

PROCEEDINGS
of the
NORTH DAKOTA
ACADEMY OF SCIENCE

Founded December, 1908

VOLUME XIV

1960

OFFICERS

President - - - - - Vera Facey
President-Elect - - - J. Frank Cassel
Secretary-Treasurer - - Ben G. Gustafson
Historian - - - - - George A. Abbott

Additional Members of Executive Committee:
Vernon Youngs
Harold Klosterman, ex-officio
H. E. Murphy

PUBLICATION COMMITTEE

Warren C. Whitman (*Chairman*)
G. A. Abbott
Ernest D. Coon
Ralph E. Dunbar
Ben G. Gustafson

*Published jointly by the University of North Dakota
and the North Dakota Agricultural College
Sponsored also by the Jamestown College*

GRAND FORKS, NORTH DAKOTA
July, 1960

TABLE OF CONTENTS

STUDENT PAPER SECTION

The Influence of Temperature on the Flight Activities of the Ant <i>Formica</i> Montana. <i>Myrna C. Anderson and Paul B. Kammowski</i>	7
Study of the Inheritance of Several Characteristics of Wheat <i>Curtis A. Hemstad</i>	7
Radio Transmission at VHF and UHF Frequencies <i>Michael B. Haverty</i>	17
Tills of Kidder County, North Dakota <i>Lee Clayton</i>	25
Modification of Morphine Hyperglycemia by Iproniazid and Reserpine <i>Sara MacDonald and B. DeBoer</i>	33
Water Surface Profiles <i>Dennis F. Meyer</i>	37
Synchronous Division of <i>Tetrahymena Pyriformis</i> in a Biphasic Medium <i>Gail L. Schuster and John W. Vennes</i>	43

PROFESSIONAL PAPER SECTION

Converting Normal Inbred Lines of Corn to the Cytoplasmic Male-Sterile Condition. <i>William Wiidakas</i>	48
Restoration of Male-Fertility to Cytoplasmic Male-Sterile Corn <i>R. E. Teschendorf, W. Wiidakas, and P. C. Sandal</i>	50
Organic Chemical Microscopy. Part X. A Review and Summary <i>Ralph E. Dunbar</i>	53
The Acetylation of Hydrocarbons With Ketene <i>R. E. Dunbar and Russell T. Arndts</i>	57
Freeliving Protozoa <i>Elizabeth Caldwell</i>	60
Development of the Secondary Bronchi in the Lungs of the House Sparrow, <i>Passer domesticus</i> (Linnaeus). <i>John W. Delphia</i>	65
Effects of Posture and Motion Upon Heartbeats Under Laboratory Conditions. <i>Ernest V. Estensen</i>	66
An All-Metal Vacuum System Utilizing an Evapor-ion Pump <i>E. N. Mitchell</i>	70
Domain Structure of Thin Iron-Nickel Films as a Function of Film Thickness <i>E. N. Mitchell and D. C. Boknecht</i>	75
Preliminary Measurements of the Magneto-resistance of Thin Iron-Nickel Films. <i>E. N. Mitchell and C. O. Heinzl</i>	79
Measurement of the Saturation Magnetization of Thin Iron-Nickel Films <i>E. N. Mitchell and T. J. Cebulla</i>	83
Dietary Cholesterol on the Cholesterol Content of Rabbit and Rat Tissues. Ether as a Carrying Agent for Dietary Cholesterol. <i>Jay W. Constantine</i>	86

TABLE OF CONTENTS

Extraction of the Antipyridoxine Factor in Flax Cotyledons <i>H. J. Klosterman, R. B. Olsgaard, W. C. Lockhart, and J. W. Magill</i>	87
Osmotic Transfer of Erythrocyte Water <i>Edwin G. Olmstead</i>	94
Malic Dehydrogenase and Diaphorase from Germinated Flax Rust Uredospores. <i>Kenneth W. Dockter and D. Stuart Frear</i>	95
The Use of Perchloric Acid as a Sole Oxidizing Reagent for Organic Matter and Chromium Oxide. <i>Donald W. Bolin and William Lockhart</i>	102
The Reaction to Particulate Matter and the Histochemistry of Certain Reticulo-endothelial Cells of the Rat's Spleen <i>Theodore Snook, Joel Linford and Robert J. Bache</i>	104
Granular Stabilization of Ion Exchange Resins from Lignite Coal <i>R. E. Dunbar, F. L. Minnear and James M. Olson</i>	105
Population Fluctuations and Cyclomorphosis in Two Species of Rotifers During the Ice-Free Period of the Year <i>G. W. Comita and Jane Ann Munro</i>	112
Urinary Excretion of Alkaline Phosphatase Following Renal Ischemia in Rats <i>John H. Lunseth</i>	113
Microbiological Assay of Yeast and Chick Inhibitors from Flaxseed <i>J. L. Parsons, J. W. Magill and H. J. Klosterman</i>	113

Notice to the Members of the North Dakota Academy of Science

The publication of the Fourteenth Volume of the Annual Proceedings is accompanied by a change in editorial policy of the Academy.

The Minutes of the Academy and the List of Members will be printed separately and issued to members only. It will be possible for responsible parties to secure copies on request to the Secretary's office.

This change in editorial policy is in keeping with the best procedures that other scientific groups are following. It is a forward step for the Academy.

BEN G. GUSTAFSON, Sec.-Treas.

The North Dakota Academy of Science Financial Statement

Receipts: Balance, Red River National Bank, Grand Forks.

April 28, 1959	\$ 726.76
From Dues	778.00
The Denison Fund	190.00
Sale of Reprints	76.10
Total	\$1770.86

Expenditures:

A.A.A.S. Dues \$12.96	
Rathmann \$50.00	\$ 62.96
Press — Printing	249.40
Postage	63.00
Scholarship Fund	219.00
Denison Prizes	90.00
Banquet Expense	30.00
Thermofax Paper (Student Papers, copies)	25.25
Office of Education (Speaker)	78.15
Stenographic Expense	150.00
Bank Exchange05
Total Expense	\$ 967.81
Bank Balance	672.55
On Hand (Dep. May 5)	130.50
Totals	\$1770.86

Records examined by the Audit Committee, May 5, 1960. Found correct.

Ernest D. Coon
Arthur W. Koth

THE INFLUENCE OF TEMPERATURE ON THE FLIGHT ACTIVITIES OF THE ANT *FORMICA MONTANA*

Myrna C. Anderson and Paul B. Kanno*

*Department of Biology
University of North Dakota*

ABSTRACT

Flights were observed during the summer of 1959 at Oakville Prairie, an outdoor biological laboratory of the University of North Dakota near Emerado, North Dakota. The effects of certain climatic factors upon the extranidal behavior of the alate females and males during the flight season were studied.

The flights took place both in early morning and late afternoon. Emergence of the alate ants from the nests in the mornings was correlated with a minimum air temperature of at least 65°F. Flights occurred in the mornings at air temperatures of 60°F. or higher. In the afternoon, alates emerged from the nest and flew at temperatures between 68° and 90° F. On mornings when the overnight minimum temperature was above 65° F., emergence did not take place until at least two hours and 20 minutes after sunrise regardless of temperature.

Apparently, temperature is a limiting factor that influences the time of alate activity each day, but is not of primary importance in initiating the activity of the alates. We believe that the ants may have an internal clock-mechanism, evidently triggered by a minimum light intensity, and that this initiates the flight activities of the alates when temperatures are above a minimum of 65°F.

*N.S.F. Undergraduate Research Participant.

A STUDY OF THE INHERITANCE OF SEVERAL CHARACTERISTICS OF WHEAT

Curtis A. Hemstad

*A Senior Student at the North Dakota Agricultural College
Fargo, North Dakota*

INTRODUCTION

Very little research has been done on the inheritance of morphological characters in wheat species. This is especially true of the durum and emmer wheats. Results are very often inconsistent for the same character, suggesting that the subject is complex in nature. (1)

The purpose of this report is to present the preliminary results

of a genetic investigation of several characteristics of wheat. The objectives of the investigation are to determine the nature of inheritance of certain characteristics and to evaluate their utility as genetic "markers" for studies involving genetic linkage. Characteristics useful as genetic markers should be discreet, simply inherited traits. The genetic analyses of the F_2 generation reported here are tentative, and observations of succeeding generations are necessary.

MATERIALS

The wheat varieties used as parental material in this study include three species, **Triticum vulgare** (common wheat), **Triticum durum** (durum wheat), and **Triticum dicoccum** (emmer wheat). The varieties C. I. 7322 and Conley are common wheats; Acme, C. I. 3255, Dwarf A, Dwarf B, Langdon, Mindum, Ramsey, Tremez Preto, and Wells are durum wheats; Khapli and Vernal are emmer wheats. The inheritance of thirteen plant characteristics were studied in selected crosses among these varieties.

A description of each variety with respect to the characteristics studied is presented below. C. I. 7322 is awnless, and has pubescent glumes and curly flag leaves. Conley is awned, and has glabrous glumes and non-curly flag leaves. C. I. 3255 has black awns, long rachis bristles, glaucous plants, amber kernels, and hollow stems. Dwarf A is a highly dwarfed selection with lemma awns. Dwarf B is slightly taller than Dwarf A and has awns arising from both the lemma and glumes. Langdon and Mindum are medium tall, and have glaucous plants, amber kernels, and hollow stems. Ramsey is tall, and has amber kernels and lemma awns. Tremez Preto has solid stems, non-glaucous plants, red kernels, and short rachis bristles. Khapli has glaucous leaves and stems, non-glaucous spikes, red seedlings, and red kernels. Vernal has pubescent leaves; Acme is non-pubescent to slightly pubescent. The crosses in which each characteristic was studied are presented in Table 1.

METHODS

Thirteen plant characteristics are described in this report. The methods and techniques used in studying these characteristics are presented below.

F_2 populations were space planted either in the greenhouse or the field. Field plantings were made at Langdon, North Dakota, where good expression of plant characteristics are obtained. With respect to awn color, plants were classified at maturity into two classes, black and straw colored. Plants were classified glaucous or non-glaucous shortly after heading. Rachis bristle length of the F_2 population was recorded as long or short by comparing the plants with the parents. Seedling color was recorded immediately after emergence. Leaf pubescence was scored with the aid of a dissecting microscope into three classes: short, intermediate and long. The

presence or absence of curly leaf and pubescent glumes, was recorded after heading. With respect to stem solidness, the 2nd, 3rd, and 4th internodes were cut diagonally and scored into five categories ranging from 1 to 5. Internodes were scored 1 if completely hollow, and 5 if completely solid. The solidness score for each plant was the sum of the scores for each internode. Height of the plants and the number of internodes per plant were measured and counted, respectively, in the dwarf study. The presence or absence of glume awns was recorded at maturity. Kernel color was classified as either red or amber in a majority of the studies, with an attempt at intermediate colors included in one of the studies. Experiments were also conducted with respect to the light and background conditions used in classifying kernel color.

Chi square and heterogeneity tests were applied to all data. Linkage was computed by the cross-over method. Probability was determined with the use of tables.

RESULTS AND DISCUSSION

The mode of inheritance of each of the 13 characteristics was studied in the F_2 generation of wheat crosses. The actual data from the various crosses are given in the discussion of each characteristic.

Awn color The inheritance of awn color was studied in the cross, Tremez Preto x C. I. 3255. The intensity of black color in the awns was variable and may have been influenced by environmental factors. All plants having a trace or more of black awn color were classified black. Of a total of 275 plants, 202 had black awns and 73 had straw colored awns. The F_2 data are presented in Table 2. According to the chi square test for goodness of fit, the ratio of black to straw color was a good fit to a 3:1 ratio. This indicates that awn color in this cross was controlled by a single gene, and that black was dominant over straw color. Black awn color is simply inherited, easily classified and a suitable marker characteristic.

Rachis bristle length Segregation for length of rachis bristles was observed in the cross, Tremez Preto x C. I. 3255. As shown in Table 2, the ratio of short to long was a good fit to a ratio of 3:1. These data indicate that Tremez Preto and C. I. 3255 differ by one gene affecting rachis bristle length. Short bristles were dominant over long bristles.

Awnedness The presence or absence of awns was studied in a cross between two common wheats, Conley and C. I. 7322. Conley is fully awned and C. I. 7322 is awnless with the exception of a few short awnlets at the apex of the spike. As shown in Table 3, approximately one-fourth of a total of 231 plants were fully awned and three-fourths were awnless or slightly awnleted. The segregation was a good fit to a ratio of 1 fully awned: 3 awnless. Apparently the fully awned condition in this cross is controlled by a single recessive factor. On the basis of these results, the awnedness character-

istic may be useful as a marker.

Pubescent glumes The inheritance or pubescent glumes was studied in the cross, Conley x C. I. 7322. The pubescence occurs on the outer glumes of the spike and its presence or absence is easily observed. The F_2 data are presented in Table 3. Of 231 F_2 plants, 175 had pubescent glumes, and 56 had non-pubescent glumes. A chi square value of 0.19 for these data indicates a good fit to the proposed ratio of 3 pubescent; 1 non-pubescent. Glume pubescence is dominant and the inheritance is controlled by a single gene.

Glaucous plants The glaucous condition of the stems, leaves, and spikes was studied in the F_2 generation of two crosses, Tremez Preto x C. I. 3255, and Tremez Preto x Langdon. The glaucous condition is caused by a whitish powdery coating on the plant surface. Glaucousness is most intense shortly before plant maturity. As shown in Table 4, the F_2 data from the two crosses were inconsistent. Of a total of 428 plants of the cross, Tremez Preto x C. I. 3255, 137 were glaucous and 291 were non-glaucous. The observed number of glaucous plants is greater than that expected on the basis of a 1:3 ratio. In the cross Tremez Preto x Langdon, 121 F_2 plants were glaucous and 355 were non-glaucous. On the basis of a 1:3 ratio, 119 glaucous and 357 non-glaucous plants are theoretically expected. These data satisfactorily fit the theoretical ratio. Apparently the segregation for glaucousness is different in the two crosses, and further study is necessary to determine the nature of inheritance. Neatby and Goulden (2) found plant glaucousness to be controlled by two independent genes.

Glaucous spike The cross, Khapli x Mindum, was used to study the inheritance of glaucous spikes. Khapli has glaucous stems and leaves, but non-glaucous spikes. Mindum has glaucous leaves, stems and spikes. The intensity of glaucousness was variable. Every F_2 plant with a trace or more of glaucousness on the spikes was classified glaucous. As presented in Table 5, the ratio of F_2 plants was 372 glaucous: 6 non-glaucous. The chi-square test shows a good fit to a 63:1 ratio. These data show that glaucousness of the spike is dominant over non-glaucousness and controlled by three genes.

Seedling color The F_2 seedlings of the cross, Khapli x Mindum, were classified for the presence or absence of red color in the first leaf. The red coloration often disappears shortly after emergence, but may remain for several days. The results of seedling color classification are summarized in Table 6. The observed ratio of 174 red: 75 green seedlings is a good fit to a ratio of 3:1. The results of this experiment indicate that the red seedling color of Khapli emmer is conditioned by a single dominant gene.

Curly flag leaf The curly flag leaf condition was studied in the cross, Conley x C. I. 7322. The expression of this characteristic is weak in that the curliness of any one plant varies from day to day. The data are presented in Table 7. Of a total of 231 F_2 plants, 96

were curly and 135 were non-curly. These data do not fit a theoretical ratio of 1 curly: 3 non curly. It is possible that this abnormality is not a genetic trait, but is caused by a seed transmitted virus.

Dwarfism Three crosses; Langdon x Dwarf A, Wells x Dwarf A, and Dwarf B x Ramsey; were used to investigate dwarfism. Table 8 shows the height distribution of the F₂ plants of each cross. In the crosses, Langdon x Dwarf and Wells x Dwarf A, a small number of segregates which were taller than the tallest parent were observed. The number of F₂ plants taller than Langdon or Wells was large enough to indicate that the added height was due to transgressive segregation. Evidence of transgressive segregation was not found in the cross, Dwarf B x Ramsey. It is evident that the inheritance of dwarfism in these crosses was complex. Torrie found that dwarfism in wheat was controlled by two complementary genes and an inhibiting gene. (3) Collection of additional data is necessary to form a genetic hypothesis.

Glume awns The dwarf parent in the cross, Dwarf B x Ramsey, has awns arising from both the lemma and the outer glume, whereas the parent Ramsey has awns arising from only the lemma. Segregation for the presence or absence of glume awns was observed in the F₂ generation. The F₂ data are presented in Table 9. Approximately one-sixty fourth of the F₂ plants had only lemma awns, and the remainder had awns on both the lemma and glumes. The observed ratio was a good fit to a theoretical ratio of 63 glume awns: 1 lemma awns. The presence of glume awns is dominant over the absence of glume awns and is conditioned by three independent genes.

Kernel color With respect to the inheritance of kernel color, only tetraploid wheats were studied. F₂ data from four crosses are presented in Table 10. The variety, Tremez Preto, was used as the female parent in crosses with C. I. 3255, Langdon, and Mindum. In each of these three crosses, the ratio of red to amber kernel color was a satisfactory fit to a 3:1 ratio. In the crosses, Tremez Preto x C. I. 3255, Tremez Preto x Langdon, and Tremez Preto x Mindum, red kernel color is dominant over amber and the inheritance is controlled by a single gene.

In the fourth cross, Khapli x Mindum, the inheritance of kernel color was more complex. Approximately 3/16 of the F₂ population had amber kernels and 13/16 had red kernels. The ratio of red to amber did not fit a 3:1 ratio, but fit a ratio of 13:3. The deviation from a 3:1 ratio may have been due to chance or to a segregation for two genetic factors apparently one gene dominant and the other incompletely dominant for red kernel color.

Pubescent leaf The cross, Acme x Vernal, was used to study leaf pubescence. The length of leaf hairs was classified in a total of 457 F₂ plants. Of this number, 29 plants had long leaf hairs, 175 had short leaf hairs, and 253 had leaf hairs intermediate in length. These data are a good fit to a ratio of 9 intermediate: 6 short: 1 long.

Apparently leaf pubescence is controlled by two genes. One incompletely dominant gene conditions pubescence, and another dominant gene incompletely inhibits pubescence. If gene A conditions pubescence, and gene B inhibits pubescence, the phenotype of the F_2 genotypes are as follows:

Long	Intermediate	Short
AAbb	AABB	AaBB
	AABb	aaBB
	AaBb	aaBb
	Aabb	aabb

Stem solidness The variety, Tremez Preto, has solid stems, whereas Mindum has hollow stems. Data pertaining to the stem solidness of the F_2 plants of the cross, Tremez Preto x Mindum, are given in Table 12. Stem of approximately $\frac{1}{4}$ of the F_2 plants were as solid as Tremez Preto, $\frac{1}{4}$ were as hollow as Mindum, and $\frac{1}{2}$ were intermediate. The satisfactory fit to a 1:2:1 ratio is evidence that stem solidness in this cross is controlled by a single, incompletely dominant gene.

Genetic associations The chi-square test for independence indicates linkage or genetic association between kernel color and plant glaucousness and between glume pubescence and awnedness. A study of the F_3 generation with respect to these characteristics is necessary for additional evidence.

SUMMARY

The nature of the inheritance of thirteen characteristics of wheat were studied in the F_2 generation of several crosses. In the crosses studied, awn color, plant glaucousness, seedling color, rachis bristle length, awnedness, glume pubescence, and stem solidness were each controlled by single genes. In each of three crosses, kernel color was controlled by one gene, but in a fourth cross the F_2 results were inconclusive. Black awns, non-glaucous plants, short rachis bristles, red kernel color, red seedling color, pubescent glumes and awnedness were dominant and stem solidness was incompletely dominant.

The two characteristics, glaucous spikes and glume awns, were controlled by three dominant genes. Leaf pubescence was controlled by an incompletely dominant gene conditioning pubescence and a dominant gene incompletely inhibiting pubescence.

The inheritance of dwarfism was complex and could not be analyzed with only F_2 data.

The analyses of the F_2 data indicated genetic linkage between kernel color and plant glaucousness and between glume pubescence and awnedness.

ACKNOWLEDGEMENTS

The author acknowledges the advice of Dr. N. D. Williams, Supervisor of the study, the North Dakota Agricultural Experiment Station for the use of its facilities, and the National Science Foundation for its aid in financing the study. (Grant #7998).

BIBLIOGRAPHY

1. Ausemus, E. R. A Summary of Genetic Studies in Hexaploid and Tetraploid Wheats Agron. Jour. 38: 1082-99. 1946.
2. Neatby, KW and Goulden, C. H. The Inheritance of Resisance to **Puccinnia graminis tritici** in crosses between varieties of **Triticum Vulgare** Sci Agr. 10: 389-404. 1930.
3. Torrie, J. H. Inheritance Studies of Several Qualitative and Quantitative Characters In Spring Wheat Crosses. Can Jour. Res Sect C 14: 368-85 1936.

