 7.6. The KIcD appears to have been separated as an intact complex as judged by the assay of DPNH formation at 340 mμ.

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RAT HEART PYROPHOSPHATE PHOSPHOHYDROLASE[1]

James F. Soodsma[2] and Robert C. Nordlie

Department of Biochemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In view of the previous studies carried out on various inorganic pyrophosphatases (1, 2) and of recent findings which indicate that inorganic pyrophosphate may serve as a factor inhibiting calcification in the aorta (3), a characterization of the activity found in the rat heart was carried out.

Subcellular distribution studies indicated that the Mg^{2+}-stimulated activity, assayed at pH 7.3, was present predominantly in the soluble fraction of the heart homogenate. The Mg^{2+}-stimulated activity in the soluble fraction was studied in more detail. While there was hydrolytic activity over a wide pH range, the enzyme exhibited maximal activity between pH 7.0 and 7.3.

Inorganic pyrophosphatase activity was determined as a function of PP_i concentration in the presence of a limiting amount (1.0 mM) and relative excess (10 mM) of Mg^{2+}. In the former case maximum activity was observed at a Mg^{2+}/PP_i ratio of approximately 2.6. In

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[1]Guy and Bertha Ireland Research Laboratory, School of Medicine. This work was supported in part by a grant from the North Dakota Heart Association.

[2]Supported by a Student Summer Research Fellowship from the Council for Tobacco Research, U.S.A.

the presence of an excess of Mg^{2+} the K_m for PP_i was found to be $1.1x10^{-4}M$.

When Mg^{2+} concentration was varied in the presence of a fixed amount of PP_i, maximal activity was found to occur with a Mg^{2+}/PP_i ratio between 2:1 and 3:1. Under otherwise identical conditions, 5.3 mM Co^{2+}, Fe^{2+}, Mn^{2+}, Ba^{2+}, Hg^{2+}, or Ca^{2+} did not replace Mg^{2+} as activating cation.

Because of possible physiological significance, as related to calcification, Ca^{2+} inhibition of rat heart inorganic pyrophosphatase was studied in more detail. A Ca^{2+} concentration of $6.0x10^{-5}M$ produced fifty percent inhibition in the presence of $3.0x10^{-3}M$ Mg^{2+}, which corresponds to a Ca^{2+}/Mg^{2+} ratio of 0.02. Subsequently the reversal of the Ca^{2+} inhibition by various compounds was attempted. Increased PP_i concentration in the presence of fixed Ca^{2+} and Mg^{2+} concentrations produced increasingly greater inhibition of the activity. Relatively low concentrations of EDTA partially or completely reversed Ca^{2+} inhibition of the system. Higher concentrations of EDTA inhibited the activity in both the absence and presence of Ca^{2+}. Increased Mg^{2+} concentrations partially reversed the Ca^{2+} inhibition. Citrate produced progressive inhibition of activity at or above 1.0 mM.

The results of a substrate specificity experiment indicate that PP_i is hydrolyzed at a much greater rate than any other compound tested.

Of a number of different hormone states, alloxan diabetes alone produced a statistically significant decreased activity.

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A STUDY OF TOLERANCE IN THE MOUSE TO SODIUM PENTOBARBITAL AND ITS THIO ANALOG THIOPENTAL[1]

L. P. Bratt and B. De Boer

Department of Physiology and Pharmacology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Experimental animals and man have been shown to develop tolerance and cross tolerance to barbiturates. In this study comparable hypnotic and "inducer" doses were determined for pento-

[1]Aided by funds from UND Institutional Grant.

barbital and its thio analog, thiopental. The rate of production, degree, and duration of tolerance and cross tolerance developed by these two barbiturate compounds were studied using male mice as the experimental animal.