TABLE 1

Crosses of wheat varieties used in the study of several genetic characteristics

Characteristic	Cross or Crosses
Awn color	Tremez Preto x C. I. 3255
Awnedness	Conley x C. I. 7322
Glaucous plants	T. Preto x C. I. 3255, T. Preto x Mindum
Glaucous spike	Mindum x Khapli
Curly flag leaf	Conley x C. I. 7322
Dwarfism	Langdon x Dwarf A, Wells x Dwarf A, Dwarf B x Ramsey
Glume awns	Dwarf B x Ramsey
Kernel color	T. Preto x C. I. 3255, T. Preto x Langdon, Khapli x Mindum, T. Preto x Mindum
Pubescent leaf	Acme x Vernal
Pubescent spike	Conley x C. I. 7322
Rachis bristle length	T. Preto x C. I. 3255
Seedling color	Khapli x Mindum
Stem solidness	T. Preto x Mindum

TABLE 2

Awn color and the length of rachis bristles in the parents and F₂ generation of the cross, Tremez Preto x C. I. 3255.

Material	Awn color		P (X ²)	Rachis Bristles		P (X ²)
	Black	Straw		Long	Short	
F ₂ observed	202	73	.50-.95 (.25)	75	200	.20-.50 (.70)
F ₂ expected	206	69		69	206	
T. Preto		all			all	
C. I. 3255		all		all		
C. I. 3255	all			all		

TABLE 3

Awn character and pubescent glumes in the parents and F₂ generation of the cross, Conley x C. I. 7322.

Material	Awn character		P (X ₂)	Pubescent Glumes		P (X ₂)
	Awnless	Awned		Pubescent	Non-Pub.	
F ₂ observed	176	55	.50-.95 (.17)	175	56	.50-.95 (.19)
F ₂ expected	174	58		174	58	
Conley		23			23	
C. I. 7322	17			17		

TABLE 4

Glaucous plants of the F₂ generation and the parents of two durum wheat crosses.

Material	Glaucous	Non-glaucous	P (X ²)
F ₂ Tremez Preto x C. I. 3255	137	291	<.01 (6.54)
F ₂ expected	107	321	
T. Preto		all	
C. I. 3255	all		
F ₂ T. Preto x Langdon	121	355	.50-.95 (.04)
F ₂ expected	119	357	
T. Preto		all	
Langdon	all		

TABLE 5

Glaucous spikes in the parents and F₂ generation of the cross Khapli x Mindum.

Material	Glaucous	Non-glaucous	P (X ²)
F ₂ observed	372	6	.95-.99 (—)
F ₂ expected (63:1)	372.1	5.9	
Khapli		9	
Mindum	13		

TABLE 6

Seedling color in the parents and F₂ generation of the cross Khapli x Mindum.

Material	Color		P (X ²)
	Green	Red	
F ₂ observed	75	174	.05-.10 (3.45)
F ₂ expected	62	186	
Khapli		7	
Mindum	3		

TABLE 7

Flag leaf characteristics of the parents and the F₂ generation of the cross Conley x C. I. 73 .

Material	Flag Leaf Characteristic		P (X ²)
	Curly	Non-curly	
F ₂ observed	96	135	<.01 (30+)
F ₂ expected	58	174	
Conley		all	
C. I. 7322	all		

Table 8

Height distribution of the parents and the F₂ generation of several durum crosses.

Material	Height (intervals of 10 cm)					
	31-50	51-70	71-90	91-110	111-130	131-150
Langdon x						
Dwarf A	3	56	110	99	18	4
Langdon			3	5		
Dwarf A	2	9				
Wells x						
Dwarf A	9	87	144	41	8	1
Wells			4	3		
Dwarf A	6	9				
Dwarf B x						
Ramsey	5	48	115	53	8	
Dwarf B	3	4	2			
Ramsey			5	2	5	

TABLE 9

Awn type of the parents and the F₂ generation of the cross Dwarf B x Ramsey.

Material	Awn type		P
	Lemma awns only	Glume awns + lemma awns	(X ²)
F ₂ observed	4	219	.50- .95
F ₂ expected			(.12)
(63:1)	3.5	219.5	
Dwarf		7	
Ramsey	9		

TABLE 10

Kernel color of the parents and F₂ generation of several wheat crosses.

Material	Color		P
	Red	Amber	(X ²)
Tremez Preto x C. I. 3255	191	66	.50- .95
F ₂ expected (3:1)	193	64	(.08)
Tremez Preto C. I. 3255	15	18	
Tremez Preto x Langdon	275	71	.05- .10
F ₂ expected (3:1)	261	87	(3.69)
Tremez Preto Langdon	12	17	
Tremez Preto x Mindum	151	49	.50- .95
F ₂ expected (3:1)	150	50	(.03)
Tremez Preto Mindum	21	6	
Khapli x Mindum	179	36	<.01
F ₂ expected (3:1)	168.7	56.3	(7.84)
F ₂ expected (13:3)	175	40	.20- .50
Khapli	6		(.49)
Mindum		4	

TABLE 11

Leaf pubescence in the parents and F₂ generation of the cross Acme x Vernal.

Material	Leaf Hair Length			P (X ²)
	Long	Intermediate	Short	
F ₂ observed	29	253	175	.50-.95 (.15)
F ₂ expected	28.5	257	171	
Acme			23	
Vernal	27			

TABLE 12

The (stem) solidness of the parents and the F₂ generation of the cross Tremez Preto x Mindum.

Material	Solidness Score (3 internodes)												P (X ²)
	4	5	6	7	8	9	10	11	12	13	14	15	
F ₂ observed	22	11	13	22	23	13	15	20	21	24	11	12	
F ₂ observed	<—46—>			<—114—>			<—47—>						.10-.20 (2.15)
F ₂ expected	52			104			52						
T. Preto										1	3	6	
Mindum	4	2	1										

RADIO TRANSMISSION AT VHF AND UHF FREQUENCIES

*Michael B. Haverty**

Department of Electrical Engineering

University of North Dakota, Grand Forks, North Dakota

Abstract

The large body of information now available on long distance transmission of radio waves with frequencies above 30 MC has been gathered in the last 15 years. It has been found that the propagation of radio waves with these frequencies is dependent upon atmospheric refraction, tropospheric scattering, and ionospheric scattering.

In the past ten years there has been a great deal of emphasis placed on obtaining experimental evidence on the long distance propagation of high frequency radio waves. At the University of North Dakota, a grant was received from the National Science Foundation for a study of the signal strength variations of very high frequency radio waves. This study was conducted during the summer

of 1959. There was found to be good correlation between theoretical predictions and experimental results.

This paper covers the "beyond the horizon" propagation theories generally accepted at the present time and their relationship to the experimental data obtained at the University of North Dakota.

I. INTRODUCTION

Since the advent of television, the terms VHF and UHF "channels" have become household words across the country. VHF is an abbreviation for very high frequency and refers to radio waves with frequencies from 30 MC to 300 MC. UHF is an abbreviation for ultra high frequency and covers the 300 MC to 3000 MC range.

Although these terms are quite common now, transmission of radio waves in these frequency regions has been quantitatively evaluated only in the past ten years. The experimental investigations have been accompanied by a considerable amount of theoretical work. Many theories have been proposed and discarded, and there is not yet complete agreement among the theoretical models proposed to explain long-distance propagation. But agreement or not, the theoretical developments have advanced to a point where they yield quite accurate predictions and can be used to meet particular circuit or systems design problems.

II. PROPAGATION THEORIES

When VHF and higher frequencies were first put to use, it was generally believed that propagation to distances beyond the horizon was governed essentially by the laws of diffraction over a smooth spherical earth surmounted by a stable homogeneous atmosphere. The useful range of these frequencies, therefore was believed to be limited to distances not much greater than line of sight. However, as more powerful transmitters, higher-gain antennas, and more sensitive receivers were developed, an increasing amount of evidence was obtained that signal levels well beyond the horizon were much higher than diffraction theory predicts, and other explanations had to be sought. As a result, it is now generally believed that high frequency radio waves are propagated beyond line of sight distances in these three ways:

1. Atmospheric refraction
2. Tropospheric scattering
3. Scattering from ionized layers or meteor trails in the ionosphere.

The major amount of atmospheric refraction takes place within a mile or two of the earth's surface. The troposphere extends from the earth to a height of seven miles and the ionosphere is located 40 to 200 miles above the earth.

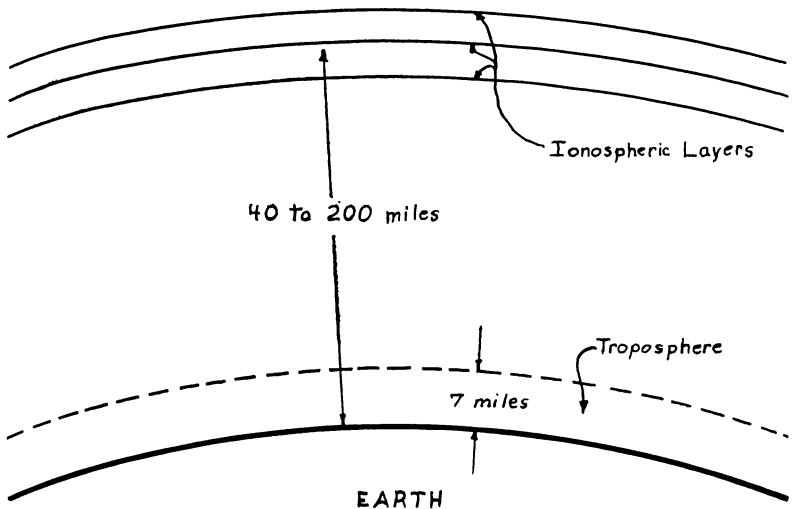


FIG. 1. Position of Atmospheric Layers

III. ATMOSPHERIC REFRACTION

First let us consider the role of atmospheric refraction in the transmission of radio waves beyond line of sight distances.

If the atmosphere were entirely homogeneous, high frequency radio waves would travel away from the transmitting antenna in almost a straight line or "line of sight" path. However, the presence of gas molecules, particularly water vapor, in the atmosphere causes it to have a dielectric constant greater than unity. Normally, changes in water vapor density, temperature, and pressure cause the dielectric constant, and thus the refractive index of the air, to decrease with altitude. The speed of an electromagnetic wave varies inversely with the index of refraction of the medium through which it passes. Thus, in traveling through the atmosphere, the upper portion of a wave travels faster than the lower portion and the wave is refracted or bent towards the earth. The amount of the bending depends upon the change in the index of refraction with height. This is called the refractive gradient. Mathematically it is:

$$\frac{dn}{dh} \quad \text{where } n = \text{refractive index of the atmosphere}$$

$$h = \text{height above the earth}$$

The net effect of the bending of the wave is to extend the distance to the radio horizon beyond that of the line of sight horizon. This can best be shown by means of a diagram—see Figure 2.

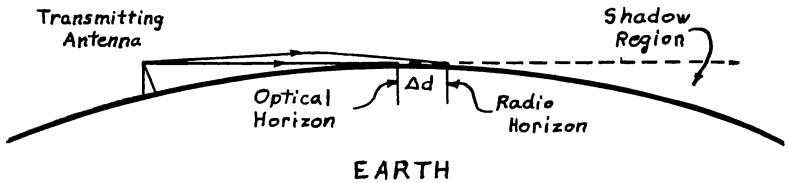


FIG. 2 Extension of the Radio Horizon Due to Standard Refraction

Where Δd is the increment of distance added to the optical horizon to obtain the radio horizon distance.

For a long time it was thought that the only transmission beyond the radio horizon would be due to diffraction. Diffraction is a property of all waves and is the spreading of the wave into its geometric shadow when it passes the edge of an obstacle. This theory would predict an exponential decrease in field intensity beyond the radio horizon.

IV. Tropospheric Scattering

At distances of 30 miles to 500 miles beyond the line of sight and with frequencies in the 40 MC to 4,000 MC range, field intensities are often measured which appreciably exceed the value calculated upon the basis of a standard atmosphere and the application of rigorous diffraction theory. The explanation which has been given the most attention has been that which hypothesizes the weak

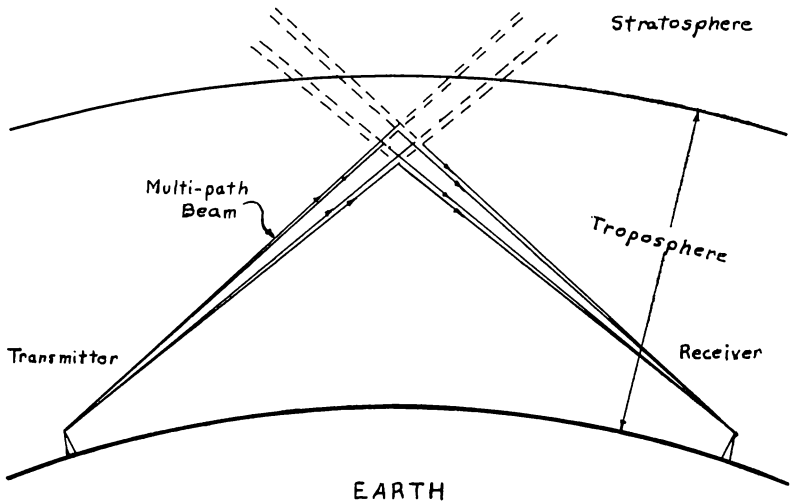


FIG. 3. Path Geometry—Tropospheric Propagation

The second type of fading is much slower and is caused chiefly by variations in the gradient of the refraction index of the atmosphere.

scattering of radio waves by turbulent departures from the mean refractive index in the troposphere. While this type of propagation is the predominant one at distances far beyond the radio horizon, some effects can be observed at distances near the radio horizon and even at short distances within the radio horizon.

Tropospheric scattering propagation is characterized by two types of fading. One is quite rapid and is due to the existence of several paths differing slightly in length.

V. Reflection from Ionized Layers of Meteor Trails in the Ionsphere

A third type of beyond the horizon propagation makes use of the scattering effect of the lower ionsphere on waves in the 30 MC to 70 MC frequency range.

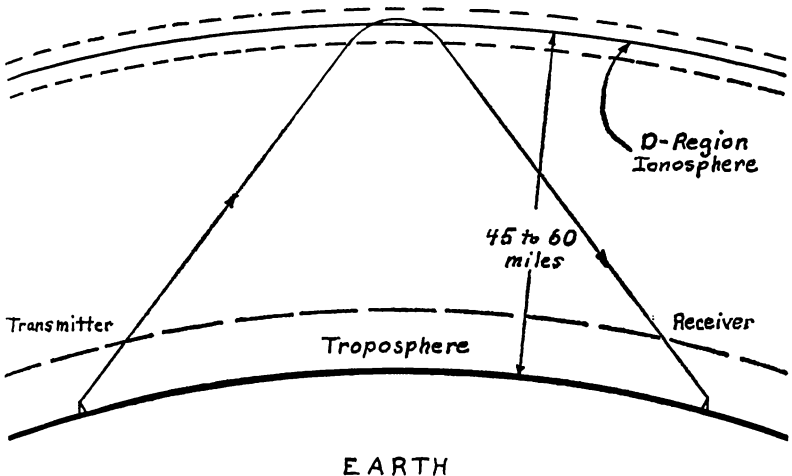


FIG. 4. Ionospheric Propagation

By making use of this effect, long range communication on the order of 1000 miles is possible.

When a wave of this frequency strikes the D Region of the lower ionsphere, most of the wave passes through, but a small percentage is returned to the earth. This signal comes back as a continuous, rapidly fading signal, with bursts of energy superimposed. The continuous signal is thought to be scatter from irregularities in ionization in the D Region and reflections from the ionized trails of many small meteors. The superimposed bursts are reflections from large meteor trails of sufficient density.

Through the use of large effective radiated power, and modern modulation detection and coding methods, the continuously present but weak and variable background scatter signal can be used as a

very reliable communications device. At present a network, using this method, had been established by NATO from Norway, through Western Europe, and to Turkey.

VI. Experimental Observations

The propagation theories covered explain very well the data obtained in an actual test. During the summer of 1959 I made a study of field intensity variations near the radio horizon. This was done by monitoring the 83.25 MC carrier wave of WDAY-TV. A field intensity meter was tuned to this carrier wave, the output fed through a D. C. amplifier and into an Esterline Angus graphic ammeter. This produced a continuous graphic recording of the strength of the carrier wave which could be easily converted to field intensity readings.

The signal strength or field intensity measurements were made at Grand Forks, North Dakota, which is located a straight line distance of 65 miles from WDAY-TV's transmitter. The distance to the radio horizon from the transmitter can be calculated by introducing a correction factor when using the radius of the earth.

The value of this correction factor or, as it is sometimes called, the effective earth radius coefficient, depends on the atmosphere's refractivity gradient. An average radio horizon distance can be calculated by using an average value for the correction factor. When the antenna heights are taken into consideration, the formula for the radio horizon distance is:

$$D = \left(\frac{2 R k h_t}{5280} \right)^{\frac{1}{2}} + \left(\frac{2 R k h_r}{5280} \right)^{\frac{1}{2}} \quad \text{miles}$$

where R = true earth's radius in miles
 k = the effective earth radius coefficient
 h_t = the transmitter height in feet
 h_r = the received height in feet

Using an effective earth's radius coefficient of 1.55, as given by the Television Allocations Study Organization Committee #5.4 for this area in the summer months, the distance to the radio horizon came out to be 63 miles. The location of the receiver was then 2 miles beyond the calculated radio horizon. This would cause the strength of the predominant signal to depend very much upon the atmospheric gradient of refraction. When this gradient was less than usual the receiver would be farther beyond the radio horizon and the signal would be propagated the last mile or two mainly by diffraction and tropospheric scattering. At all times there was a fairly small rapid fluctuation in the field intensity. This would be due to the rapid-varying portion of the signal reaching the receiver due to tropospheric scattering.

A complete calculation of the refractivity gradient along the path traveled by the radio wave would require a vast quantity of information. B. P. Bean and F. M. Meaney made some field in-

tensity studies in 1954 in which they calculated ΔN , the difference between the ground level radio refractivity and its value one kilometer above the earth's surface. The "radio refractivity" is usually used for ease in calculations and is simply: $N = (n - 1) \times 10^6$, where $n =$ index of refraction.

A comparison of ΔN and the transmission loss present in their tests produced a .07 coefficient of correlation. They also found a very good correlation between this "gradient" and the ground level radio refractivity.

The ground level radio refractivity is given by the formula:

$$N = \frac{77.6}{T} \left(P + \frac{4810 e_s RH}{T} \right)$$

where $P =$ atmospheric pressure in millibars
 $T =$ temperature in degrees Kelvin
 $e_s =$ saturation vapor pressure in millibars
for the temperature T
 $RH =$ relative humidity

In the light of these findings, an examination was made to determine the correlation between the variables in the refractivity equation at the receiving location and the slow varying portion of the field intensity. Very little correlation was found to exist between ground level temperature and field intensity. This is to be expected because in most cases when the ground level temperature rises, the temperature of the atmosphere also rises, thereby not affecting the gradient of refractivity substantially. A marked correlation was found to exist between the field intensity and the ground level relative humidity at the receiver location. The overall coefficient of correlation, using readings taken each day at 10:00 A.M., at 5:00 p.m., and at 10:00 p.m., was .61. Breaking this down, at 10.00 a.m., the coefficient of correlation was .42, at 5:00 p.m. it was .70, and at 10:00 p.m., it was .45. These are considered high coefficients of correlation and show that there is a marked relationship between the relative humidity and the field intensity.

An 83.25 MC radio wave is considered to have too high a frequency to be affected by ionospheric disturbances. This was verified very well in the tests during the period from July 6, 1959 to July 20, 1959. At that time a great increase of solar activity produced radio blackouts at lower frequency ranges, tremendous disturbances in the earth's magnetic field, and noteworthy increases in cosmic rays. No noticeable changes in field intensity were recorded during this time.

Severe meteorological disturbances in the form of violent storms also had a marked effect on the field intensity when these storms were located in the area between the transmitter and the receiver. Three times during the period monitored a storm such as this occurred. At these times the field intensity fluctuated violently and the average value was lower than usual.

VII. Conclusion

It has been pointed out that propagation using tropospheric scattering and ionospheric scattering is characterized by weak signals whose intensity varies over quite wide ranges over a period of time. This of course creates problems in reliability and quality when using these types of propagation. In order to overcome the fading difficulties, it is necessary to use transmitter powers up to 50 kw., free space plane wave antenna gains as high as 40-45 db., and space-diversity reception. Using circuit parameters of this order, single-hop circuits can be designed to carry 10 to 12 information channels over distances of some 500 miles. There is also evidence that reasonably satisfactory transmission characteristics can be obtained for standard black and white television signals at distances of 150-200 miles. At the present time the military services are using VHF and UHF long distance transmission. The greater cost required in the installation and operation relative to other means of long distance communication has limited its commercial attractiveness. The low frequency bands are becoming more and more crowded every day, therefore it is a great possibility that before too long it will become an absolute necessity to put the high frequency portion of the spectrum to more extensive use.

BIBIOGRAPHY

- Baris, A. P., "Some Aspects of Tropospheric Radio Wave Propagation", **IRE Transactions on Broadcast Transmission Systems**, pp. 1-10, October, 1956.
- Bean, B. P. and F. M. Meany, "Some Applications of the Monthly Median Refractivity Gradient in Tropospheric Propagation", **Proc. IRE**, Vol. 43, pp. 1419-1431, October, 1955.
- Bullington, K., "Radio Propagation Fundamentals", **The Bell System Technical Journal**, Vol. 36, pp. 593-625, May, 1957.
- Bullington, K., "Characteristics of Beyond the Horizon Radio Transmission", **Proc. IRE**, Vol. 43, pp. 1174-1180, October, 1955.
- Johns, H. B. and P. I. Wells, "Some Tropospheric Scatter Propagation Measurements Near the Horizon", **Proc. IRE**, Vol. 43, pp. 1336-1340, October, 1955.
- Joint Technical Advisory Committee, "I. Ionospheric Scatter Transmission, II. Long Range Tropospheric Transmission", **Proc. IRE**, Vol. 48, pp. 5-44, January, 1960.
- LaGrone, A. H., "Report of TASO Committee #5.4 on Forecasting Television Service Fields", **1959 IRE Convention Record**, Vol. 7, Part 7, pp. 159-164.
- Supplementary to Report TA-11 on Solar Activity, High Altitude Observatory, Boulder, Colorado, July 24, 1959.
- Terman, F. E., **Electronic and Radio Engineering**, New York: McGraw-Hill, 1955.

*Undergraduate Research Participants.

TILLS OF KIDDER COUNTY, NORTH DAKOTA

*Lee Clayton***Department of Geology**University of North Dakota, Grand Forks, North Dakota*

INTRODUCTION

During part of the summer of 1959 the author worked with a North Dakota Geological Survey party that mapped the glacial geology of Kidder County, North Dakota. During mapping, the problem came up of differentiating tills of different ice advances. There is a special set of problems in the study area—a large blanket of outwash covers much of the county and an irregular preglacial topography makes correlating of end moraines confusing. Thus differentiation and correlation on the basis of landforms is difficult. The purpose of this study was to find ways of lithologically differentiating the tills of the glacial advances.

Kidder County is on the Coteau du Missouri in the glaciated Missouri Plateau section of the Great Plains province of south-central North Dakota (2). The elevation of the county varies from 1700 to 2300 feet above sea level. The county has a short grass native vegetation and lies near the edge of the Chestnut soil zone (16).

Flint's "Pleistocene Geology of Eastern South Dakota" is the most complete work on the glacial geology of the end moraine complex in this general area (Kidder County is 50 miles from the South Dakota boundary). Considerable reference will be made to it. Age designations are from Lemke and Colton (10). (See table 1.) All field observations and laboratory analyses were made by the author. The generalized glacial map of the county (fig. 1) is taken in part from the unpublished work of Dr. Jon L. Rau, Wallace E. Bakken, James C. Chmelik, and Barrett J. Williams, members of the Survey mapping party.

During six weeks in June and July of 1959, 125 spot samples of till and loess were collected from throughout the county. Size analyses were made by means of a slightly modified American Society for Testing Materials (1) hydrometer procedure D422-54T. The course sand (Wentworth scale) fractions separated during the size analyses were studied and the percentages of shale grains recorded. Color was determined by comparing the samples with the Munsell soil color charts. Differential thermal analysis was used to identify dolomite and gypsum in two samples.

Summary of the glacial geology of Kidder County

The Wisconsin glaciers that entered Kidder County had moved southwest across North Dakota over older drift and nearly 200 miles of Pierre shale. The ice advance came to a halt after moving

over the escarpment at the east edge of the Coteau du Missouri. In Kidder County the topography of the end moraine complex was complicated by the irregular topography developed on bedrock of Fox Hills sandstone. What appears to be end moraine is often only an irregular preglacial topography covered with a thin blanket of drift. Till behind the end moraines is often in the form of confusing "stagnation" or "dead ice" moraine (11). Much of the till is covered with one of the largest outwash bodies in the United States (7). Therefore true relationships between the tills of different glacial advances are often difficult to determine using only the till sheets.

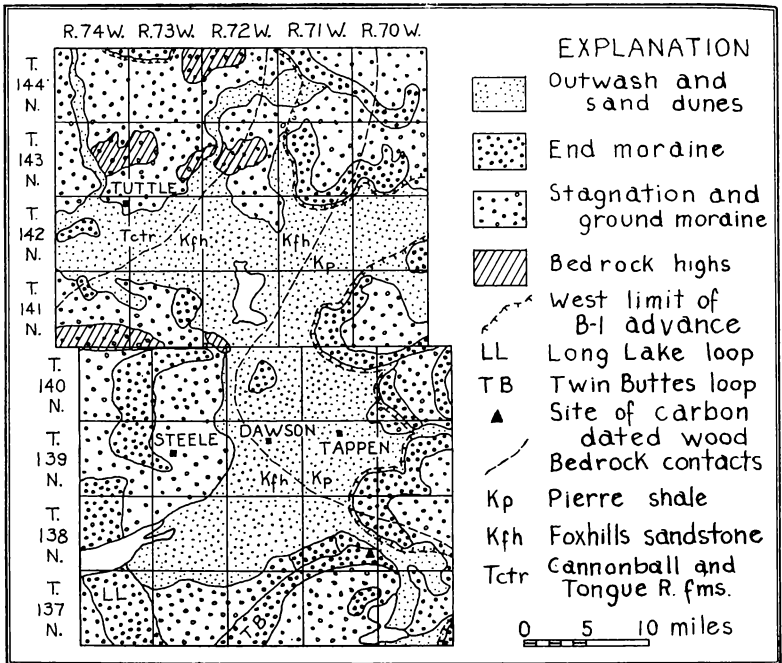


FIGURE 1—Generalized map of surface geology of Kidder County, N. Dak.

The till of the end moraines on the northern and eastern sides of the county (fig. 1) belongs to the post-Cary advance number 1 of Lemke and Colton, which corresponds to Flint's B-1 Mankato advance in South Dakota. The rest of the county is covered by till of the post-Tazewell—pre-Two Creeks substage, which corresponds to Flint's A-1 Mankato advance. For convenience, the two advances will be referred to as the A-1 and B-1 advances. Some till of the Tazewell drift sheet may be exposed in the county. (See p. 10.)

TABLE 1
Classification of the Wisconsin stage

From Lemke and Colton, 1958, table 1	Lemke and Colton (1958, fig. 3) in N. Dak.	Flint in S. Dak. (Lemke and Colton, 1958, fig. 3.)	This report
Valders substage			
Two Creeks interstadial			
Mankato substage	Post-Cary	B-1 and later Mankato advances	B-1 Mankato advance
	Post-Tazewell—pre-Two Creeks	A-1 Mankato advance	A-1 Mankato advance
Interstadial			
Cary substage	Not present	Cary	
Interstadial			
Tazewell substage	Tazewell (?)	Tazewell	Tazewell
Interstadial			
Iowan substage	Iowan (?)	Iowan	

The end moraine of the B-1 advance has markedly arcuate loops with closely spaced subparallel ridges of high relief. The "Twin Buttes" loop of the A-1 advance has high relief but few subparallel ridges. The rest of the end moraine of the A-1 advance in the central and west part of the county has low relief and wider spaced subparallel ridges; in the central part of the county much of it is covered with outwash from the B-1 advance. Not only does this outwash hide much of the glacial geology of the county, but part of it was probably incorporated into the advancing ice, making the resultant till more heterogeneous.

Yellow loess was observed in only a few isolated exposures in the county; one of these is described on page 8. From ½ to 3 feet of dark loesslike material covers much of the outwash and till of the A-1 advance in the south two-thirds of the county. It is fine sand (the same size as that in active dunes in the south-central part of the county) with a dark brown, organic silt matrix; it is not

bedded and is fairly well consolidated (5). This is probably the same material found above the spruce wood (in the Twin Buttes loop) that was radiocarbon dated at $11,480 \pm 300$ years B. P., which is the age of the Two Creeks interstadial (13). Lemke and Colton (9) stated that "because of the present lack of knowledge of the stratigraphic relations of the deposits overlying the radiocarbon dated material [in the Twin Buttes loop], it cannot now be ascertained whether [the drift of the B-1 advance] antedates or post dates the Two Creeks interstadial." None of the loesslike sand was observed on till of the B-1 advance. If this windblown sand were deposited during or shortly after the deposition of the B-1 outwash, the B-1 advance occurred about $11,480 \pm 300$ years ago.

RESULTS

Field appearance.—No significant difference in color exists between the tills of the A-1 and B-1 advances. On the Munsell scale they are generally 2.5Y-5Y 5-7/2-4 when dry and 2.5Y-5Y 4-5/2-4 when wet. The wet color can generally be described as olive or olive brown. Most of the till has reddish-yellow spots, probably caused

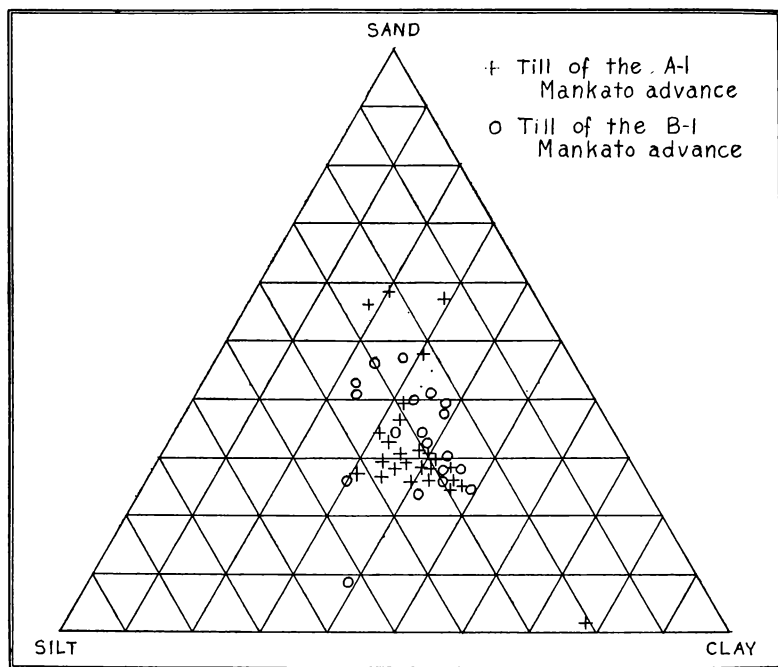


FIG. 2.—Triangular diagram of sand-silt-clay composition of 45 samples of Mankato till.

by the oxidation of fragments of iron oxide concretions from the Pierre shale, and a white mottling, due to an irregular concentration of calcium carbonate. In several places in the southwest corner of the county the till contains "geodes" filled with one-eighth inch crystals of gypsum. Also observed within the till in the southwest corner were salmon-colored dolomitic lenses and sheets (at the northwest corner of sec. 30, T. 137 N., R. 74 W.; and three miles into Burleigh County, 0.1 mile north of the southeast corner of sec 28, T. 139 N., R. 75 W.). Horizontal fissility was noted at a few exposures. Except for one exposure (described on page 9), the tills have no significant visual differences that might be used for correlation.

Grain size analysis.—Shepps (15) made size analyses on about 75 till samples from Tazewill, early Cary, and late Cary till sheets in several counties in northeast Ohio. He found that the sand-silt-clay ratios plotted on a triangular diagram were very useful in correlation the till sheets. Triangular plots of 45 samples of till from Kidder County are shown in figure 2. No major difference exists between the size compositions of the A-1 and B-1 tills. Most plots fall within a small area on the triangular diagram. The very clayey sample is probably contaminated with lake clay. Nearly all the samples that have greater than average percentages of sand have been contaminated with outwash. Histograms of complete size analyses of 11 till samples showed no correlation or differentiation of A-1 and B-1 tills.

An attempt was made to plot the sand and clay percentages on isopleth maps. Only a random distribution of the sand and clay percentages was noted. Closer spaced samples, however, might show significant relationships between outcrops of Fox Hills sandstone, outwash deposits, and the amount of sand in the till. It might be expected that the till west of the contact of the Fox Hills sandstone and the Pierre shale would have a higher percentage of sand. However the difference is not great: 14 samples from east of the contact average 31% sand, and 33 samples west of the contact average 33% sand.

Stone counts.—Stone counts have been used to differentiate till sheets by determining differences in provenance. Flint (6) made stone counts at 34 localities in South Dakota and found no significant differences in composition of stones in till of different glacial advances. Fourteen pebble counts were made on A-1 and B-1 tills, and no significant differences were noted in pebble composition of the tills of the two advances.

Flint noted (6) that the true percentage of shale in pebbles is hard to determine because the shale pebbles disintegrate easily. The coarse sand fractions separated during size analysis were saved and the percentages of shale grains determined. Again no significant trends were noted. It would be expected that the till east of the

contact of the Fox Hills sandstone and the Pierre shale would have a higher percentage of shale grains. However, shale averaged 27% of the coarse fraction in samples from east of the contact, while west of the contact, shale averaged 31% of that fraction. Dilution with large amounts of shale-free outwash may be the reason the till east of the contact has less shale.

Stratigraphy.—At least two localities were observed in the county that had more than one till exposed. In a road cut 8½ miles south of Tappen, 0.3 mile north of the southwest corner of sec. 22, T. 138 N., R. 71 W., the following section was observed:

	Feet
6. Fine sand, dark brown, fine grained; wind blown; loesslike, with silt matrix	1.9
5. Till, pale olive (5Y 6/3) when dry; 5 mm iron oxide spots; 1 ft thick lime concentration near middle. (Probably is till of the Twin Buttes loop of the A-1 Mankato advance.)	3.8
Sharp contact.	
4. Loess, pale yellow (5Y 7/4) when dry; inconspicuous vertical jointing; with tube shaped ferruginous concretions, 2 mm in diameter	0.2-0.8
Sharp contact.	
3. Gravel, with a few boulders up to 1 ft in diameter	0.2
Sharp contact.	
2. Till, pale olive (5Y 6/3) when dry; 5 mm iron oxide spots; 0.1 ft. thick lime concentration at top; well compacted. (Probably is till of a recessional of the Lake loop off he A-1 Mankato advance)	5.0
Concealed interval	7.0
1. Till, pale yellow (5Y 7/3) when dry; 5 mm iron oxide spots. (May be same as unit 2.)	2.0
Base concealed.	20.7

Flint noted tube-shaped ferruginous concretions in many South Dakota losses (4). They probably developed around roots of the plants that caught the wind-blown silt. Lemke and Kaye (12) found a lime concentration associated with a boulder concentration between two tills in north central North Dakota. If Lemke and Colton were correct in saying that both the Twin Buttes and Long Lake loops belong to the A-1 advance of the Mankato, the interval represented by the gravel concentrate and loess must be fairly short. However, other evidence indicates that the till north and west of the Twin Buttes loop is Cary rather than Mankato in age.

The second exposure with two tills is 13 miles south of Dawson, 0.3 mile north of the southeast corner of sec. 16, T. 137 N., R. 72 W., on either side of State highway 3:

	Feet
3. Till, pale yellow (5Y 7/3) when dry; 5 mm iron oxide spots; lime mottling; contains more pebbles than unit 2. (Till of the Twin Buttes loop of the A-1 Mankota advance.)	3-8
Sharp irregular unciniformity.	
2. Till, pale yellow (2.5Y 7/4) when dry; very sticky when wet, hard when dry; iron and manganese oxide coatings on the faces of small, irregular, 1 in. spaced joints. (Probably Tazewell.)	10
Contact gradational.	
1. Till, similar to unit 2 but greyer. (Probably the unoxidized equivalent of unit 2.)	5
Base concealed.	maximum 25

The till of unit 2 is the only one seen in the county that differs conspicuously from all the others. Iron and manganese oxide on small irregular joint faces was also noted in the older (Iowan or Tazewell) of two tills described by Lemke and Kaye (12). It was also noted by Flint (3) on joint surfaces of Iowan till in South Dakota. Because this till is considerably different from other tills in the county, it is probably not of the A-1 or B-1 Mankato advances but is Tazewell in age (Tazewell (?) is exposed 30 miles southwest of this exposure). Flint found that most pre-Wisconsin till is more prominently jointed.

CONCLUSIONS

Tills of different glacial advances in the end moraine complex in south-central North Dakota are difficult to differentiate by lithology alone. Little difference exists between tills of the A-1 and B-1 advances of the Mankato substage. If the major direction of glacial flow in the different substages had varied, the till lithologies might have been different. In northwestern North Dakota, where the ice approached from more than one major direction, Howard (8) outlined the contact between two different tills by using stone counts. Stone counts also might be used in south-central North Dakota to differentiate substages. (Stone counts were made only on till of the Mankato substage in Kidder County.) However, the major glacial movements were probably all southwest, over nearly 200 miles of a single bedrock lithology. Secondary changes, such as oxidation and iron and manganese oxide on joint faces, may be of considerable help in distinguishing between Mankato and Tazewell tills. (Very strong jointing would suggest pre-Wisconsin till.) Except for the geomorphology of the till sheets, stratigraphic relationships, where they can be observed, are the most helpful in differentiating Wisconsin tills in south-central North Dakota.

A second conclusion that was reached concerns Shepps' statement (14) that the importance of the local source of till has been

overestimated. He thinks only 20 to 30% of till in northeastern Ohio has been locally derived. If by "local" he meant an area the size of Kidder County, the same conclusion can probably be reached for the tills of this county. Except for the thin till sheets with much incorporated outwash, little significant difference was seen in the percentage of shale in the coarse sand fraction of samples taken from up to 15 miles on either side of the contact of the Fox Hills sandstone and the Pierre shale. And, except for the influence of outwash, the percentages of sand, silt, and clay doesn't vary significantly on either side of the contact. If the local source were important, more sand should be found in tills of the Fox Hills-Pierre contact and more clay east of it.

LITERATURE CITED

1. American Society for Testing Materials, Nomenclature and definitions, standards methods, suggested methods, Philadelphia, p. 83-93, 1958.
2. Fenneman, N. M., Physiography of western United States, New York, McGraw-Hill Book Co., 1931.
3. Flint, R. F., Pleistocene geology of eastern South Dakota, U. S. Geol. Survey Prof. Paper 262, p. 35, 1955.
4. *Ibid.*, p. 39 and 97.
5. *Ibid.*, p. 128.
6. *Ibid.*, p. 136-137.
7. Flint, R. F., and others, Glacial map of the United States east of the Rocky Mountains, Geol. Soc., America, 1959.
8. Howard, A. D., Till pebble isopleth maps of parts of Montana and North Dakota, Geol. Soc. America Bull., v. 67, p. 1199-1206, 1946.
9. Lemke, R. W., and R. B. Colton, Summary of the Pleistocene geology of North Dakota in *Midwestern Friends of the Pleistocene*, N. Dak., Guidebook 9th Ann. Field Conf., p. 49, 1958.
10. *Ibid.*, table 1 and fig. 3.
11. *Ibid.*, fig. 5.
12. Lemke, R. W., and C. A. Kay, Two tills in the Donny brook area, North Dakota in *Midwestern Friends of the Pleistocene*, N. Dak., Guidebook 9th Ann. Field Conf., p. 94-95, 1958.
13. Moir, D. R., Occurrence and radiocarbon date of coniferous wood in Kidder County, North Dakota in *Midwestern Friends of the Pleistocene*, N. Dak., Guidebook 9th Ann. Field Conf., p. 109-110, 1958.
14. Shepps, V. C., Correlation of the tills of northeastern Ohio by size analysis, *Jour. Sed. Petrology*, v. 23, p. 46-47, 1953.
15. *Ibid.*, p. 35.
16. U. S. Department of Agriculture, Yearbook 1941, p. 117.

*Undergraduate Research Participants.

MODIFICATION OF MORPHINE HYPERGLYCEMIA BY IPRONIAZID AND RESERPINE

Sara MacDonald and B. DeBoer*

*Department of Physiology and Pharmacology
University of North Dakota, Grand Forks, North Dakota*

INTRODUCTION

It has been demonstrated repeatedly that morphine in doses of 2.5 mg/kg or higher is accompanied by a marked rise in blood sugar in the cat, dog, and rabbit. The peak effect occurs within 2 hours after a single injection and blood sugar levels are usually within the normal range after four hours. In the course of studies of the effect of several drugs on morphine hyperglycemia, De Boer, Engelstadt, and Gray (1), reported that iproniazid (Marsilid) or reserpine, given at the same time as morphine, reduced the level of morphine hyperglycemia. They administered different drugs to the same animals at weekly intervals without considering the possibility that the effect of a given drug might persist for as long as a week.