Comparable hypnotic doses were found to be 80 mg/kg of thiopental and 60 mg/kg of pentobarbital. An effective "inducer" dose was found to be 15 mg/kg for both drugs. After six daily "inducer" injections, all animal groups showed tolerance or cross tolerance, as indicated by a reduction in sleeping time following administration of hypnotic doses of the barbiturates. Intermediate degrees of tolerance were noted in most groups receiving several daily inducer doses. With cessation of daily inducer injections, tolerance decreased and sleeping times returned toward normal levels within one week. Although thiopental appeared to be slightly more effective than pentobarbital in inducing tolerance and cross tolerance the difference was not statistically significant.

CROSS TOLERANCE BETWEEN PENTOBARBITAL AND OTHER BARBITURATES IN MICE[1]

Katharine O. De Boer, Barbara Cushing and B. De Boer
Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The average sleeping time of young adult female mice given pentobarbital 60 mg/kg intraperitoneally was found to be 70 minutes. The duration of sleep was recorded as the period of loss of the righting reflex. Groups of animals were given intraperitoneal injections of 30 mg/kg of amobarbital, barbital, hexobarbital, methitural, pentoberabital, secobarbital, talbutal or thiopental for 6 consecutive days. The seventh day pentobarbital 60 mg/kg was injected and sleeping time determined.

The sleeping time of the control group was the same as the previous week. Experimental groups which had been given daily "inducer" doses of barbiturate slept 21 to 70% of the original sleeping time. Pentobarbital was the least effective and thiopental the most effective tolerance-inducing agent in this series. Within 48 hours after the second sleeping time, the animals were injected with pentobarbital 60 mg/kg and sacrificed 30 minutes later. Liver weights of control and experimental animals were essentially the same. Studies of liver homogenates and blood barbiturate levels did not reveal any significant differences between control and experimental animals. Brain barbiturate analyses suggested that pentobarbital levels were higher in control than in experimental groups.

[1]Aided by a grant from Abbott Laboratories, Chicago, Illinois and UND Institutional Grant.

JUGLONE TOXICITY IN DOGS[1]

J. P. Ries 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 chemical substance originally isolated from the walnut tree, has not been investigated to any great extent. Preliminary reports revealed that juglone has a depressant effect on mice, rats and rabbits when administered intraperitoneally and is lethal in large doses.

This study was performed to determine the relative toxicity of juglone to dogs when administered intravenously. The fourteen adult mongrel dogs used were subdivided into three groups. Two groups of five each received 3 mg/kg and 5 mg/kg of juglone respectively in isotonic glucose. The remaining four animals served as controls, receiving corresponding volumes of 5% glucose only. The animals were anesthetized with Na Pentobarbital (32.5 mg/kg) and infused with the juglone solution through the left femoral vein. Four hours after the infusion was completed the animals were sacrificed and tissues of heart, lung, kidney and liver obtained for pathological examination.

The pathological reports indicate that juglone is toxic to the lungs and kidneys of dogs at both doses employed. Histological examination reveals a moderate to marked atelectasis of the lung alveoli and early degeneration of the proximal tubules of the kidneys. Sections of the heart and liver of the experimental animals were unchanged. The tissues of the control groups appeared normal in all instances.

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[1]Guy and Bertha Ireland Research Laboratory, School of Medicine
Supported in part by PHS Grant HE 09652-01

PRELIMINARY DATA ON THE CRYSTAL STRUCTURE OF GLUTARONITRILE COPPER(I) CHLORIDE

J. O. Martin and H. L. Haight

Department of Chemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In recent years interest in the aliphatic nitrile complexes of transition metals has increased. Some of these are believed to be intermediates in catalytic polymerizations and in the process of dyeing polyacrylonitrile fibers.

Recently the preparations and stoichiometries of several nitrile

complexes of cuprous chloride have been reported (1). It was found that with dinitriles, such as malononitrile, succinonitrile, glutaronitrile and adiponitrile, complexes of the type LCuX (X=Cl or Br) (L=nitrile ligand) were obtained with the exception that the adiponitrile CuBr complex contained two moles of CuBr per mole of nitrile.