It has been observed that reserpine has its greatest tranquilizing effect only after several days of medication. It has also been shown that reserpine effects are maintained for a considerable period after cessation of the drug. It was decided, therefore, to study the possible delayed effect of this drug on morphine hyperglycemia. It has also been shown that the effects of iproniazid are long lasting. They were included in this study.

MATERIALS AND METHODS

In the present study New Zealand rabbits weighing 2 to 4 Kilo were used. A control blood sample was taken and the animal then given a single intravenous injection of saline or the desired drug or drugs. Following this, blood samples were taken after $\frac{1}{2}$, 1, 2, and 4 hours. Blood glucose was determined by the Somogyi-Nelson technique (2). Morphine was given in a dosage of 5.0 mg/kg, iproniazid 10.0 mg/kg and reserpine 0.5/kg (Two animals were given 1.0 mg/kg., but this and higher dosages were fatal in a few days). Concentration of drugs was adjusted so that the required dose was administered by 0.5 ml/kg intravenously.

RESULTS

In the group of animals reported here there was considerable individual variation in the hyperglycemic response to morphine. Some animals consistently gave low levels and others high levels of blood sugar following the same dose of morphine. However, since the same group of animals was used on successive weeks, the general trend of changes can be seen by the average readings.

The results are summarized in Table 1. In general the older animals (those used in the months September through December) showed lower blood sugar levels than the younger animals. Because the iproniazid series was run in the fall, these levels should be compared with morphine controls run at that time, since the controls ran lower in the fall than during the summer. The reserpine studies covered both summer and fall and these results are compared with the averages of all morphine controls.

It can be noted from Table 1 that the saline controls showed no change in blood sugar. The morphine controls showed the usual hyperglycemia. The average maximal blood sugar was 182 for the total group while the animals run in the fall had a peak reading of 128.

The average peak reading for the animals given both morphine and reserpine was 188 which is no decrease from the control. The hyperglycemic period was prolonged. Six of the 14 animals had readings of 150 mg% or higher after 4 hours, at which time the morphine series had only 2 still approximately 120 mg%. Morphine 3-7 days after reserpine showed peak readings much reduced but the hyperglycemia was not prolonged. When tested with morphine 2-4 weeks after reserpine or 6-8 weeks after reserpine the average blood sugar was below the morphine controls. In one case during this time was there a prolonged effect.

Iproniazid alone did not result in hyperglycemia. Morphine given with iproniazid resulted in a marked reduction in the blood sugar throughout the 4 hour period. One animal had a peak above 150 mg%. Morphine given 5-7 days after iproniazid produced some return in hyperglycemia with an average in maximal readings of 127 mg%. Animals tested at 2-3 week intervals showed an additional return toward the usual morphine response. However, those animals tested 4-5 weeks after iproniazid had blood sugars almost identical with values obtained the time they were first given both morphine and iproniazid.

DISCUSSION

The hyperglycemia of morphine has no clinical significance. However, it is of use and of interest in studying the pharmacodynamics of morphine itself. It is almost 100 years ago that Coze reported that carbohydrate metabolism was influenced by morphine. How morphine produces this effect is still unknown. In recent years it has been shown that many synthetic analgesics (pain killers) such as meperidine (Demerol), levorphanol, etc. also produce hyperglycemia and nalorphine, a morphine antagonist, inhibits the blood sugar changes. (3) (4).

It has been suggested that these changes are due to the release of epinephrine into the blood stream. Epinephrine (Adrenalin) injected into animals will result in hyperglycemia. Removal of the

adrenal medulla has been partially effective in reducing the hyperglycemia of morphine. Adrenergic blocking agents (those drugs which paralyze the nerve endings producing norepinephrine) also seem to have some action in reducing the blood sugar response of opiates. None of these attempts have been conclusive.

Reserpine has been shown to enhance the depressant effects of hypnotic agents. However, it appears to antagonize the analgesic effects of morphine. Its effects are long lasting. In this investigation we were unable to confirm the earlier report of De Boer, Engelstadt and Gray (1) of obliterating the hyperglycemic response of morphine. Rather reserpine given at the same time as morphine did not affect the amount of rise in blood sugar immediately, but in some cases even prolonged the hyperglycemic period. A decrease in hyperglycemia was noted after 3-7 days and continued to the end of the study, about 8 weeks. Campos (5) has very recently reported that in cats 48 hours after high doses of reserpine the catecholamine content (this includes all epinephrine-like compounds in the body) of the brain is low and the hyperglycemic response to morphine is reduced. However with lower doses of reserpine the catecholamine content of the brain was also low, but the hyperglycemic response to morphine was not reduced. Our results would suggest that reserpine depletes some compounds in the body faster than it itself is depleted and a period of time is necessary in order to rebuild them up.

Iproniazid is an amine oxidase inhibitor (one of a group of drugs which prevents the breakdown of epinephrine by removal of the amine (NH) group), and makes the material relatively inactive. A substance such as iproniazid should prolong the action of epinephrine within the body and thus prolong the effects of morphine hyperglycemia if these effects were due to the release of epinephrine into the system and its subsequent action upon the liver. It causes the liver to release glycogen which is then converted into blood glucose. Earlier reports (1) had indicated that iproniazid depressed the hyperglycemia of morphine. Our investigation confirms this work and indicates that subsequent injections of morphine result in a hyperglycemic response. However, 4-5 weeks after iproniazid the blood sugar response to morphine was again reduced. This needs further study. More recently it has been shown that the metabolism of epinephrine in vivo probably does not depend upon the amine oxidases in the body since breakdown of the catechol portion of the neurohormone takes place more rapidly.

SUMMARY

Iproniazid, an amine oxidase inhibitor, did depress morphine hyperglycemia when given concomitantly with morphine but may prolong the blood sugar rise. However during the subsequent 8 weeks after reserpine morphine hyperglycemia was decreased.

TABLE 1
EFFECT OF RESERPINE AND IPRONIAZID ON MORPHINE HYPERGLYCEMIA
BLOOD SUGAR MEAN (in mg%)

No. of Rabbits	Drugs	Dosage	Previous Drug	Elapsed Time	Control	½ hr.	1 hr.	2 hr.	4 hr.	Av. of Peak Read.
23	Saline				75	78	77	79	73	
16	Morphine	5 mg/kg			70	137	154	157	89	182
6	Morphine	5 mg/kg			75	95	120	121	107	128
	(fall)									
	Concomitant Drugs:									
14	Morphine	5 mg/kg			71	113	162	174	140	188
	Reserpine	0.5 mg/kg								
11	Morphine	5 mg/kg			78	86	105	108	90	114
	Iproniazid	10 mg/kg								
	Delayed effect:									
15	Morphine	5 mg/kg	Reserpine	3-7 da.	93	115	133	99	93	138
16	Morphine	5 mg/kg	Reserpine	2-4 wk.	75	110	125	130	91	140
11	Morphine	5 mg/kg	Reserpine	6-8 wk.	67	103	114	126	77	130
11	Morphine	5 mg/kg	Iproniazid	5-7 da.	65	93	120	112	87	127
11	Morphine	5 mg/kg	Iproniazid	2-3 wk.	78	98	118	127	87	139
18	Morphine	5 mg/kg	Iproniazid	4-5 wk.	76	105	104	101	89	114

Iproniazid an amini oxidase inhibitor did depress morphine hyperglycemia as previously reported. The effect did not appear to last a week, but the morphine hyperglycemia 4-5 weeks after iproniazid was less than normal.

BIBLIOGRAPHY

1. De Boer, B. O. Engelstadt, and Dorothy Gray, **Inhibition of morphine hyperglycemia**, Fed. Proc. Vol. 18, p. 382, 1959.
2. Somogyi, M. J., **A new reagent for the determination of sugar.**, J. Biol. Chem. Vol. 160, pp. 61-68, 1945.
3. Keith, E. F. and B. De Boer, **The effect of N-Allylnormorphine on narcotic-induced hyperglycemia**, Arch. Int. Pharm. et Therap. Vol. 101, pp. 481-487, 1955.
4. Keith, E. F. **A study of analgesic hyperglycemia**, Thesis U.N.D., pp. 1-69, 1956.
5. Campos, H. A., **Role of central nervous system catecholamines in morphine hyperglycemia**, Fed. Proc., Vol. 19, p. 272, 1960.

*Undergraduate Research Participants.

WATER SURFACE PROFILES

Dennis F. Meyer

Agricultural Engineering Department

North Dakota Agricultural College, Fargo, North Dakota

This report contains a step by step hydrologic analysis of a typical flat land area in the Red River Valley where flooding is a major problem. From this analysis the area inundated by the different CMSs may be measured thus providing a basis for estimating damage caused by flood waters.

The first step is to determine a number of Kd values ($Kd = 1.486AR^{2/3}$

_____) for the X-sec's* of the flood plain so that Kd curves

N
may be plotted. From these curves it is possible to solve the equation $Q = Kd S^{1/2}$, through trial and error procedures, $S^{1/2}$ and Kd being two dependent variables. Having found the slopes of a water surface, the elevation of the water surface at the X-sec may be determined. When this has been done for a representative number of X-sec's, the elevation for corresponding CSMs at the X-sec's are connected by a continuous line. This line defines the area inundated by the CSM in question.

This method is restricted to flat areas where the approach velocity to constrictions are negligible.

WATER SURFACE PROFILES

Water, the basic element for all life, may if not controlled do

considerable damage. It is the purpose of this report to show one of the procedures used in hydrologic analysis of a typical flat land area where flood water is a problem. This method is only applicable on flat land areas where flow velocities are negligible in considering discharge through constrictions and over roadways and determining subsequent elevations of flood level. At elevations obtained for various stages of flow, water surface profiles are drawn based on Manning's Equation. These successive profiles up-channel for corresponding CSMs represent the area inundated at the respective flood water stages.

From Manning's Equation for discharge:

$$Q = \frac{1.486AR^{2/3} S^{1/2}}{N}$$

Q = volume flow of H₂O in Ft³/sec.
 A = cross-sectional area*
 $R = A/P = \frac{\text{X-sec. area}}{\text{wetted perimeter}}$
 S = slope
 N = coefficient of roughness

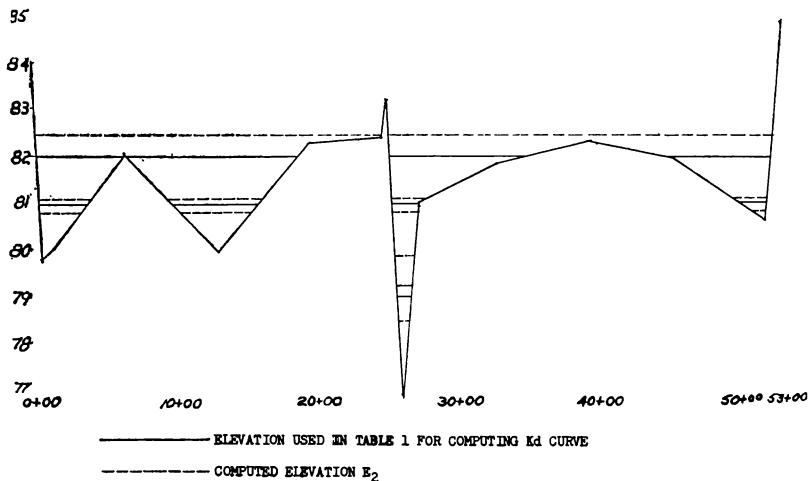
By definition, the dependent variable K_d is equal to $\frac{1.486AR^{2/3}}{N}$

and from Manning's Equation $Q = K_d \cdot \frac{\text{ft}^3}{\text{sec}}$.

$CSM = \frac{\text{ft}^3}{(\text{sec})(\text{miles}^2)}$; therefore (CSM) (miles²) = ft³/sec. or CFS.

Cross-sections of the flood plain** are obtained from a topographic map of the area. These X-sec's are drawn so they are as nearly perpendicular to the flow of H₂O as possible. From the

TYPICAL CROSS SECTION



sample X-sec. in fig. 1, the unknowns of the Kd equation can be determined or calculated except Manning's N which can be assumed for the condition of the channel. To determine the elevations at which Kd values for a Kd curve are desired requires some judgement. These points should be at an elevation where another area begins to contribute to the flow or any change in the shape of the channel occurs. When the Kd values have been determined (Table 1), they are plotted as a function of elevation on log-log paper, fig. 2. In flat land areas, where there isn't a well defined channel, one cycle is adequate vertically for elevations. Horizontal scale of Kd values will generally require four or five cycles. Listed in Table 1 are the Kd values computed as shown on the X-sec. (Fig. 1).

TABLE 1

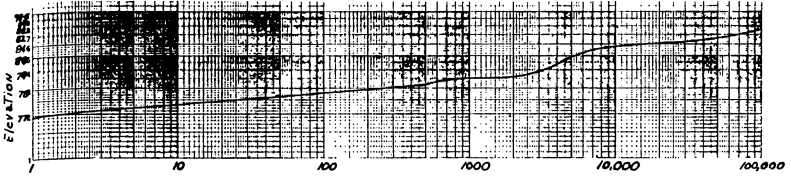
Elev. water surface	Area Sq. Ft. "A"	Wet. per. "p"	Hyd. Radius R = A/P	"N"	Kd = $\frac{1.486AR^{2/3}}{N}$
976.9	0	0	0	.06	0
79.0	79.2	70	1.13	.06	2,134
79.8	145	95	1.53	.06	4,781
80.4	350	600	.58	.06	6,040
81.0	898	1240	.72	.06	17,905
82.0	3128	3410	.92	.06	73,474

By starting at the lower end of the watershed, elevations of the water surfaces for corresponding CSMs will increase. A trial Kd value for an elevation (E_3) is selected from Figure 2. From the equation $Q = Kd S^{1/2}$ or $S = \left(\frac{Q}{Kd}\right)^2$, the slope (S) of the water surface may be obtained as shown in Table 2. Slope is then multiplied by the distance (L) between two successive X-sec's and this becomes H_1 . When E_3 , which is H_1 plus the elevation of the water surface at the previous X-sec. (E_1) is equal to E_3 the correct Kd value has been selected. These elevations are indicated on the X-sec. in Figure 1. When $E_3 > E_2$ a lower Kd must be selected or if $E_3 < E_2$ the Kd value must be increased, again using the corresponding elevations.

TABLE 2

CSM	DA Mi. ²	Q CFS (DA)(CSM)	L ft.	E ₁ ft.	E ₃ Kd. el.	Kd	S ^{1/2} Q/Kd	S (Q/Kd) ²	H (L)(S)	E ² E ₁ +H ₁
1	33.25	33.25	2000	76.03	78.5	950	.035	.00122	2.45	78.48
3	33.25	99.75	2000	76.90	79.2	2925	.0341	.00116	2.33	79.23
5	33.25	166.25	2000	77.45	79.8	4850	.0343	.00117	2.35	79.8
10	33.25	332.5	2000	78.40	80.8	9500	.035	.00122	2.35	80.85
20	33.25	665.0	2000	79.70	81.14	24,500	.0217	.00073	1.47	81.17
50	33.25	1662.5	2000	82.18	82.5	140,000	.0118	.00014	.28	82.46

It is evident that the slopes for a X-sec. change very little for CSM flows of increasing magnitude where channel conditions remain



uniform. This situation then enables one to make accurate approximations of the elevations of subsequent CSMs by two methods. (1) Add the change in elevation for one of the CSMs (this would generally be the first CSM since it is the first to be solved) to the elevation of the water surface of the previous X-sec. The resulting elevation, if not correct, may then be changed by changing the Kd

values so $E_2 = E_3$. (2) From the equation $Kd = \frac{Q}{S^{1/2}}$, Kd is determined.

It is then possible to obtain the corresponding elevation from the Kd curve. Adding S times L to the elevation from the Kd curve E_2 is obtained. If E_2 does not equal E_3 the necessary corrections may be made by adjusting the Kd value. The second method is preferred because changes that occur in slope generally establish a trend. It is then possible to predict the slope more accurately and quickly for subsequent CSMs by this method. (Table 2).

When a number of CSMs of adequate range are used, the results of the water surface profiles may be correlated with hydrographs for the area.

The elevations calculated, for a typical X-sec., Table 2, may be affected by an obstruction such as, a road containing bridges or culverts which may produce three conditions.

- (1) Culverts and bridges may have the required capacity so no constriction exists.
- (2) Culverts and bridges may reach full flow to produce orifice flow.
- (3) Culverts and bridges may reach full flow to produce orifice flow plus weir flow over the road.

Weir flow may be calculated by the equation:

$$Q = CLH^{3/2}$$

C = coefficient of discharge
length of flow over road

$$L = \frac{2}{H}$$

H = maximum flow depth

Orifice flow may be calculated by the equation:

$$Q = CMA_2 h^{1/2}$$

h = distance between water surface to the center of the orifice.

C = coefficient of discharge

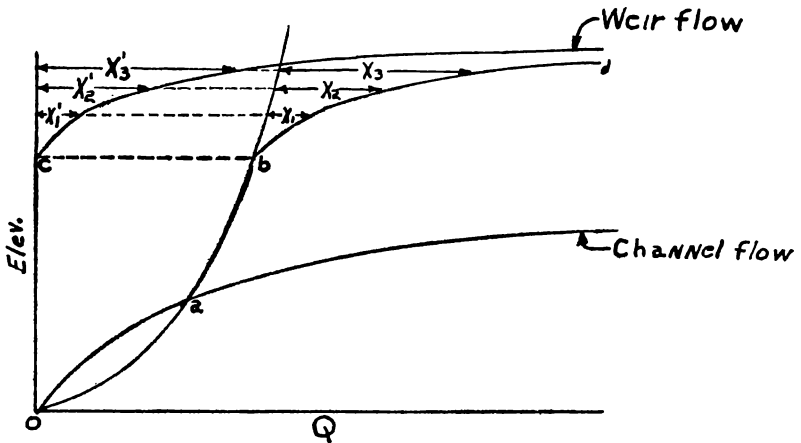
A_2 = area of orifice

A_1 = area of channel

g = acceleration due to gravity

$$M = \left[\frac{2g}{\left(1 - \frac{A_2}{A_1}\right)^2} \right]^{1/2}$$

Plotting the results of these calculations, the following curves are obtained.



Typical flow curve Figure 3.

From the shape of the curves, it can be observed that orifice discharge does not increase nearly as fast for an incremental rise in elevation as does weir discharge.

Point (c) is the elevation where weir flow over the road starts. Channel and orifice flow intersect at point (a) which indicates the beginning of the constriction effect. At point (b) which is at the same elevation as point (c) simultaneous weir and orifice flow begins. Observe that the distances X_1' , X_2' and X_3' are equal to X_1 , X_2 and X_3 and are at the same respective elevations. This transposes the weir flow curve and when added to the orifice flow curve as was done, the net flow is determined for various elevations. Curve (o a b d) becomes the discharge curve for the X-sec. and the constriction effect on the elevation becomes the vertical distance between the channel flow curve and curve (a b d) for those values of Q which apply.

The final elevations which are the computed elevations (E_2) plus the constriction effect, if one exists, are transferred to the topographic map. When the elevations, which are determined for corresponding CSMs, are connected by a continuous line the area inundated between the X-sec's. becomes defined. It is then possible to planimeter the area to get square feet or acres inundated.

REFERENCES

- Handbook of Hydraulics — McGraw Hill
- Hydraulics — George E. Russell
- Hydraulics Handbook — U S D A, Soil Conservation Service

*Cross-section may also be noted X-sec.

*Cross-sectional area may also be noted X-sec. area.

**Flood plain is that area of the potential inundation adjacent to streams.

SYNCHRONOUS DIVISION OF *TETRAHYMENA* *PYRIFORMIS* IN A BIPHASIC MEDIUM

Gail L. Schuster and John W. Vennes*

Department of Bacteriology

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

Due to its ability to grow axenically in a chemically defined medium, its accessibility in nature, and its adaptability to a variety of ecological situations, the ciliate *Tetrahymena* has become increasingly popular for growth and nutritional experiments (1, 2, 3, 4).

Within recent years the experimentation with the normal growth and size of the organism (7, 10, 6) led to temperature-induced synchronization of cell division by Scherbaum and Zeuthen (8, 9). Synchronization of cell division has contributed to the advancement in the field with studies of cell division, the mitotic process, and the overall process of growth and division with emphasis on individual cell growth (7).

An attempt was made in this investigation to obtain synchronization by temperature shifts (8, 9) in a biphasic medium (11). Viable rather than direct counts were made in the course of the experiment as a more reliable criterion of cell numbers (12). The system consisted of 100 ml. of broth medium rather than the smaller amounts used by others (8, 9) so that large numbers of cells, all in the same phase of division, would be available for other studies.

MATERIALS AND METHODS

The organism used was *Tetrahymena pyriformis* strain S obtained from Dr. G. R. Seaman, University of Texas, Galveston and was grown in 250-ml. flasks containing sterile liquid or biphasic medium. The liquid medium, in 100 ml. amounts, contained peptone 1%, glucose 1%, and yeast extract 0.1%. The biphasic system consisted of 50-ml. agar layer (peptone 1%, glucose 1%, yeast extract 0.1% and agar 2%) overlaid with 100 ml. of broth minus the agar.

Inoculated flasks were placed on a rotary type shaker at 28-33C. Stock cultures were transferred weekly and were grown at the variable room temperatures of 25-33C. in 50-ml., screwcap tubes

*Undergraduate Research Participation Program Fellow of the National Science Foundation.

containing 20 ml. of the following sterile medium: saline 0.85%, proteose peptone 1% and glucose 1%.

The dilution medium consisted of 99 ml. of 1% peptone. Plate counts were made at regular intervals with a semi-solid agar mixture containing agar 0.5%, yeast extract 0.1%, and sodium caseinate 0.75%. To prevent casein hydrolysis, the agar and the yeast extract and the casein were sterilized separately, cooled, and kept in a 42 C. water bath. The two liquids were mixed prior to pouring the plates. A black surface and an oblique light source aided the counting of the zones of precipitation. Only those plates containing 10-200 zones of precipitation were counted.

In the treatment for induction of synchronous division, culture flasks were partly submerged in a water bath for the 34 C. level. The lower temperature of 29 C. was obtained by placing the flask in a pan of water in a 29 C. incubator.

In preparation of starter culture for the experiment, flasks of biphasic medium were inoculated with 1 ml. of static stock culture, 48-120 hours old, and incubated at 30 C. for 66 hours. One ml. (ca. 10^8 organisms) of this culture was transferred to the flask of biphasic medium. This medium contained Penicillin (100 units/ml.) and Streptomycin (1 mg/ml.). The flasks were then treated for 6 hours with intermittent heat shocks ($\frac{1}{2}$ hour high and $\frac{1}{2}$ hour low) with these variations (29 ± 0.5 C.) (34 ± 0.02 C.) and then incubated on a rotary shaker. Counts were made every hour by pipeting 0.1 ml. of the culture into the first set of sterile Petri dishes for the 10^{-1} plates, 1 ml. of culture into 99 ml. of peptone for the 10^{-2} plates, and 0.1 ml. of this dilution for the 10^{-3} plates. The agar and casein were mixed and poured into the dishes. Gentle rotation of the plates was used to equally distribute the organisms, and the plates were incubated at 30 C. for 15-20 hours. Counts were made and growth curves were plotted.

Normal growth curves (no heat treatment) were also made.

RESULTS AND DISCUSSION

The use of biphasic medium for growth of *Tetrahymena pyriformis* strain S increased the number of viable organisms per ml. 20-30 fold over those grown in broth (12). The advantage of the prolonged rate of multiplication in a biphasic system (figure 1) suggested that this system may offer advantages over an ordinary broth culture. Although the synchronous division experiments were carried on for only 35 hours, this prolonged rate may have been a factor in the excellent results obtained.

The addition of Penicillin and Streptomycin had no effect upon *Tetrahymena* growth as noted by Loefer (5) but did inhibit chance bacterial contamination.

In the synchronous division experiments there was a period of simultaneous division followed by a levelling-off phase. Each level

is clearly defined by the doubling of the cell number as noted in table 1. The length of each level (figure 2) was from 4-6 hours as compared to 4.85 and 5.20 hours, the generation times for the same period in a normal growth curve.

Non-agreement with Scherbaum and Zeuthen (8,9) was found in the heat treatment of the first 6 hours. In this time a doubling of cell number did not occur but a pattern of a rise from 0-2 hours, a drop from 2-3 hours, a second rise from 3-4 hours, a second drop from 4-5 hours, and a final rise from 5-6 hours was observed.

The advantage of viable over direct counts (counting chamber) was that all cells counted were alive. Figure 3 shows a photograph of a plate containing thirteen zones or colonies of protozoa.

TABLE 1
Synchronous growth of *Tetrahymena pyriformis* strain S in a medium

Hours	Viable counts per ml broth ($\times 10^8$)	Average	Theoretical
		Counts $\times 10^8$	
0	0.8		
1	1.2		
2	1.6		
3	1.1		
4	1.3		
5	1.1		
6	1.3	1.2	1.5
7	2.7		
8	2.9		
9	3.0		
10	2.8	2.9	3.0
11	5.8		
12	5.5		
13	5.1		
14	5.6		
15	6.6	5.7	6.0
16	13		
17	11		
18	12		
19	13		
20	13		
21	*	12	12
22	21		
23	24		
24	23		
25	24		
26	21	23	24
27	45		
28	*		

29	47		
30	*		
31	46		
32	52		
33	44	47	48
34	77		
35	81	79	96

*No count

To increase accuracy, five plates of each dilution were made. However, if only two plates were used, the deviation in counts was not greater than that obtained with five plates.

Due to frequent withdrawals, less than 100 ml. of broth was not practical for our use. We have shown that six successive levels of division can be attained with large amounts of medium. This division synchronism would make available a large amount of cells for use in other growth and nutritional experiments. It is expected that 1-2 liters of broth could be used with this biphasic system.

SUMMARY

Tetrahymena pyriformis strain S was grown in a biphasic medium and counts of cell numbers were obtained by a viable count method. Six synchronous divisions were obtained by the use of temperature shifts. A relatively large amount of medium was used (100 ml.) so that sufficient cells in any stage of development might be obtained.

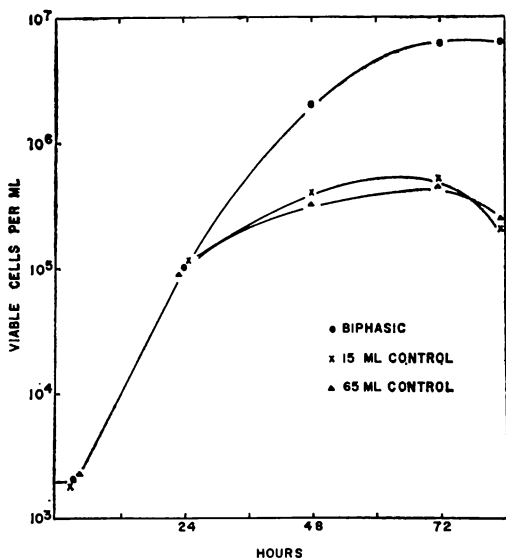


FIGURE 1. Growth curves of *Tetrahymena pyriformis* in broth and biphasic media.

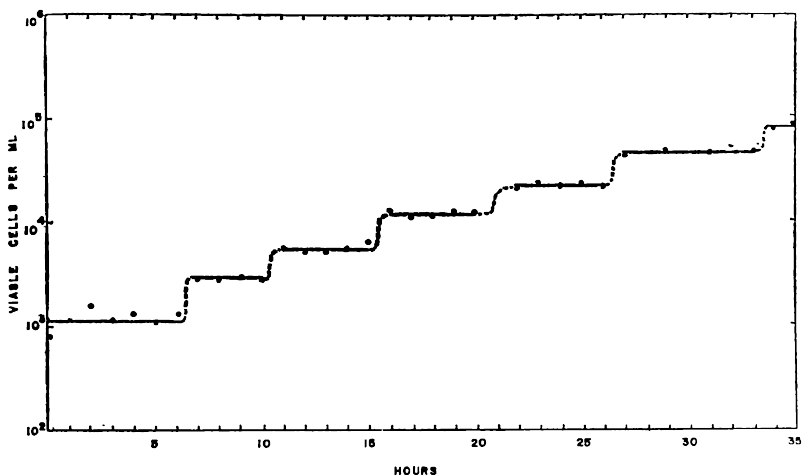


FIGURE 2. Growth curve of heat-treated *Tetrahymena pyriformis* in a biphasic medium.

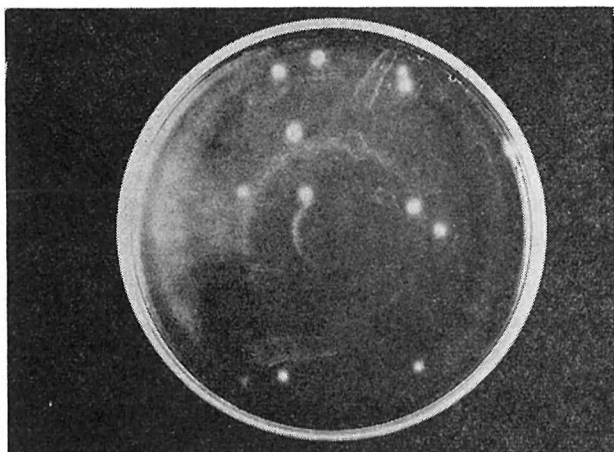


FIGURE 3. Colonies (zones of precipitation) of *Tetrahymena pyriformis* on a casein-agar medium.

REFERENCES

1. Corliss, J. O. 1954 The literature on *Tetrahymena*: Its history, growth, and recent trends. *J. Protozool.*, **1**, 156-169.
2. Elliott, A. and Nanney, D. L. 1952 Conjugation in *Tetrahymena*. *Science*, **116**, 33-34.
3. Elliott, A. 1959 A quarter century exploring *Tetrahymena*. *J.*

- Protozool., **6**, 1-7.
4. James, T. W. 1959 Synchronization of cell division in Amoebae. *Ann. of N. Y. Acad. Science*, **78**, 501.
 5. Loefer, J. B. 1949 Penicillin and Streptomycin sensitivity of some protozoa. *Texas J. Science*, **1**, 92-94.
 6. Loefer, J. B. 1952 Some observations on the size of Tetrahymena. *J. Morphol.*, **90**, 407-414.
 7. Prescott, D. M. 1957 Relation between multiplication rate and temperature in *Tetrahymena pyriformis* strain HS and GL. *J. Protozool.*, **4**, 252-256.
 8. Scherbaum, O. and Zeuthen, E. 1954 Induction of synchronous cell division in mass cultures of *Tetrahymena pyriformis*. *Expt. Cell Research*, **6**, 221-227.
 9. Scherbaum, O. and Zeuthen, E. 1955 Temperature-induced synchronous divisions in the ciliate protozoan *Tetrahymena pyriformis* grown in synthetic and proteose-peptone media. *Expt. Cell Research*, **Suppl. 3**, 312-325.
 10. Scherbaum, O. 1957 The division index and multiplication in a mass culture of Tetrahymena following inoculation. *J. Protozool.*, **4**, 257-259.
 11. Tyrell, E. A., MacDonald, R. E., and Gerhardt, P. 1958 Biphasic system for growing bacteria in a concentrated culture. *J. Bacteriol.*, **75**, 5-8.
 12. Vennes, J. W. 1958 The use of bacteriological methods for obtaining viable counts and increased growth of protozoa. *Bacteriol. Proceedings*, 139.
-

CONVERTING NORMAL INBRED LINES OF CORN TO THE CYTOPLASMIC MALE-STERILE CONDITION

William Wiidakas

The effect of the cytoplasm on the inheritance of male-sterility was speculated before the rediscovery of Mendel's Law. It was observed that in rare cases the development of the sperm nucleus of A was not possible in the cytoplasm of B, but the B sperm developed in the cytoplasm of A.

The cytoplasm influences or controls the inheritance of a wide variety of phenotypes in the plant and animal kingdoms. Six categories of cytoplasmic male-sterility listed by Edwardson are:

1. Intergeneric crosses
2. Interspecific crosses
3. Intraspecific crosses
4. Occurring apparently spontaneously
5. Doubtful cases
6. Occurring in the animal kingdom

Male-sterility inheritance in plants are classified as cytoplasmic,

cytoplasmic-genetic and genetic. This report, however, deals with one phenotype of male-sterility, which is influenced by hereditary mechanism in the cytoplasm.

Occurring apparently spontaneously. Perhaps the first spontaneous occurrence of the cytoplasmic inheritance of male-sterility in corn was demonstrated by Rhoades in 1931, 1933. This character was transmitted purely maternally. Cytoplasmic male-sterility occurs naturally in numerous varieties of corn. However, the most stable, known and useful sources of cytoplasmic male-sterility are Texas type-T reported by Roger and Edwardson (1952) which was discovered in several closely related Texas corn varieties. Same type of male-sterility was found by R. C. Mangesdorf in Mexican June variety and subsequently in Golden June and numerous other related varieties. Another USDA type-S, originally found in teoped crossed by linkage testers. These two types of cytoplasmic male-sterility may be classified as genic-cytoplasmic interactions which controls sterility or fertility. This cytoplasmic male-sterile condition is caused by pollen or spore abortion or the formation of a small shriveled pollen, probably due to the failure of starch production in the pollen grain following the meiosis.

At Fargo the conversion of ten normal inbred lines to the Texas type of cytoplasmic male-sterility began in 1952. After the initial cross of the cytoplasmic male-sterile stock by normal, backcrossings with the original normal pollen parent were continued for seven generations. Six inbred lines have been converted to a complete cytoplasmic male-sterile condition. One inbred is only partially male-sterile, while three inbreds have produced some fertile, semi-sterile and completely sterile plants. (Table 1). This type of sterility is influenced by environmental conditions and requires testing lines under a range of moisture and temperature conditions.

Some male-sterile inbreds tend to be shorter than their normal counterparts. Internodes are shorter above the ear and especially the last internode, the peduncle of the tassel. A few cytoplasmic male-sterile lines are more vigorous than their normal counterparts, probably due to chromogenic heterozygosity. Additional research is needed to determine the genetics conditioning variation among the male-sterile derived lines.

Among various operations in producing hybrid corn seed, detasseling, pulling the tassels from female parent plants in a crossing field is truly the most troublesome. Available help to detassel at the right time during all kinds of weather is difficult and expensive. By introducing cytoplasmic male-sterility into the seed parent, the detasseling process can be eliminated. However, the cytoplasmic male-sterile condition is transferred from an inbred line to the seed parent single cross and then to the final commercial hybrid corn. Without pollen production no seed set occurs in commercial fields. A source of pollen in the final crop may be introduced by one of

two ways: (a) mixing normal, fertile hybrid seed with sterile hybrid seed, or (b) restoring pollen fertility by incorporating pollen restoring chromogenes into the pollen parent used in the production of commercial hybrid seed. The latter is difficult to accomplish because the pollen fertility restorer genes must be transferred to the otherwise agronomically desirable inbred lines.

TABLE 1
Range in Fertility of Back-Cross
Ear Progenies of T Male-Sterility %

Inbred	F.	Sst.	St.	F.	Sst.	St.
ND167	0	0	100	0	3	97
ND203	0	0	100	0	1	99
ND255	0	0	100	0	1	99
ND283	0	0	100	0	0	100
CV3	0	0	100	0	0	100
WM13	0	0	100	0	1	99
A111	0	0	100	0	60	40
ND211	0	0	100	75	15	10
ND230	0	5	95	20	50	30
A9C	0	0	100	65	10	25
Io153 Reed.	0	0	100	0	0	100

RESTORATION OF MALE-FERTILITY TO CYTOPLASMIC MALE-STERILE CORN

R. E. Teschendorf, W. Wiidakas and P. C. Sandal

Department of Agronomy

North Dakota Agricultural College, Fargo, North Dakota

INTRODUCTION

The detasseling process of female rows in hybrid seed corn production can be eliminated by using cytoplasmic male-sterile seed parents. The Texas type (3) of cytoplasmic male-sterility, one of the most widely used sources, was used to convert several North Dakota adapted inbred lines to the sterile condition. This type is conditioned by the interaction of a sterile cytoplasm and non-fertility restoring genes. This condition can be transferred to normal inbred lines of corn by crossing a sterile line with a fertile inbred and subsequent backcrossing to the normal inbred sufficient times so that the newly derived line is like the original inbred agronomically but is cytoplasmically male-sterile. These inbreds serve as female or seed parents in crossing plots with normal inbreds used as pollinator parents. An undesirable feature of this method of producing hybrid seed is that the resulting hybrids are often male-sterile and

will not shed pollen in commercial fields. Thus, to utilize cytoplasmic male-sterile female lines requires a source of corn lines which, when used as male parents in crosses, will restore fertility to F_1 hybrids.

Current studies emphasize the relationship between nuclear genes and plasmagenes. Dodson (1) states that some geneticists consider the plasmagenes to be replicas of nuclear genes, which have been released into the cytoplasm, where they function as self-reproducing physiological agents. Thus the relationships between cytoplasmic and nuclear elements in heredity provide an important field of research. Duvick (2) studied segregation of restoring chromogenes in crosses containing WF9T sterile cytoplasm and found that there were at least two dominant complementary genes plus one or more dominant, duplicate modifying genes necessary for full pollen production under environmental conditions prevailing in Iowa during the test. Restoration of fertility in all crosses containing various inbred restorers with either Ind. 33-16 or Texas cytoplasm was found by Josephson (5) to be due apparently to a major dominant gene contributing to complete fertility plus at least one modifying gene contributing to partial fertility. The environment appeared to influence partial fertility. Jones, Stinson, and Khoo (4) observed progenies that were completely sterile in the field but were highly fertile when grown in the greenhouse during the winter. They thought that this was due possibly to differences in length of day.

Materials and Methods

The Texas type of cytoplasmic male-sterility was incorporated into several North Dakota adapted inbred lines by backcrossing for a sufficient number of years. The new lines obtained (like the original inbreds agronomically but cytoplasmically male-sterile) are referred to as male-sterile counterpart lines of these various inbreds. Eleven completely sterile counterpart lines of these seven inbreds were selected and crossed with three potential restorer inbred lines. Nine lines were crossed with Line 52, one with Line 153 and one with Line 211. The F_1 and F_2 progenies of each cross were grown in the field in 1959. The average F_1 population from each cross was about 150 plants whereas the F_2 populations contained from 300 to 500 plants each.

Individual plants within progenies were classified as male-fertile or male-sterile during the pollination season. Plants with anthers shedding pollen were recorded as male-fertile. Plants without discharged anthers and those possessing a few empty anthers were classified as male-sterile. Plants classified as fertile were verified at random by selfing from five to ten ears per row and obtaining good seed set.

RESULTS AND DISCUSSION

All the F_1 progenies were male-fertile indicating that the restorer lines carried dominant restorer gene complements for male-fertility. The number of fertile and sterile plants observed in the F_2 progenies derived from eleven F_1 lines are shown in Table 1.

TABLE 1

Frequency distribution of fertile and sterile plants in the F_2 generations of male-sterile X restorer crosses.

Cross	Number of F_2 Plants			Ratio	Chi ²	P
	Total	Fertile	Sterile			
A	312	231	81	3:1	.153	.50-.70
B	532	303	229	9:7	.1070	.70-.80
C	499	288	211	9:7	.4350	.50-.70
D	409	221	188	9:7	.8150	.30-.50
E	397	224	173	9:7	.0048	.90-.95
F	446	249	197	9:7	.0320	.80-.90
G	455	157	298	81:175	1.726	.10-.20
H	463	286	177		No Ratio	
I	318	218	100		No Ratio	
J	413	262	151		No Ratio	
K	362	253	109		No Ratio	
Total	4606	2692	1914			

A segregation pattern fitting an expected ratio of nine male-fertile to seven male-sterile plants in the F_2 of five crosses, (B, C, D, E, and F) indicated that two factor pairs conditioning male-fertility were inherent in the restorer lines. A dominant gene at each restorer-gene locus was necessary for restoring male-fertility. The observed F_2 segregation in cross A revealed that one gene pair with dominance controlled pollen restoration. In cross G, four factor pairs with dominance controlling the pollen-restoration phenomenon were identified. In this case, a dominant gene at each of four loci was necessary to restore male-fertility. The observed F_2 segregations in four crosses (H, I, J and K) did not fit normally expected ratios assuming dominance for male-fertility restoration in association with an hypothesis of one, two, three or four factor pair control. Apparently in these crosses a more complex genetic mechanism accounted for the restoration of fertile pollen.

SUMMARY

The use of cytoplasmic male-sterile seed parents in hybrid seed corn production eliminates the laborious and costly detasseling process. However, the male parents must contain restoring gene complements so that F_1 hybrid progeny will produce fertile pollen.

The Texas type of male-sterility was incorporated into various North Dakota inbred lines by the backcross method of plant breed-

ing. Sterile counterpart lines of these inbreds were crossed with three potential restorer lines.

All the F_1 off-spring were male-fertile indicating that the restorer lines carried dominant restorer genes for fertile pollen production. The segregation patterns of the F_2 progenies of seven crosses indicated that one, two or four factor pairs conditioned male-fertility. A dominant gene at each restorer gene locus was, in each case, necessary for restoring male-fertility. The inheritance of pollen restoration in four crosses was not determined.