Previous work (2) on the succinonitrile, glutaronitrile and adiponitrile cuprous nitrate complexes has shown these to be of the type L_2CuNO_3 (L=nitrile ligand). X-ray structure studies on these compounds indicate that the dinitriles act as bridging ligands between two copper ions to form complex polymeric cations. This arrangement requires the 2:1 stoichiometry observed in the cuprous nitrate complexes. Since the cuperous chloride complexes are LCuCl rather than L_2CuCl, it is of interest to determine their structures to see how the two types of complexes differ.

Preliminary single crystal X-ray data indicate that the glutaronitrile-cuprous chloride complex crystallizes in the tetragonal system with unit cell constants a = 8.54 A and c = 20.48 A. Weissenberg photographs show systematic absences when h + k + l is not equal to 2n for hkl data and when l is not equal to 2n for okl data showing the unit cell to be bodycentered and limiting the possible space groups to I4cm, $I\bar{4}c2$, or I4/mcm. The measured density of the complex is 1.77 g/cc indicating that there are eight $(C_3H_6N_2)CuCl$ units per unit cell.

Intensity measurements have been made using a diffractometer equipped with a Eulerian Cradle and a scintillation counter. After Lorentz, polarization, and absorption corrections were made, two and three dimensional Patterson maps were drawn. From these the positions of copper and chlorine atoms were deduced and subsequently verified by preliminary structure factor calculations.

In the proposed structure there are two kinds of copper atoms. Four of the eight copper atoms are bound in $CuCl_2^-$ anions while the other four are in a $[Cu(nitrile)_2]_n^{n+}$ cation with the nitrogen atoms located tetrahedrally about the copper. The nitrile molecules appear to bridge two copper atoms in an infinite two dimensional network very similar to that found for the glutaronitrile cuprous nitrate complex.

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THE EFFECT OF TWO GROWTH REGULATORS ON THE INITIAL PHOTOSYNTHETIC REATIONS IN ISOLATED SPINACH CHLOROPLASTS

Murray E. Duysen
Department of Botany
North Dakota State University of Agriculture and Applied Science,
Fargo, North Dakota

ABSTRACT

The effect of gibberellic acid (GA_3) and (2-chloroethyl) trimethylammonium chloride (CCC) on the photosynthetic mechanism of isolated spinach chloroplasts was determined. Spinach, *Spinacia oleraceae*, L. 'Bloomsdale', was grown in the greenhouse in washed river sand. Single dosages of GA_3 and CCC were applied separately to 7-week old plants with the nutrient solution at concentrations of 10^{-7}, 10^{-6}, 10^{-5}M GA_3, and 10^{-4}, 10^{-3}, 10^{-2}M CCC. In addition, growth regulators were applied together at dosages of 10^{-6}M GA_3 + 10^{-3}M CCC, 10^{-6}M GA_3 + 10^{-2}M CCC, 10^{-5}M GA_3 +10^{-3}M CCC, and 10^{-5}M GA_3 + 10^{-2}M CCC. Hypotonically-broken chloroplasts received most of the GA_3 and CCC dosages given to whole plants (10^{-7}, 10^{-6}, 10^{-5}M GA_3, and 10^{-4}, 10^{-3}M CCC). The activity of isolated chloroplasts was determined by the synthesis of adenosine triphosphate (ATP), production of oxygen, and reduction of nicotinamide-adenine dinucleotide phosphate (NADP).

Photophosphorylation was more sensitive to growth regulator treatment than either photoevolution of oxygen or photoreduction of NADP. GA_3 caused an apparent increase in ATP synthesis when applied through the root system or directly to chloroplasts. High concentrations of CCC and most of the combination treatments increased the production of ATP. Photoevolution of oxygen was slightly stimulated by GA_3 at low (direct chloroplast treatment) and intermediate dosages (root system application). Oxygen evolution was decreased by CCC and was either not influenced or was decreased by the combination treatments. Photoreduction of NADP was slightly increased by (1) GA_3 applied through the root system, intermediate dosage, (2) GA_3 applied directly to chloroplasts, low dosage, and (3) the GA_3-CCC combination treatments. NADP reduction was slightly decreased by CCC.