BIBLIOGRAPHY

1. Dodson, E. O. 1956 Cytoplasmic inheritance. *Genetics, the Modern Science of Heredity*. pp 215-223.
2. Duvick, D. N. 1956. Allelism and comparative genetics of fertility restoration of cytoplasmic pollen sterile maize. *Genetics* 41:544-565.
3. Edwardson, J. F. 1955 The restoration of fertility to cytoplasmic male-sterile corn. *Agronomy Journal* 47:457-461.
4. Jones, D. F., Stinson, H. T. Jr., and Khoo, V. 1957 Pollen restoring genes. *Conn. Agr. Expt. Sta. Bul.* 610.
5. Josephson, L. M. 1959 Gene-cytoplasm interactions in 33-16 type male-sterility in corn. *Agronomy Abstracts, American Society of Agronomy*. p 60.

ORGANIC CHEMICAL MICROSCOPIC PART X*

A REVIEW AND SUMMARY

Ralph E. Dunbar

School of Chemical Technology

North Dakota Agricultural College, Fargo, North Dakota

The qualitative and quantitative identification of inorganic and organic compounds has long been, and undoubtedly will always remain, an important activity of professional chemists. The more or less stereotyped inorganic procedures have long plagued unsuspecting freshmen and sophomores in every university from coast to coast. The more fortunate elite, who have been exposed to traditional courses in organic qualitative analyses, soon discover that every unknown is a challenge that taxes the ingenuity of the analyst to the utmost. No set procedure of analysis is possible, and each attack is best based on past observations and a broad knowledge of organic functional behavior. But herein lies the fascination and future potentialities of the science.

Let us briefly consider the classical approach to the identification of a strictly new organic compound. Assuming the same to have been newly synthesized or recently isolated from natural

sources, we proceed to purify the substance by traditional methods including distillation, crystallization, sublimation, dialysis, or similar techniques. We can conveniently check the effectiveness of our attack by noting the sharpness and magnitude of the melting point, boiling point, density, index of refraction or other significant physical constants. A qualitative and quantitative elementary analysis, together with molecular weight determination, will enable us to assign a probable and logical formula. Tests for dominant functional groups will help in characterizing the probable class of organic compound involved. However, there still remains the last and two most difficult assignments; namely, the degradation and subsequent synthesis of the suspected compound, before its identity is firmly established.

Obviously the above approach would be too tedious and time consuming for practical use with known organic compounds that have already been adequately characterized. In such instances a more abbreviated procedure is frequently followed. This usually involves a preliminary physical and chemical examination as to purity and uniformity, followed by a determination of several significant physical constants. A qualitative elementary analysis, followed by a test of solubility behavior and classification reactions will usually reveal the type of substance involved. Consultation of comprehensive tables of physical constants now usually limits the unknown to a relatively few possibilities. The final confirmation usually comes with the preparation of a related derivative. The melting point, and the melting point only, is usually the only additional check made on the prepared derivative. These techniques are adequately outlined in numerous standard texts dealing with organic qualitative analyses (3, 11, 8).

It has long been recognized that the inclusion of any additional characteristic constant, as applied to an organic unknown compound or its derivative, adds greatly to the ease and reliability of the identification. For several years our laboratories have been exploring and expanding the use of organic chemical microscopy as an adjunct to organic qualitative analysis. Results to date have been most encouraging and fortunately many of the findings are now available in recent issues of the "Microchemical Journal" (4). Other releases are to appear in future issues. Additional research activity is now progressing or is contemplating for the immediate future. The possibilities for study in this area are almost unlimited.

Chemical microscopy is a term which was introduced by Emile Chamot (2) in order to differentiate between microchemical methods and microscopic methods. Microchemistry refers to chemical experimentation on a small scale, whereas microscopy deals with the observation of crystalline behavior and any other properties which are readily adaptable to examination through the chemical microscope. Photomicroscopy is a branch of microscopy which deals with

the photographic reproduction of various materials through the microscope. This is not to be confused with microphotography, the process of reducing ordinary photographs to microscopic size.

Perhaps the most significant and early comprehensive treatment of chemical microscopy should be attributed again to Emile Chamot (7) who organized the available data of this type near the turn of the century. This material was subsequently expanded and developed into a comprehensive system of inorganic qualitative analysis (2). Organic chemists soon began to make use of microscopy, although it must be admitted with shame, that we have long trailed the use and progress created by bacteriologists, pharmacists, geologists and many other workers in natural science. As early as 1897, Knorr (6) photographed the picrates of the ethanolamines. Putt, (10) in 1912, published results on microchemical tests for alkaloids. Microscopy soon found wide use in the testing and analysis of drugs and medicinals. Keenan (5) photographed the crystals and determined the optical properties of several amino acids. Bryant (1), over a period of years, has published several articles on the use of the microscope for optical crystallography. Recently quantitative determinations have been made through the use of chemical microscopy (9). The field has recently been surveyed in an eight-page symposium report in the April, 1949, issue of "Analytical Chemistry." Interest in this approach to analytical organic identifications appears to be increasing for a surprisingly large number of brief references to the technique are appearing in recent journals.

The original work in this laboratory was initiated by Ferrin (4) some twelve years ago, when he prepared, tabulated and photographed numerous derivatives of alcohols, phenols, amino acids, amines and metallic salts. Similar subsequent contributions were made by Knuteson (4) in his study of amine picrates; Moore (4) with anilides of carboxylic acids; Aaland (4) with semicarbazones of aldehydes and ketones; King (4) with aromatic halide derivatives; Farnum (4) with the amides of carboxylic acids; and Moore (4) with the *p*-toluidides and phenylhydrazides of carboxylic acids. Further work is either contemplated or under study dealing with urethanes, additional carboxylic acid derivatives, and other possible series. Another distinct advantage of chemical microscopy over many other older traditional methods of analysis is the extreme ease and speed of the techniques involved.

When it is remembered that at least thirty-five different derivatives have been recommended for the amines alone (3); sixty-nine for carboxylic acids; forty-one for aldehydes and ketones; forty for alcohols and related compounds; fourteen for aromatic halides; some fifty for amino acids; and a comparable number for almost any other organic series that might be named, it is rather obvious that there is still much work to do. We welcome sympathetic collaboration from all interested parties. It is predicted that with ade-

quate time and research this phase of organic qualitative analysis will develop into a comprehensive system, comparable to that now employed in inorganic chemistry and mineralogy. We propose to maintain an active interest and participation in this development.

A few typical photographs of characteristic derivatives will more adequately illustrate the fascinating possibilities than numerous words. These pictures are typical of hundreds that have accumulated during the course of the total study. It is hoped that these and many others may eventually be made available in a comprehensive volume dealing with "Organic Chemical Microscopy."

The techniques employed in this approach are not basically different from those used in most traditional courses of qualitative organic analysis using the several standard texts (3,8,11). It is merely proposed to add a microscopic examination to former procedures. A comparison can then be made with suspected knowns or a series of related photomicrographs. Usually a small amount of the organic derivative, already available, can be transferred to the microscope slide and examined. At most only one additional recrystallization is required. Another distinct advantage of this approach is the possibility of locating two or more related compounds simultaneously in a typical mixture. As this new technique develops, it is proposed to add other optical properties such as structure, extinction angle, density, index of refraction, and crystal angles.

In conclusion, this study has been a stimulating experience. Derivatives so far investigated and tabulated include the following:

- Semicarbazones of aldehydes.
- Semicarbazones of ketones.
- Amine picrates.
- Acid amides.
- Acid anilides.
- Acid *p*-toluides.
- Acid phenylhydrazides.
- 3,5-Dinitrobenzoates of alcohols.
- Dibenzofuran sulfonic acid metallic salts.
- Dibenzofuran sulfonic acid amino acid salts.
- Dibenzofuran sulfonic acid amine salts.
- Nitrated aromatic halides.
- Amides of acid halides.
- Amides of acid anhydrides
- Halogenated aromatic acids.

LITERATURE CITED

*For Part IX, see **Microchem. J.**, 4, 167-179 (1960).

1. W. M. D. Bryant, "Optical Crystallographic Studies with the Polarizing Microscope. I. Identification and Semiquantitative Determination of Acetic and Propionic *p*-Bromo-anilides in their Binary Mixtures," **J. Am. Chem. Soc.**, 60, 1394-7 (1938).

2. E. M. Chamot and C. W. Mason, "Handbook of Chemical Microscopy, Vols. I and II," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1940.
3. N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," Interscience Publishers, Inc., New York, N. Y., 1957.
4. R. E. Dunbar, et. al., "Organic Chemical Microscopy." **Microchem. J.**, **1**, 17-37 (1957); **1**, 209-22 (1957); **2**, 113-27 (1958); **3**, 65-82 (1959); **3**, 143-53 (1959); **3**, 491-505 (1959); and **4**, 59-72 (1960).
5. G. L. Keenan, "The Optical Properties of Some Amino Acids," **J. Biol. Chem.**, **62**, 163-72 (1924).
6. L. Knorr, "Ueber den Amidoathylalkohol (1,2-Aethanolamin)", **Ber.**, **30**, 909-18 (1897).
7. C. W. Mason, "The Services of Emile M. Chamot to Chemical Microscopy," *Ind. Eng. Chem., Anal. Ed.*, **11**, 341-3 (1939).
8. S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1945.
9. J. Mitchell, Jr., "Microscopic Identification of Organic Compounds," **Anal. Chem.**, **21**, 448-61 (1949).
10. E. B. Putt, "Micro-chemical Tests for the Identification of Some of the Alkaloids," *J. Ind. Eng. Chem.*, **4**, 508-12 (1912).
11. R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956.

THE ACETYLATION OF HYDROCARBONS WITH KETENE

R. E. Dunbar and Russell T. Arndts

School of Chemical Technology

North Dakota Agricultural College, Fargo, North Dakota

Previous studies in this laboratory have involved the acetylation of acids (5), alcohols (4), mercaptans (3), amides (7), imides (6), carbohydrates (4), and glycols (4), with ketene to form anhydrides, esters, thioesters, imides, triacyl ammonias, and polyacetates respectively. However, very little work has been reported on the acetylation of hydrocarbons with ketene.

Packendorff, Zelinskii, and Leder-Packendorff (9) reacted benzene and toluene with ketene, reduced the semicarbazones of their products, and obtained ethyl benzene and *p*-methylethylbenzene. Spring and Vickerstaff (11) repeated the acetylation of benzene with ketene and found not only acetophenone, but also *p*-ethylacetophenone among their reaction products. The ethyl group, in the latter product, was derived from the action of ethylene, a by-product in the production of ketene. Hurd (8) succeeded in acetylating naphtha-

lene and anisole. From naphthalene he obtained alpha- and beta-methylnaphthyl ketone and dinaphthyl. Anisole reacted with ketene to form three products: *o*-, *p*-, and *m*-methoxyacetophenone. Recently a more thorough investigation was conducted by Williams and Osborn (13), in which they obtained more inclusive results and improved yields. In addition to benzene, they acetylated naphthalene, tetralin, and diphenyl. The acetylation of naphthalene produced methylnaphthyl ketone, while reaction with tetralin yielded both alpha- and beta-tetrahydronaphthylmethyl ketone, and diphenyl reacted to form *p*-phenylacetophenone.

All the reactions described above were of the Friedel-Crafts type and used large amounts of aluminum chloride as catalyst. In order to avoid rearrangements due to the presence of the aluminum chloride, Williams and Osborn (13) conducted the reactions at zero degrees.

In the Friedel-Crafts reaction, acetyl chloride and acetic anhydride have been found to acetylate many substituted aromatic compounds to form methyl ketones. These same methyl ketones can also be produced by reacting these substituted aromatic compounds with acetic acid under proper conditions. Since ketene resembles acetyl chloride, acetic anhydride, and acetic acid in many of its acetylating properties, it appeared probable that ketene should react similarly to form these same methyl ketones. Likewise, ketene has been previously shown to react with benzene, toluene, naphthalene, tetralin, and diphenyl, and it seemed quite probable that it should also react with other substituted benzenes. For these reasons a study was made of the acetylation of substituted benzenes with ketene. Also the use of other catalysts was investigated briefly.

EXPERIMENTAL

The ketene for this study was generated by the pyrolysis of acetone in a Hurd-type lamp (12). All acetylations were conducted in a closed reaction vessel equipped with mechanical stirrer, combined gas absorption tube (1), with attached reflux condenser, and two inlet tubes for the addition of catalyst and ketene.

The several acetylations of aromatic hydrocarbons were achieved by placing 100 ml. of the liquid compound in the reaction vessel and diluting with 250 ml. of carbon disulfide. The reaction flask was maintained at 0° by immersion in an ice bath. During the operation of the ketene generator, 10 g. portions of anhydrous aluminum chloride were introduced every 15 minutes until a total of 180 g. had been added. An excess of ketene was added during 8.5 hours. The reaction mixture was then poured into 1000 g. of chipped ice containing 10 ml. of concentrated hydrochloric acid to hydrolyze the aluminum chloride and facilitate the isolation of the final product. The organic layer was separated from the water layer, the solvent removed by distillation under vacuum, and the residue

carefully fractionated under reduced pressure. The final methyl ketones were identified by means of their 2,4-dinitrophenylhydrazones (2), oxime or semicarbazone (10).

Ketene acetylations were satisfactorily achieved with toluene, ethylbenzene, propylbenzene, and isopropylbenzene. Yields were disappointingly low, however, largely because of mechanical losses in the isolation of the final product, and formation of other isomers. Other attempted ketene acetylations that require additional effort or improved techniques involved the use of fluorene, and mesitylene. Preliminary results indicate other catalysts including ion exchange resins, boron trifluoride, sulfuric acid, and sodium acetate may be more effective in promoting hydrocarbon acetylations with ketene.

DISCUSSION

This research extends the list of hydrocarbons that have been successfully acetylated with ketene. This was achieved by a Friedel-Crafts type of reaction which is similar to the traditional acetylations with acetyl chloride and acetic anhydride. The solvent, the catalyst, the temperature conditions, and the purification of the final product are also similar to the procedures used in the usual Friedel-Crafts reaction. Toluene reacted predominately in the para-position to produce *p*-methylacetophenone. Ethylbenzene similarly gave the *p*-methyl ketone. Propylbenzene and isopropylbenzene also acetylated preferentially in the para-position. The melting point for the 2,4-dinitrophenylhydrazone of *p*-propylacetophenone was found to be 154-156°, and apparently is reported for the first time.

LITERATURE CITED

1. Luther Bolstad and Ralph E. Dunbar, "Gas-Absorption Apparatus," *Ind. Eng. Chem., Anal. Ed.*, **15**, 498 (1943).
2. N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," Interscience Publishers, Inc., New York, N. Y., p. 391, (1947).
3. R. E. Dunbar and A. N. Bolstad, "The Acetylation of Mercaptans with Ketene," *J. Am. Chem. Soc.*, **77**, 4672 (1955).
4. R. E. Dunbar and Luther L. Bolstad, "The Acetylation of Organic Hydroxy Compounds with Ketene," *J. Org. Chem.*, **21**, 1041-3 (1956).
5. R. E. Dunbar and Floyd C. Garven, "Acetylation of Monocarboxylic Acids with Ketene," *J. Am. Chem. Soc.*, **77**, 4161 (1955).
6. R. E. Dunbar and Wayne R. Swenson, "Acetylation of Imides with Ketene," *J. Org. Chem.*, **23**, 1793-4 (1958).
7. R. E. Dunbar and Gerald C. White, "Acetylation of Amides with Ketene," *J. Org. Chem.*, **23**, 915-6 (1958).
8. C. D. Hurd, "Ketenes in the Friedel and Crafts Reaction," *J. Am. Chem. Soc.*, **47**, 2777-80 (1925).
9. K. Packendorff, N. D. Zelinskii and L. Leder-Packendorff, "Be-

- havior of Ketene in the Friedel-Crafts Reaction." *Ber.*, **66**, 1069-73 (1933).
10. R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., p. 351, (1956).
 11. F. S. Spring and T. Vickerstaff, "The Behavior of Ketene in the Friedel-Crafts Reaction," *J. Chem. Soc.*, **1935**, 1873-4.
 12. J. W. Williams and C. D. Hurd, "An Improved Apparatus for the Laboratory Preparation of Ketene and Butadiene," *J. Org. Chem.*, **5**, 122 (1940).
 13. J. W. Williams and J. N. Osborn, "Ketene in the Friedel-Crafts Reaction. I. Direct Acetylation of Aromatic Hydrocarbons with Ketene," *J. Am. Chem. Soc.*, **61**, 3438-9 (1939).
-

FREELIVING PROTOZOA OF CENTRAL GRAND FORKS COUNTY, NORTH DAKOTA

Elizabeth W. Caldwell

University of North Dakota, Grand Forks, North Dakota

The purpose of this work has been the classification and study of the freeliving protozoa of central Grand Forks County, North Dakota. I have dealt with only those protozoa which are found in water; this work doesn't include the varieties found in soil and other media. The only work of this kind on North Dakota waters to ever be published, and so far as I know the only one ever to be done at all, is that of C. H. Edmundson, who did a study of the Devils Lake Complex. His findings appeared in the **Transactions of the American Microscopical Society** in July 1920.

Bausch and Lomb Optical Company has made it possible for me to continue my work through the winter by lending me a very fine STA 629 microscope for the year. Their help has been invaluable.

There are two major reasons for undertaking this work. First, since there is no sharp distinction between protozoa and protophyta or between protozoa and metazoa or even between the major classes of protozoa themselves, progress in the taxonomy of this phylum contributes to the advancement of knowledge of the probable steps by which living things in general evolved.

Secondly, in genetics a knowledge of protozoa and often the protozoa themselves are used in the study of heredity and variation. It therefore stands to reason that if protozoa are to be of the greatest possible help in other fields, they themselves must first be classified and known.

There is quite a little difference of opinion among protozoologists as to whether the Mastigophora are protozoa or protophyta. They have a distinct nucleus which is a protozoan feature; protophytan

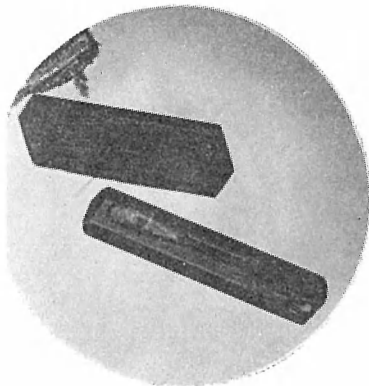
nuclei are not as readily discernible. Many Mastigophora reproduce by longitudinal binary fission, another protozoan characteristic. But some do so by multiple fission or budding. However, the method of reproduction is not a clear distinction between phyla because many members of Class Ciliata reproduce by transverse binary fission, which is a protophytic characteristic. The Mastigophora do contain chromatoplasts, a decidedly protophytic characteristic, but many of them are saprophytic and/or holozoic as well. I choose to include Mastigophora under phylum Protozoa since it appears to me that the protozoan characteristics outweigh that of chlorophyll content, which is the only protophytic characteristic found consistently throughout the class.

During the past year I have classified protozoa found in water and encysted in ice from thirteen various sites in the area, Townships 149-152 North, Ranges 49-56 West. Included in this classification are classes Mastigophora, Sarcodina, Ciliata, and Suctorina. Thus far I have identified species from all of these except Suctorina.

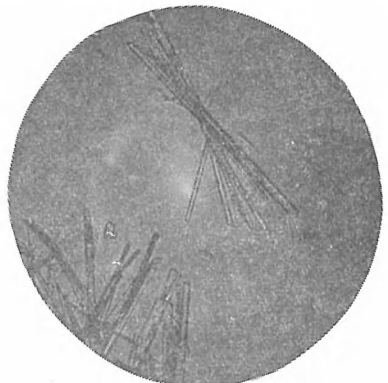
I have identified thirty species from orders: Amoebina, Euglenoidina, Holotricha, Peritricha, and Spirotricha; from families: Amoebidae, Amphileptidae, Astasiidae, Colepidae, Euchelinidae, Holophryidae, Loxodidae, Oxytrichidae, Parameciidae, Stentoridae, Strobilidiidae, Tracheliidae, and Vorticellidae.

In addition to about two dozen animals which I have been unable to identify at all, there are seven species, one each from genera Stentor, Strobilidium, Dinamoeba, Holophrya, Loxodes, Paramecium, and Vorticella, which I have thus far been unable to identify.

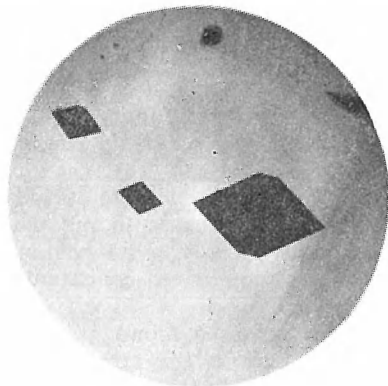
There is also an animal found in Townships 150 and 152 North, Range 50 West in early winter which is of special interest. It possesses the several flagella characteristic of Subphylum Ciliophora, Class Mastigophora and the adoral zone characteristic of Subclass Euciliata. It has the counter-clockwise adoral zone of Order Peritricha



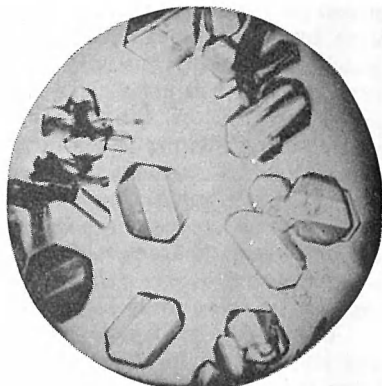
Aniline picrate



m-Bromoaniline picrate



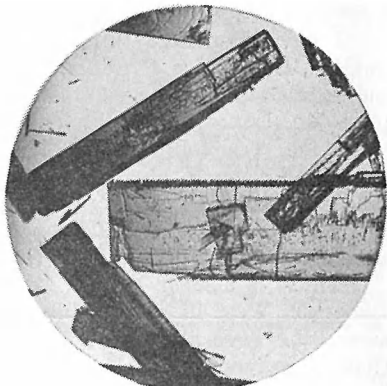
p-Bromoaniline picrate



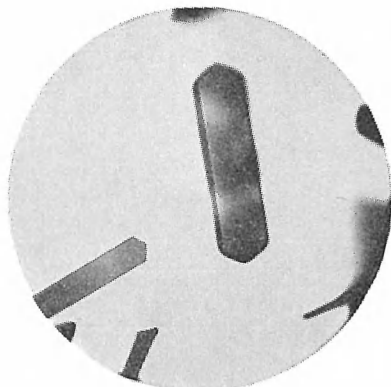
o-Chloroaniline picrate



2,5-Dichloroaniline picrate



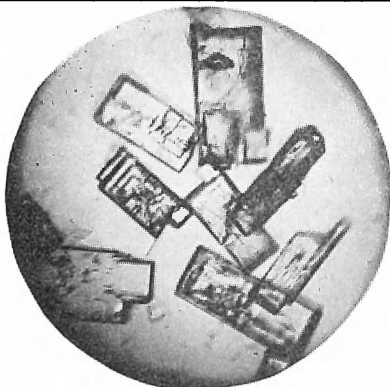
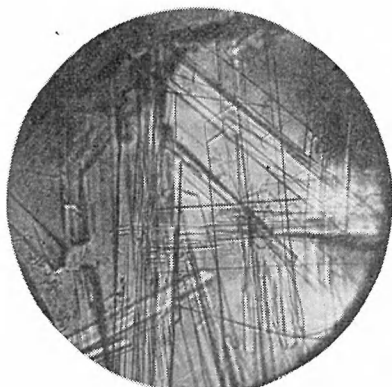
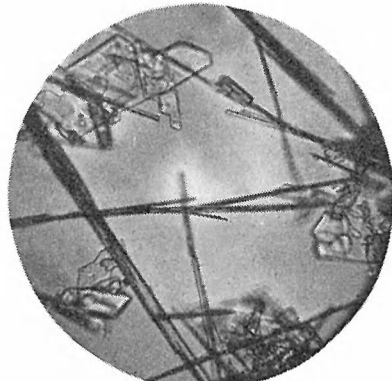
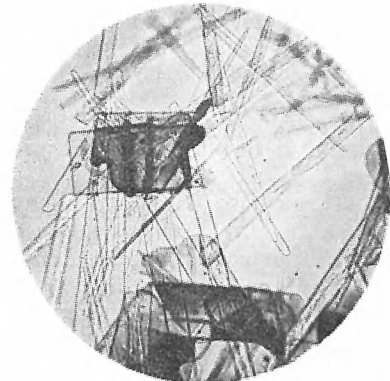
N-Ethylaniline picrate



N,N-Diethylaniline picrate

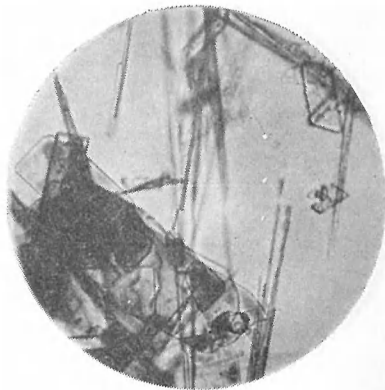


B-Naphthylamine picrate

**dl-Alanine****1-Hydroxyproline dibenzofuran-2-sulfonate****dl-Threonine dibenzofuran-2-sulfonate****dl-Phenylalanine dibenzofuran-2-sulfonate****Propyl 3,5-dinitrobenzoate
Benzyl 3,5-dinitrobenzoate****Isopropyl 3,5-dinitrobenzoate
sec-Butyl 3,5-dinitrobenzoate**



Propyl 3, 5-dinitrobenzoate
Isopropyl 3, 5-dinitrobenzoate



sec-Butyl 3, 5-dinitrobenzoate
Propyl 3, 5-dinitrobenzoate
Benzyl 3,5-dinitrobenzoate

but lacks the enlarged disk-like anterior region and does not have the barrel shape typical of the non-stalked members of this order. Aside from the adoral zone, it has the characteristics of Order Spirotricha, Suborder Hypotricha. It is flattened, with strong cirri restricted to the ventral surface. It has six frontal and five anal cirri on the dorsal side. These and the flexible ellipsoid shape of the body are typical of Hypotricha. However without exception the members of this order and suborder have clockwise adoral zones. These facts lead me to think that perhaps this is a new protozoa heretofore unclassified. However, before drawing any definite conclusions about this I would like to culture the animal and observe more closely its feeding habits and reproduction. So far I have been unable to sustain a culture of this strain.

C. H. Edmundson reported in 1920 that he had found no members of Order Radiolaria in the Devils Lake Complex. He attributed this to the decided lack of oxygen in the ooze at the bottom of the waters he studied. And so it is interesting to note that so far neither have I found any Radiolaria in the waters of Central Grand Forks County. I, however, am not prepared to offer any explanation of this.

Throughout the year I have noted a definite progression of orders. In May 1959 there was a profusion of Holotricha, Peritricha and Spirotricha. By June Peritricha alone were in abundance, although Holotricha and Spirotricha were still also present. Throughout July, August and September, Holotricha were predominant, but again the others were present. Gradually the number of Holotricha declined until in October I observed them only rarely. However, Spirotricha were again abundant. In November Family Oxytricha were the only Spirotricha present, but they were profuse. Here, too,

Eugenoidina appeared in abundance. These were present in lesser degree in all samples taken during the winter. In ice samples taken in December were many Amoebina. January and February's ice contained many members of Order Spirotricha, Family Strobili-diidae, Genus Strobilidium. Now that the ice is thawed I find once again the Holotricha and Peritricha with many Vorticella.

It puzzles me that ice which presumably froze at approximately the same time should yield different orders in profusion at various times throughout the winter. I have found no satisfactory explanation of this and I feel it is a phenomenon which deserves more observation and study. Also, on the experimental side, the Hydrogen Ion Concentration, pH, of the various samples taken ranges from 6.2 to 7.8, an optimum range for most protozoa.

There are a number of things aside from continued classification which need to be done. The temperatures of the various depths throughout the year should be determined as should the mineral, bacterial, and vegetable content. The possibly new animal and the progression of orders found in ice during the winter need more study. Thus far, I have identified thirty species of freeliving protozoa from the waters of central Grand Forks County and have determined a definite progression of orders throughout the year.

DEVELOPMENT OF THE SECONDARY BRONCHI IN THE LUNGS OF THE HOUSE SPARROW, *PASSER DOMESTICUS* (LINNAEUS)

John M. Delphia, Ph. D.

North Dakota Agricultural College, Fargo, North Dakota

The purpose of this paper is to present the origin and development of the secondary bronchi in the house sparrow. Embryos were studied by means of serial sagittal, frontal and transverse sections at twelve hour intervals from three through thirteen days incubation (hatching time). Camera lucida tracings, photomicrographs and tracings of direct projections were used in the construction of diagrammatic reconstructions of the bronchi in the lungs at regular intervals during incubation.

The secondary bronchi are divided into three groups according to the place of origin and general destination of the bronchus within the lung. There are in the house sparrow five mesio bronchi, nine dorsobronchi and nine lateroventrobronchi. The time of origin, place of origin and general destination of each secondary bronchus is established. Tertiary and lesser bronchial subdivisions related to airsac origins or connections of the lungs to the airsacs are also described.

The time of origin, place of origin and general relationships of the secondary bronchi in this species are compared with their counterparts in the domestic fowl and domestic duck.

EFFECTS OF MOTION AND POSTURE UPON HEARTBEATS UNDER LABORATORY CONDITIONS

Ernest V. Estensen

Department of Education and Psychology

North Dakota Agricultural College, Fargo North Dakota

This experiment originated in the need for designing a series of undergraduate laboratory activities focusing upon the various forms of human sensitivity. Most research dealing with the human senses has centered upon vision and hearing, with considerable material also available for experiments dealing with gustation, olfaction, the four skin senses, kinesthesia, and vestibular sensitivity. Current textbooks in general psychology also mention, but barely mention, organic sensitivity, limited perhaps to a paragraph or a page.

Munn devotes half a page to organic sensitivity, and the following extract from this source may serve as a representative definition of organic sensitivity:

Organic sensitivity is sensitivity of the visceral and other internal organs of the body cavity. The viscera include the stomach, intestines, internal sex structure, and kidneys. Non-visceral inner structures are the throat, lungs, and heart. . . . Activities of the internal organs excite sensory fibers, sending nerve impulses into the central nervous system. (2)

Some of the experiences associated with this sensitivity, as listed in the same source, are thirst, hunger, nausea, bladder and intestinal tensions, sexual cravings and thrills, suffocation, and the feeling of fullness.

In explaining organic sensitivity, Dashiell (1) refers to "receptive nerve-endings in tissues of the alimentary canal, lungs, heart, and liver." He associates nausea with the digestive tract; suffocation with the respiratory system; heart panic with circulation; sex appetite with the sex apparatus; and other experiences involve distention of hollow organs such as the stomach, colon, and bladder.

In setting up an experiment to illustrate organic sensitivity, it was clear that the problem would have to be severely delimited for a laboratory experiment; thus, the heartbeat became the focus of the experiment. Since heartbeats are of the very essence of life, inherent interest in heart action assured good motivation for the experiment.

Woodworth and Schlosberg (4) define **heartbeat** as follows: "a wave coursing through the arteries which can be felt and counted without instrumental aids or recorded in detail by use of the sphygmograph." Their extensive review of the literature suggests various factors which impinge upon the present experiment. Assuredly, one's heartbeat is "exceedingly sensitive to all sorts of psychological

changes." It is suggested that a **basal heart** rate should be that of a person lying awake in the morning, which was found to be 61 for males and 70 for females. For ordinary indoor occupations, it was found that the male average was 78 and the female was 84, with wide variations. Studies are cited to show effects of startle, of surprise, of shifts of attention, and of a loud whistle or a pistol shot to which the heart responds with a sudden jump. Mere expectancy also raises the pulse rate. A study at the University of California is cited in which children ran up four flights of stairs, which resulted in greatly increased heart activity. Mental activity, if there is some muscular activity involved, results in an increase, although mental arithmetic is said to result in no noticeable increase unless competition against others or against time is introduced. One study reported that the heart rate of 13 students was very high just before and after a six-hour examination. Another study dealt with 17 college seniors whose pulse rose 23 before and five after the first day of a two-day comprehensive examination; 16 before and 4 after the second day of the examination. In another study, a trick chair was used to study the effect of surprise. As for the effect of sex, a man listening to a phonograph record of a song of his loved one upped his pulse from 64 to 121. Other procedures dealt with movies of love scenes, of anger-provoking scenes, and of fear-arousing scenes and their effects upon the heartbeat.

Needless to state, the present experiment was decidedly non-medical, the data submitted are based on student reports, and, as previously stated, the experiment was designed primarily to illustrate one form of organic sensitivity, of which the heartbeat by definition is at least one phase of organic sensitivity. This report consists of two sets of data, as indicated by the title: (1) effects of motion upon the heartbeats of 185 Ss, and (2) effects of variations in posture upon the heartbeats of 32 athletes.

As revealed in Table I data, the effect of motion was determined on the basis of climbing two flights of stairs in old Science Hall. (Incidentally, this involves 32 risers of 7" each on 12" base.) After suitable orientation, the mean normal pulse of 185 Ss was

TABLE I

Effects of Stair-Climbing Upon Heartbeats of 185 Ss

Pulse	Mean
Normal	74.8
Pulse After Climb	98.3
Increase	23.5
Pulse after 120	75.3
Difference	0.5

computed and found to be 74.8, which should represent a fair sampling of college population as to age and sex. Immediately after climbing the two flights of stairs, the mean normal heartbeat mounted to 98.3, an increase of 23.5 heartbeats. After returning to the laboratory within two minutes, the mean normal heartbeat was again determined and found to be 75.3, indicating only a negligible difference of .5, or half a heartbeat. Averages, of course, do not reveal deviates of whom there were a few, one of whom counted 19 above normal for more than five minutes, and another who used a special technique to slow down his count, thus offsetting each other in the means. To apply statistical formulas to determine the significance of a change of 23.5 would be to elaborate upon the obvious. In part 2, however, the formulas (3) are needed and used.

As indicated in Table II data, the normal pulse of 32 athletes was recorded, after which they were tested in varying postures. Each position was held for one minute. Normal relaxed sitting pos-

TABLE II

Effects of Variations in Posture Upon Normal Heartbeats
of 32 Ss After One Minute

Mean Normal Pulse of 32 Ss	72.7
Position	Mean Change
Sitting (relaxed)	-1.9
Sitting, Feet Up	-3.0
Lying Down (prone)	-5.1
Standing (at attention)	+8.9

ture showed a decrease of 1.9, which by statistical formula was found to be slightly more than chance. Sitting with feet at level of buttock showed a decline of 3.0, significant at the 5 per cent level. The prone position showed the greatest reduction with a mean of 5.1, significant also at the 5 per cent level, but almost at the one per cent level. The standing position showed a mean **increase** of 8.9, definitely significant at the one per cent level.

Incidentally, the mean heartbeat of the 32 athletes, all men, was 72.7, or 2.1 less than of the 185 Ss in the first experiment, which might have been expected, since men's hearts are known to beat more slowly than women's hearts.

Some Ss become concerned about the pulse count. Is there a norm? At 18 breaths per minute, with four pulsations per breath, the count is 72. But patterns of heartbeats are complex and variable and do not conform to such simple arithmetic, as indeed the data in these experiments have amply indicated.

To draw the conclusion that the variations in heartbeats as recorded in these laboratory experiments arose solely from changes

in motion or posture, as one might be tempted to do, would hardly be justified in the light of numerous other factors previously mentioned. The normal pulse of 74.8 might have been slightly affected by expectancy of the Ss although they were accustomed to laboratory orientation. Limiting the second part of the experiment to 32 athletes necessarily suggests slower heart action. In short, however, one may at least infer that motion and posture do play a part in variations of heartbeats.

With submission of data, one should perhaps be safer to draw a concluding 30 to his report, but commentary is hard to resist. For example, the tired businessman who puts his feet desk-high may be answering the silent voice of his heart for some kind of respite. Moreover, since heart ailments of various kinds constitute the first cause of death, there may be some lifelong patterns of behavior which cause male hearts to fail sooner than women's hearts. A recent statistic showed a 7-year difference against the male. To close the gap, perhaps potions of siesta, fiesta, and manana might be effective. Certainly, 65-year-old men wisely avoid golfing in mile-high Colorado. Instead of escalators in this hallowed hall of learning, where we meet today, the old-timers learn to climb at measured beat and slow. Too, the predestination thinker comments that to each man is allotted a certain number of heartbeats, so why use them too soon. Against this view is that of those who insist that exercise of the heart tends to keep it strong, even into the mature stages of life.

If these observations be unjustifiably tangential, the reason is that the heart was the center of this experiment; if conclusions seem unwarranted because of the numerous factors involved, consider that the heart is the center of life's activity. In any event, the main purpose was to design an experiment which would illustrate organic sensitivity, regardless of outcomes and with or without conclusions or implications.

REFERENCES

1. Dashiell, J. F., *Fundamentals of General Psychology*, Houghton Mifflin Co., N. Y., 1949, p. 249.
2. Munn, Norman L., *Psychology*, 3rd Ed., Houghton Mifflin Co., N. Y., 1956, p. 385.
3. Underwood, Benton J., et al, *Elementary Statistics*, Appleton-Century-Crofts, Inc., N. Y., 1954, pp. 114-117, 170, 230.
4. Woodworth, R. S., and Harold Schlosberg, *Experimental Psychology*, Rev. Ed., Holt, N. Y., 1954, pp. 160-173.

AN ALL METAL VACUUM DEPOSITION SYSTEM UTILIZING AN EVAPOR-ION PUMP

E. N. Mitchell

Department of Physics

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

The impetus for making the vacuum system which is to be described here was a desire to produce thin ferromagnetic films which are made by vacuum deposition and condensation of molten metal onto a heated substrate (usually glass). These films are the order of one centimeter in diameter and vary in thickness from several hundred to several thousand Angstroms. This work was initially instigated on permalloy (82% nickel, 18% iron) because these films appear to be useful in computer applications, particularly for memory storage purposes.² The films as made normally are anisotropic in their magnetic properties in the plane of the film. If an orienting field is applied parallel to the plane of the film, then the hysteresis loop of the film along the direction of the orienting magnetic field (easy direction) is essentially rectangular. At right angles to this direction and in the plane of the film (difficult direction) the hysteresis loop is the normal nearly closed loop of bulk ferromagnetic materials.² The coercive force is usually of the order of Oersteds as is the field required to saturate the films in any direction in the plane of the film. The speed with which the film can be switched from one remanent state in the easy direction to the other, and the rectangular nature of the loop are the basic reasons for their potential as memory elements. Our interest in the films is a scientific one in which we are trying to understand the quasistatic properties of the films. It is to supply the films for this investigation for which this vacuum system was designed.

If the physical investigations are to be meaningful, the films should be made in a minimum of residual atmosphere. This will require first, that a low ultimate vacuum be obtained. In addition, because the film is made by melting and evaporating metal from a melt, the pump must be able to handle large gas loads during the time of the deposition of the films. Finally, if a vapor diffusion pump is used, the system will have to have a cold barrier (cold trap) between the pump and the vacuum chamber. This is commonly done with a re-entrant chamber which is cooled by liquid nitrogen which is not readily available in our laboratory.

In consideration of all of these factors, it was decided to build a vacuum system about a relatively new type of pump called an evapor-ion pump.¹ This pump utilizes two principles in its operation: chemical action of an evaporated metal, and ionization. Most of the

pumping action at pressures above 10^{-7} mm. Hg. is accomplished by chemical action of titanium vapor with the chemically active constituents of the environment. The vapor is produced by evaporating titanium wire which is fed onto an electron bombardment heated metal post. After chemical action, the vapor condenses on the cooled walls of the pump. In order to pump the inert gases and to reach pressures below 10^{-7} mm. Hg., ionization grids are oriented in the system so as to ionize these residual gases which are then driven to the walls of the pump chamber by a potential gradient where they are then plated over by the titanium metal. This type of pump eliminates the necessity of a cold trap inasmuch as the metal can be plated out on a baffle at room temperature. One needs a fore pump (mechanical) to rough the system down, but once this is accomplished, the fore pump can be valved off from the system and all oil kept out of the evaporation chamber.

APPARATUS

Figure one shows a schematic of the vacuum system as incorporated in our laboratory. The upper part of the cylinder to the left represents the vacuum cell. Directly below it is the vapor pump. To the right, the roughing pump line leads to the roughing

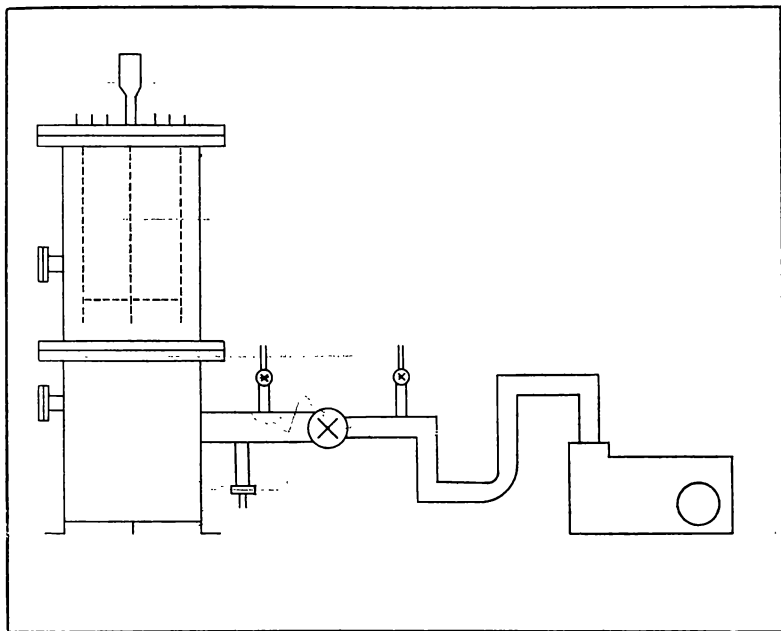


FIGURE I—Schematic diagram of all metal vacuum deposition system.

valve, the fore line cold trap which is cooled with a solution of dry ice and alcohol, and the fore pump. Once the vapor pump is in operation, the roughing valve is closed and this source of contaminant is removed from the system.