Since it is known that chlorophyll system II regulates the photoproduction of oxygen, it is assumed that the growth regulators, particularly CCC, interferes with the transport of electrons from the first photoreaction. The increased ATP synthesis can be generally ascribed to the stimulation of cyclic photophosphorylation since it usually occurred without a concomitant increase in the production of oxygen and $NADPH_2$. In some treatments both cyclic and non-cyclic photophosphorylation could account for the increased synthesis of ATP, oxygen, and $NADPH_2$.

PREPARATION AND PURIFICATION OF HUMIC ACID AND ITS CONDUCTIVITY AS A FUNCTION OF ALKALINE-ACIDIC NEUTRALIZATION IN AQUEOUS MEDIUM[1]

Stephen O. Johnson[2] *and Wasyl S. Hnojewyj*

College of Chemistry and Physics

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

INTRODUCTION

Humic acid (H-Ac) has attracted the attention and interest of several researchers and research institutions in North Dakota because of possible economic importance. Youngs and Frost (1) give the definition of humic acid and an account of the history of and some of the industrial research on this subject. Hnojewyj (2), and Bakken and Hnojewyj (3), in this laboratory, reported on some basic research concerned with the adsorptivity and electroconductivity of H-humate. The conclusion of the last investigators was that humic acid is a large molecule(s) containing various functional groups which cause the appearance of characteristic data (slopes in sorptions and conductivities). Therefore, for the purpose of furthering the investigation of sorption and conductivity, it was desirable to prepare a specimen of humic acid with maximum surface, and to compare the results obtained from such a sample with those already reported.

This paper reports two methods of preparing a humic acid of high surface area, and the results of conductometric titrations utilizing samples prepared as described herein.

PREPARATION-PURIFICATION OF HUMIC ACID

A granular, black-brown sample of H-humate obtained from the Baroid Division of National Lead Company, Houston, Texas, was used. This commercial substance, upon being mixed with water, forms a visible suspension which slightly colors the water, then disaggregates and approaches a colloidal state of dark brown color upon addition of base. Subsequent acidification causes the formation of a suspension which appears to be of finer consistency than that of the original substance. These traits of H-humate were used to prepare samples of H-Ac with highly developed surface area by the following methods:

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

[2]Senior in Physics, North Dakota State University.

a) *Method of alkaline treatment followed by dialysis and lyophilization: Product, humic acid-(denoted)* $H\text{-}Ac_{Na}$[3]

Approximately 10 g of H-humate was suspended in 200-300 ml of distilled water. The pH of the suspension, as measured by a Beckman Zeromatic pH meter, stabilized around 3.40 after an hour of stirring. Then 0.10 N NaOH was added in one ml quantities with time allowance for equilibrium as determined by the pH meter. After 100 ml of NaOH had been added in small quantities, large quantities of NaOH and water were added until a total of 500 ml and pH of 10.8 was reached. At this point, the original suspension turned into a dark brown colloid. A few suspended particles in this colloid, which seem to be of mineral nature, were removed by suction-filtration through glass wool. The filtrate, containing excess NaOH and Na-humate in a colloidal state, was subjected to dialysis.

The dialysis consisted of allowing the excess NaOH to diffuse from the colloid into distilled water through a membrane. The filtrate-colloid sodium humates with excess NaOH was poured into a dialyzing tube (No. S-25275-KC, Sargent and Co.), closed at both ends and immersed in a bath containing 10 leters of stirred distilled water. The tube permitted the passage of NaOH, but not that of the colloidal humate molecules. This could be seen from the fact that the bath water did not become colored. After equilibrium had been reached, which was ascertained by periodic measurement of the pH outside of the dialysis tube, the bath water was discarded and replaced by fresh distilled water. In the first water-bath, the distilled water initially had a pH of 6.7, and thirteen hours later equilibrium was reached at a pH of 10.20. The water was changed and another equilibrium was reached as still more excess NaOH diffused through the membrane into the bath. The equilibrium pH steadily decreased until after 19 days, during which 24 changes were made, an equilibrium pH of 6.96 was obtained, and the dialysis was assumed to be complete. The $H\text{-}Ac_{Na}$ inside the tube was found to have a surprising value of pH = 5.50, which is less than the pH to which the H_2O had risen. This pH of $H\text{-}Ac_{Na}$ seems to be the final equilibrium reached under these conditions, as shown in Figure 1.

The dialyzate was diluted to 0.50 - 1.0% and then lyophilized for the purpose of obtaining a highly developed surface of H-Ac. This final step consisted of filling round glass flasks one-third full of the colloid-dispersion and placing them in acetone-dry ice to freeze. The flasks were then placed under a vacuum of about 0.50 - 0.01 mm to sublime the water, leaving the dry humic acid as the final product with a greatly increased surface. This dark-yellow powdery substance was used for further investigations, and is denoted $H\text{-}Ac_{Na}$.

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[3] $H\text{-}Ac_{Na}$ and $H\text{-}Ac_{H}$ are abbreviations for humic acid and Na and H indicate the method of preparation.

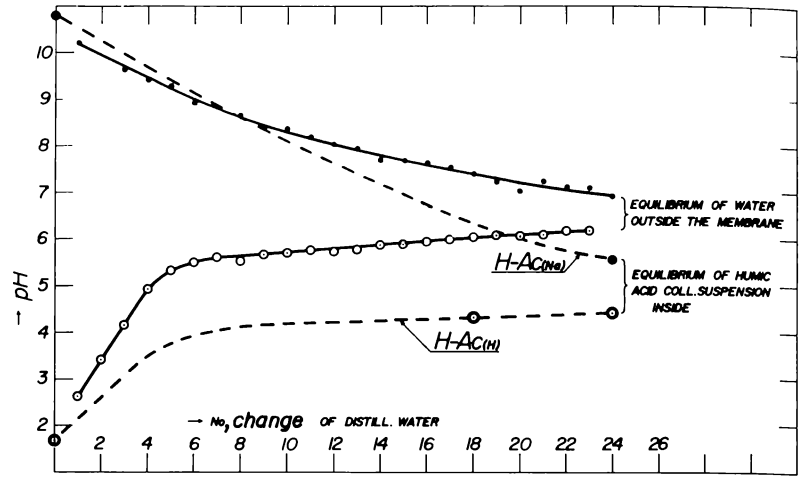

FIGURE 1—Dialysis of humic acids against distilled water.

b) *Method of alkaline and acidic treatment followed by lyophilization: Product humic acid-(denoted)* $H\text{-}Ac_{II}$

This is a variation of the method described in full above. Again about 10 g of H-humate was suspended in distilled water and 0.10 N NaOH was added to pH 9.0. It was then filtered through glass wool and filter paper with suction to remove a few large particles. The filtrate, a dark-brown colloid, was then slowly neutralized with 0.10 N HCl during intensive stirring. Then HCl was added in an excess until a pH value of 1.7 was reached, and diluted with water to a final volume of about 2 liters. Dialysis followed as before, where the progress of diffusion of excess HCl into the bath was controlled using a pH meter. The equilibrium of pH of the final change of water (the 24th change) was 6.20, while the colloid-suspension inside the tube had a pH = 4.40, as shown in Figure 1. Similar lyophilization of this dialyzate produced humic acid, denoted $H\text{-}Ac_{II}$. It appeared similar to $H\text{-}Ac_{Na}$ but it was less finely divided and more dense, exhibiting a tendency toward a semicrystalline aggregate state.[4]

CONDUCTIVITY OF LYOPHILIZED HUMIC ACID

Conductivities, as function of alkaline and acidic neutralization, were measured on lyophilized species of humic acid prepared by both of the above methods described.

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[4]A different method of dialysis, used in this laboratory on different substances, was a great time-saver. A continuous stream of distilled water flowed outside the tube while N_2 bubbled through the tube for agitation on the inside. The tube was suspended in a vertical pyrex cylinder 10 cm in diameter and 15 cm high.

A conductivity cell (platinized platinum electrodes) with auxiliaries, and method of measurements as described earlier (3), were used. However, an oscilloscope, used as an indicator, was replaced by a potentiometer.

For each run, a sample of about 115 mg was suspended-disolved in 600 ml of distilled water previously stabilized at 30°. After equilibration the electro-resistance was measured in the course of forward titration done by adding in small portions (0.20 ml) 0.04825 N NaOH until a final pH of 10.0 was reached. The back titration followed, adding 0.04845 N HCl in the same manner. For every addition of titrant, a time of 2 - 5 min. was provided for equilibration.

The resistances in ohms vs volumes in ml of titrant were plotted on a large scale. Data of these plots were used for recalculations of the conductivity in ohm^{-1} = MHO vs amount of titrant in mmoles/g of dry humic acid, the moisture content of which was determined previously. These data were plotted as shown in Figures 2 and 3.

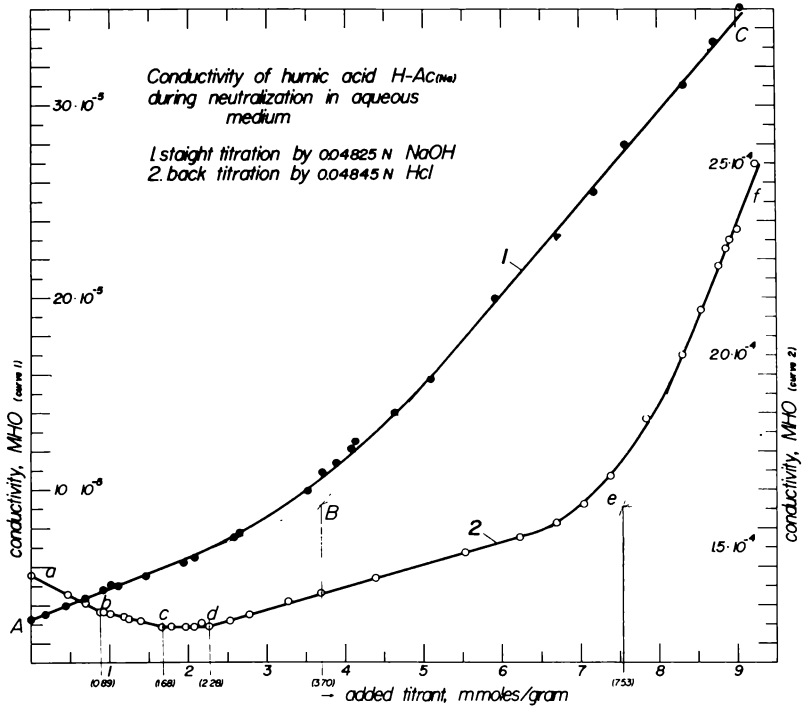

FIGURE 2—Conductivity of humic acid - $H\text{-}Ac_{Na}$. Curve 1 represents the straight titration by 0.04825 N NaOH and curve 2 represents the back titration by 0.04845 N HCl.

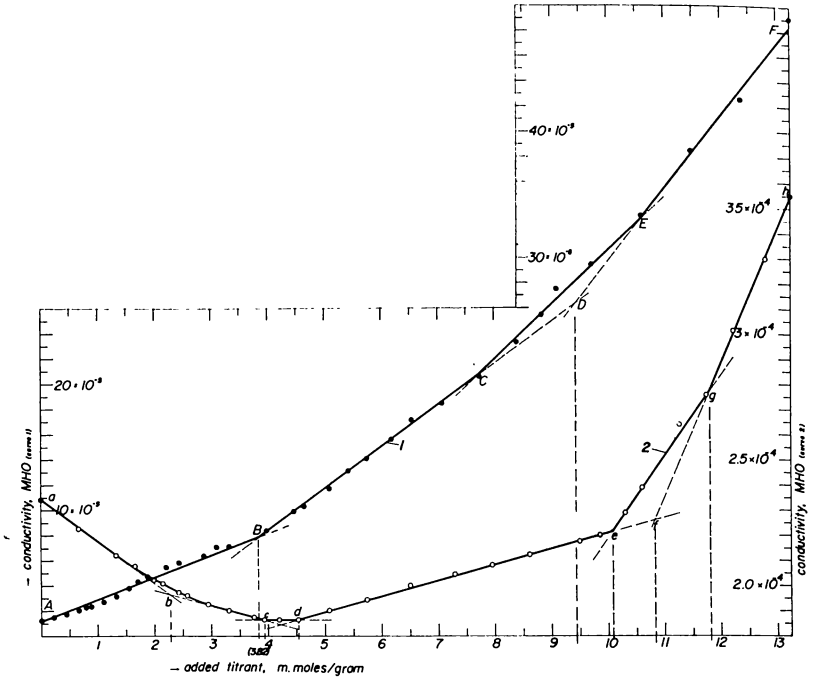

FIGURE 3—Conductivity of humic acid - H-Ac$_{II}$. Curve 1 represents the straight titration by 0.04825 N NaOH and curve 2 represents the back titration by 0.04845 N HCl.

RESULTS AND DISCUSSION

a) *Preparation-Purification of Humic Acid*

In the first method of preparation (H-humate treated with NaOH followed by dialysis) is established the fact, that the Na-humate inside of dialyzing tube was regenerated to the acid form at pH = 5.50 (see Figure 1).

This lead to conclusion that the cations which had bonded onto acid sites of the H-Ac molecule(s), and H^+ ions from H_2O exchanged places, regenerating the acid, thus freeing Na^+ and OH^- ions to diffuse out through the membrane and to raise the pH outside the dialysing tube. This means that the colloid in the acid form seems to be the most stable form of humic acid.

It was strongly suggested (2) that humic acid may be the only organic substance present in lignite: It could be in free acid or salt-humate forms, which are mixed with a variety of minerals of Fe, Al and Si origin. These would complicate a preparation of chemically pure humic acid or its salts, the importance of which is obvious for structural studies.

Consequently, although the preparation and purification presented here gives a product that is characterized by having a very large surface area, the purity of the sample is limited in the following respects.

1. H-humate, which is treated by NaOH in the 1st method, gives Na-humate in true collidal form. When diluted with water, it may be considered as molecularly disaggregated. This is later regenerated to acid form by dialysis, which still is of finely distributed consistence.

The presence of some cations of mineral origin (especially Fe^{3+}) may cause hydroxide formations, which may not be separated by dialysis. Presence of such hydroxides as impurities in H-Ac_{Na} may affect true data of the investigations (sorptivity of H_2O, conductivity).

It should be mentioned that the ash of H-Ac_{Na} found to be 7.0% on dry substance, which showed definite lowering in comparison with that of H-humate. The color of ash is red (like "scoria") indicating the presence of Fe_2O_3.

2. In the 2nd method of preparation, a treatment for H-humate by NaOH (for disaggregation) was followed by addition of an excess of HCl, which supposedly completely regenerated humic acid and destroyed hydroxides through formation of chlorides of the cations present. Furthermore, HCl may block all the basic functionalities of humic acid, especially the amino groups, as it was oberved for the natural proteins (5). Therefore, H-Ac_H in water should exhibit a lower pH than H-Ac_{Na}, as was readily observed and demonstrated in Figure 1. Naturally, the behavior (sorptivity, conductivity of H-Ac_H) may be somewhat different.

Again, the ash content in this sample (H-Ac_H) found to be 3.9% on dry substance, that is notably lower indicating high purity. It means also that the chlorides of cations present are more removable through a membrane by dialysis.

b) *Conductivity of H-Ac_{Na} in aqueous medium*

In the course of a straight titration by NaOH an increase of conductivity was observed. This occurs in two stages, represented by two definite slopes, as shown in Figure 2, curve 1. The slope AB represents a real neutralization of humic acid indicating a value of 3.70 mmoles/g of dry humic acid, while the slope BC appears due to excess of NaOH. It seems that excess of NaOH basically contributes to conductivity and is involved in breaking of cross bonds in humic acid, disaggregating its molecule(s).

The back-titration with HCl was usually followed by a delay of time in starting conductivity, which resulted in a shift of equilibrium due in part to sorption of CO_2 from air. Curve 2 in Figure 2 represents a reproducible and typical back-titration indicating at least five stages characterized by different slopes. The initial three

slopes "ab", "bc" and "cd" seem to result from neutralization c excess NaOH as well as interaction with hyroxides mentioned i the methods of preparation. Slope "de" may be considered as inter action of Na-humate with HCl. Its value is estimated as equal to 5.2 mmoles of HCl per g of dry humic acid. Slope "ef" appears as a result of HCl in excess, which is characterized by a final value of pH about 2-3.

It should be mentioned that the difference in values for neutralization (3.70 mmoles/g for straight titration by NaOH and a 5.25 mmoles/g for back titration by HCl) is not a suprise because of the presence of NaCl in the last one. Note that the ratio of the two titrations is: (straight/back) = 3.70/5.25 = approx. 0.70.

c) *Conductivity of H-Ac$_{II}$ in aqueous medium*

In Figure 3, curve 1, is shown the conductivity during the course of a straight titration which contains four slopes. Slope AB indicates the actual neutralization of humic acid by NaOH, which has a value of 3.82 m moles/g, slightly higher than for H-Ac$_{Na}$, because of its higher purity (lower content of ash).

In the course of a back titration with HCl, analogous to the previous one, the slope "de" should be referred to as an interaction of Na-humate with HCl. Its value is estimated to be equal to 5.40 mmoles/g, also somewhat higher than for H-Ac$_{Na}$. The ratio of the two titrations, (straight/back) = 3.82/5.40 = approx. 0.7, the same as was for H-Ac$_{Na}$.

CONCLUSION

Comparing the shapes of conductivity curves obtained on both lyophilized samples, their similarity is evident even though additional slopes can be distinguished in the forward and back titration of H Ac$_{II}$ runs. From the small differences between the numbers of millimoles reacting during both straight titrations, it can be seen that the two methods of prepartion affect the data very little. The differences can be considered essentially as experimental errors and these data can be averaged: 3.76 mmoles of NaOH/g react during straight titration and 5.32 mmoles of HCl/g react during back titration. Hence H-Ac is practically not altered by these methods of preparation and can be analytically recovered completely, even in the presence of the foreign cations as impurities.

If these results are compared with a previous investigation of H-humate (not lyophilized (3)), they demonstrate an advantage in quantitative determination due to the highly developed surface, resulting in the elimination of imperfection of equilibrium caused by high aggregation in the original H-humate.

SUMMARY

1. A method for the prepartion of humic acid from H-humate is presented. This method includes the following operational steps:

disaggregation in aqueous medium by treatment with NaOH (followed by HCl treatment as a variation), filtration, regeneration by dialytic exchange through a membrane against distilled water to constant pH, and lyophilization from a 0.5 - 1.0% aqueous medium. 2. Conductivity data concerning the titration of humic acid, prepared and purified by the above mentioned method, are presented.

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