The vacuum cell is a non-magnetic stainless steel cylinder 30 inches long and 12 inches in diameter to which flanges of the same material have been welded. Provision is made on the side for a viewing port similar to one in the pump. The top of the cell consists of a disk of non-magnetic stainless steel through which holes have been made to accommodate electrical feedthroughs and a vacuum gauge. Three stainless steel rods are attached to the underside of this disk which serve as the mounting framework for the evaporation deposition experiments when the system is to be opened, the top disk is unbolted and the entire assembly lifted out of the cylinder.

The windows, the vapor pump and vacuum cell, and the cylinder and top disk are fastened together by flanges in which aluminum wire is used as the gasket material. The electrical feedthroughs are of the flanged Kovar type and are fastened into the top disk in a similar manner by means of hold-down rings. The components in

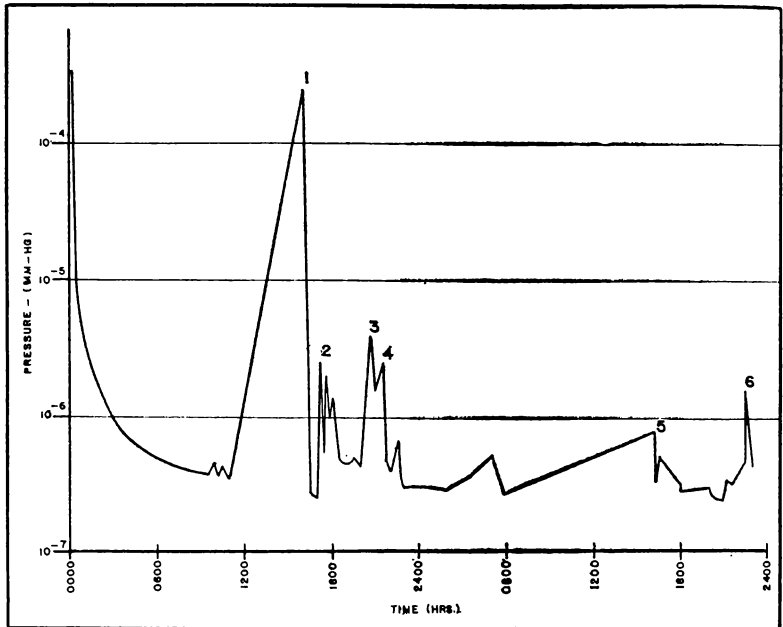


FIGURE 2—Typical pumping curve for all metal vacuum deposition system.

the fore-line are threaded and sealed with glyptol. The valves are either of needle type or utilize neoprene gaskets in the seats. This

means that the high vacuum side of the system is either of metal or glass and, hence, suitable for outgassing by bakeout techniques. In addition, the high vacuum side of the system is free of organic materials which would be a source of high vapor pressures and contaminants. It is to be recognized, however, that while the low vacuum side of the system is a source of some organic vapors (neoprene valve washers and glyptol paint), these vapors will not readily get up into the evaporation cell proper.

The fore pump in the system is of the compound type. Two vacuum gauges are incorporated into the system. One, a thermocouple gauge which measures nominally from atmospheric pressure down to 10^{-3} mm. Hg. pressure, is placed in the foreline and the second, an ionization gauge which measures nominally from 10^{-3} mm. Hg. down to 10^{-9} mm Hg. is placed in the top disk of the vacuum cell. This arrangement has proven more than adequate for the desired measurements.

RESULTS

This system has been operating successfully in our laboratory with a moderate amount of maintenance (on the evapor-ion pump). In order to demonstrate the operation of this system, a typical run was made in which the system was pumped down, outgassed, and then the operations carried out which would be required to make a metal film. This run was atypical in the sense that it was extended over a period of 48 hours, whereas our normal runs are usually of about eight hours duration. Figure two shows the pressure of the system as a function of time. This curve starts with the starting of the evapor-ion pump. Prior to this, about an hour was spent in roughing the system down to the required starting pressure for the evapor-ion pump. As can be seen from the curve, after ten hours of pumping the system was down to 3.8×10^{-7} mm. Hg.; however, the system had not been outgassed and the application of any heat to the interior parts would cause the pressure to rise rapidly. These occluded gases had to be removed before the evaporation of the alloy could be performed in a good vacuum. The spikes that appear in the diagram are in general due to various outgassing operations or controlled changes in the pumping condition. Some of these spikes will now be discussed in detail.

The large rise which is designated (1) in the figure occurred during the night when no one was present to tend the apparatus and a momentary power failure caused safety relays to turn the system off. As can be seen, it took less than one hour to get the system back down to 3×10^{-7} mm. Hg. The sharp rise in pressure at (2) is due to the heating of the substrate holder which held the slides on which the evaporation was to be made. The sharp rise

in pressure at (3) was due to the heating of the filament used for the evaporation of the metal. The increase in pressure at (4) was due to the heating of the body of the pump. In each of these cases the rise was due to occluded gases which were on the surface of the pump parts and driven off by the heating of these surfaces. The rise in pressure at (5) was due to the fact that the pump was being pumped by the ion process alone (no titanium metal being evaporated). As the metal on the pump surfaces was being covered by impurity atoms the pressure rose and then fell sharply again as more metal was evaporated.

As we started to prepare for the evaporation at about 2100, the pressure did not rise above 5×10^{-7} mm. Hg. until the metal was actually melted and being evaporated (6). The highest pressure during the evaporation was 1.6×10^{-6} mm. Hg. at the end of the evaporation.

CONCLUSIONS

It is concluded that this system, as it stands, is capable of producing films in an environment of the order of 10^{-9} mm. Hg., if adequate bake-out precautions are taken. If the bake-out procedure is not followed, the pressure rise is to the order of 5×10^{-5} mm. Hg. In this sense, the system seems to accomplish the goals set for it. It is felt by the author that an even more thorough bake-out procedure, which might include a technique called ion-scrubbing, should result in being able to produce films in an environment of the order of 10^{-7} mm. Hg. It is realized that this vacuum does not approach that achieved by sealed glass systems (of the order of 10^{-9} mm. Hg.) but the flexibility of this system, its ease of mounting and demounting, and its large size are felt to be worth the sacrifice.

Plans have been made and the parts are on order for including the ion-scrubbing process in the cleaning procedure for our vacuum system. This process removes occluded gas from the surface of the system by first ionizing the residual atmosphere and then bombarding the surfaces with these ions by means of an accelerating potential appropriately introduced into the system.

The author wishes to thank his colleagues who have helped in the construction of the apparatus and the University of North Dakota for its cooperation in this venture. Credit is also due the National Science Foundation who in part supported this work.

BIBLIOGRAPHY

1. R. H. Davis and A. S. Divatia, *Rev. Sci Instr.* **25**, 1193 (1954).
2. C. D. Olson and A. V. Pohm, *J. Appl. Phys.*, **29**, 274 (1958).

DOMAIN STRUCTURE OF THIN IRON NICKEL FILMS AS A FUNCTION OF FILM THICKNESS

(A Progress Report)

E. N. Mitchell and D. C. Bolknecht

Department of Physics

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

The domain structure of thin films of iron and nickel (in this case 83% nickel and 17% iron) are quite simple. In the remanent state, they are very nearly a single domain, and in any case if the film is less than the order of four thousand Angstroms thick the films are one domain thick. In a partially demagnetized state the film breaks into several large domains, as in Figure one at the top where the easy axis of the film is as indicated. The shaded sections are found to be magnetized in one direction (say from left to right) and the unshaded sections in the opposite direction. Such a film can be changed from one remanent state to the other by wall motion on the application of a D.C. field of appropriate magnitude and direction. If the field is applied from left to right, the shaded domains will increase in size and the unshaded portions decrease in size.

These domain structures have in general been observed using two techniques. In one, the Bitter method,¹ colloidal magnetite is placed on the surface to be examined and the magnetite settles out on the wall boundaries where the concentrations of magnetite can be observed as fine lines when examined with a low-powered microscope. An alternative method is to take advantage of the Kerr effect.³ If one reflects plane polarized light from the surface of a material which is magnetized along the plane of incidence, the plane of polarization will be rotated and the direction of rotation will depend on the direction in which the film is magnetized. An analyzer appropriately oriented and used for observation can hence detect the direction in which the various parts of the surface are magnetized.

The question which is to be considered here is that of how the domain structure varies with the thickness of the film. In particular it would be desirable to establish the thickness at which the film ceases to be one domain thick and becomes two domains thick. Fowler and Fryer³ have made some observations on this phenomena and state that films of nominally 80% nickel and 20% iron were essentially one domain thick in the interval from 500 to 5000 Angstroms thick, but were unable to observe any domain structure in films 10,000 and 20,000 Angstroms thick.

APPARATUS

The films which are to be discussed here were made in the apparatus described in the last paper.¹ The substrate was micro-

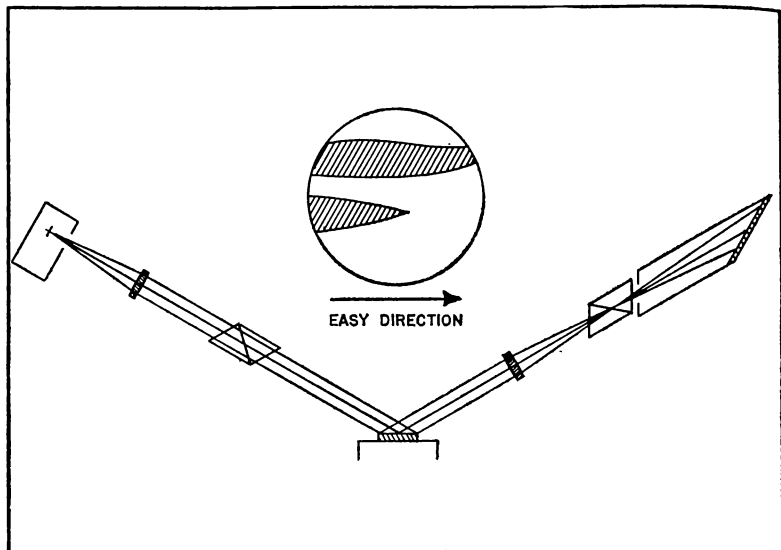


FIGURE 1. At the top is a typical domain pattern of an iron nickel film in a partially demagnetized state; at the bottom is a schematic of the Kerr apparatus.

scope slide glass which had previously been cleaned with a detergent and rinsed in distilled water. Prior to deposition, the substrates were heated in a substrate holder to nominally 300° C. for additional cleaning. No orienting magnetic field was used during the deposition of the films but the films still exhibited some anisotropy in the plane of the film (that is they exhibited an easy and a hard direction). Iron and nickel wire, making an average composition of 83% nickel and 17% iron, was wound around a tungsten filament, and this was used as the evaporation source when heated. The evaporations were of the order of one minute duration, and the vacuum during deposition was better than 5×10^{-5} mm. Hg.

The magnetic domains of these films were studied utilizing the Kerr effect which was described earlier. The sketch of the apparatus (Figure one) shows the essential components of the system. Light from a small source was placed at the focus of the lens system at the left. The resulting parallel light passed through the polarizer and this plane polarized light was reflected from the film in the center, passed through an optical system which formed a virtual or real image depending on whether observation was being made visually or photographically, and finally passed through the analyzer at the right. The plane of polarization was rotated when the light was reflected from the film, its direction of rotation depending on the direction of magnetization in the particular part of the film

under observation. This difference was detected by the analyzer, and the difference in magnetization was observed as areas of light and dark contrast.

In these preliminary measurements, the thickness was measured using a hysteresis loop display apparatus.² This apparatus displayed the hysteresis loop of the film in such a manner that the ordinate was proportional to the dipole moment of the film. An absolute calibration of this apparatus has been made using a torque magnetometer and assuming a bulk value for the saturation magnetization; an estimate of the physical thickness can be made if the film is driven to saturation. The films which are to be discussed here were the order of 5,000, 10,000, and 18,000 Angstroms thick.

RESULTS

In general, the domain structure of these films has been observed with the easy direction of the film in the plane of incidence of the reflected light. With the film in either saturated state, no domain structure was observed, but the film surface appeared either completely dark or completely light. If the film was in an intermediate state, one saw the structure shown in Figure one. This was accomplished by applying a small constant magnetic field of appropriate magnitude along the easy axis of the film.

In order to determine whether the film was or was not more than one domain thick, the domain structure on the front and back surface of the film was observed. Observation of the back surface was made more difficult because one must look through the glass substrate which was strained and hence optically active. In addition, if a film was two domains thick, the most probable domain structure in the intermediate state was one in which the domain structure on the back surface was a mirror of that on the front with the exception that the areas appearing dark on the front surface were light on the back and vice versa. This was because the demagnetization energy of the film would be minimized in such a state. It is also true that one might have this mirrored structure without the reversal in contrast in a film which is any odd number of domains thick, but experience dictates that films of less than 5,000 Angstroms thick will be at most two domains thick.

Films which were of the order of 5,000 Angstroms thick were found to exhibit in the intermediate state identical domain structures on the front and back surfaces and hence are concluded to be one domain thick. Films which were of the order of 10,000 Angstroms thick were found to exhibit the same domain structure on the front and back surfaces, but the contrast was reversed when front was compared to back. These films are tentatively identified as being two domains thick, though at this date one cannot state the relative thickness of the domains. Some films of approximately the same thickness showed domain structures front and back which were

not mirrors, and these films would also be classed as two domains thick.

It was not possible to establish an intermediate state in films which were of the order of 18,000 Angstroms thick. This means that either the film was an odd number of domains thick, or that the domains were oriented perpendicular to the plane of the film. Because of the behavior of these films in the hysteresis loop display apparatus, it was felt that the latter was the case. Such an orientation would be unlikely considering the high demagnetization energy of such a configuration, but considering the expected composition of the films, the magnetoelastic energy could be high enough to cause this configuration to occur.

CONCLUSIONS

One concludes tentatively that films made in this manner from a melt of 83% nickel and 17% iron up to 5,000 Angstroms thick are one domain thick, for films somewhere between 5,000 and 10,000 Angstroms thick the films are two domains thick, and for films somewhere between 10,000 and 18,000 Angstroms thick the domains become oriented perpendicular to the plane of the film. One might explain the results of Fowler and Fryer by observing that their films may be even more magnetostrictive than the ones with which we are dealing.

It is planned now to do at least three things to improve and complete this experiment. The thickness of the films used will be measured more precisely and directly using multiple beam interferometer techniques. Films will be made of an appropriate composition so that the magnetostriction will not cause the domains to align perpendicular to the plane of the film. Finally the domains of a very thick film of such composition will be observed as a function of the thickness of this film. The thickness will be varied by etching away material with a non-preferential etch. In this manner, it should be possible to observe how the domain structure changes as the film is made thinner.

The authors wish to thank their colleagues whose help was invaluable and the University of North Dakota for its cooperation in this work. Credit is also due the National Science Foundation who in part supported this research.

BIBLIOGRAPHY

1. F. Bitter. **Phys. Rev.**, **38**, 1903 (1931)
2. E. C. Crittenden, Jr., A. A. Hudimac and R. I. Straugh, **Rev. of Sci. Instr.**, **22**, 872 (1951)
3. A. Fowler, Jr., and E. M. Fryer, **Phys. Rev.**, **94**, 52 (1954)
4. E. N. Mitchell, **Proc. No. Dak. Acad. of Sci.** (1960)

PRELIMINARY MEASUREMENTS OF THE MAGNETORESISTANCE OF THIN IRON NICKEL FILMS

E. N. Mitchell and C. O. Heinzel

Department of Physics

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

If one measures the resistance in a given direction of bulk isotropic ferromagnetic material as a function of the direction and magnitude of the applied magnetic field, one finds that the resistance is in general not constant.¹ In particular, if one applies an increasing field along the direction of measurement of resistance in nickel one finds that as the field is increased, the resistivity increases quite rapidly by about 1.8% for an applied field of 2000 Oerstseds and then the change decreases to 1.4% of the resistance in the demagnetized state for an applied field of 18,000 Oerstseds. In addition, it has

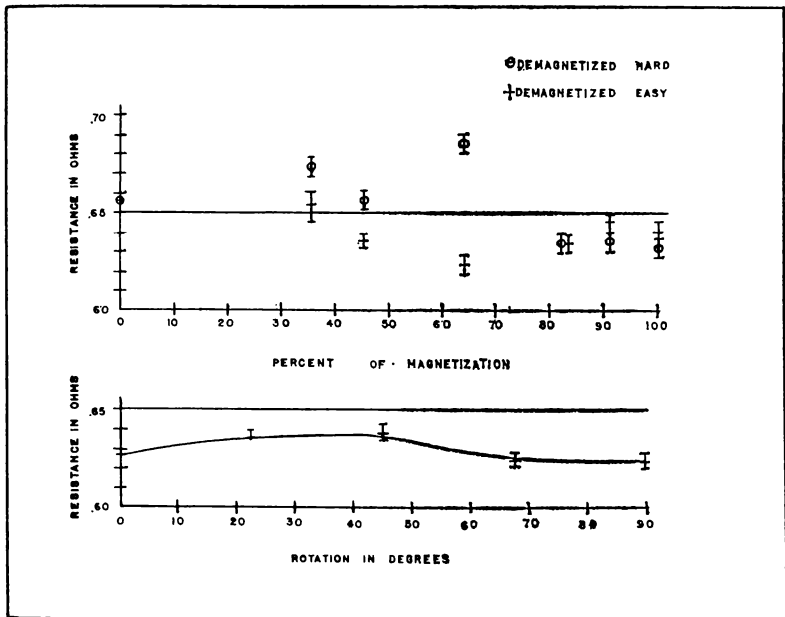


FIGURE 1—The top graph shows the resistance of the sample measured as a function of the percent of magnetization with the method of demagnetization as a parameter. The bottom shows the resistance along the easy direction of a saturated domain as a function of the direction of saturation magnetization of the domain.

been observed that the resistance in nickel decreases as the field is increased in a direction perpendicular to the direction along which the resistance is measured. An alloy of 80% nickel and 20% iron shows a maximum change in resistance in bulk polycrystalline material of about 3.5%.⁴ The purpose of this paper is to present some results on the measurement of the magnetoresistance of thin films of an alloy of 83% nickel and 17% iron.

APPARATUS AND MEASUREMENTS

The films examined were prepared much as those in the last paper.³ The substrate (microscope slide glass) was washed in a detergent, rinsed in distilled water, placed in a substrate heater, and heated in a vacuum to about 300° C. The evaporation was performed by heating metal wires of iron and nickel wrapped on a tungsten wire. The pressure during deposition was of the order of 5×10^{-5} mm. Hg. and the time for the evaporation the order of a minute. The films were made in the form of two centimeter squares and were approximately 1500 Angstroms thick on the basis of hysteresis loop measurements.³ The films were prepared in the presence of a D.C. orienting field of 24 Oersteds which was parallel to one side of the square films. These films exhibited the usual magnetic anisotropy in the plane of the film though this anisotropy was not as marked as that usually seen² in films made in this manner. Essentially, a four terminal method was used to measure the resistance of the film. Aluminum foil strip contacts along opposite edges of the film fed current across the film and two point contacts .95 centimeters apart were used to measure the potential drop at the center of the film along the direction of current flow (the magnetic easy direction). Both the current and potential drop were measured using a potentiometer. In the case of the current, the potential drop across a standard resistor was measured. In order to keep heating effects in the film at a minimum, the current through the film was maintained at the order of three milliamperes. This resulted in a voltage drop of the order of two millivolts which required using the potentiometer out near the limit of its range in order to detect changes in resistance of the order of three per cent which were expected.

While much of the data reported heretofore has been given as a function of the applied field, actually the results are more meaningful if they are plotted as a function of the internal field. This is especially true in a material such as the films we considered, for the internal field was not proportional to the driving field along the easy direction. In order to establish the magnitude of the internal field for a given applied field, the hysteresis loop display apparatus was used³ and the field necessary to drive the film to a given fraction of its saturation magnetization was observed at sixty cycles per second. After the film had been suitably demagnetized by applying a slowly decreasing alternating field to the film, the previ-

ously determined constant driving fields was applied to the film under the assumption that the constant field would magnetize the film to the same degree that the alternating field had magnetized it. This was not a bad assumption in this case, inasmuch as the alternating field was changing slowly at its maximum applied value and inasmuch as the reversal time for this type of film was short compared to the period of the sixty cycle per second driving field of the hysteresis loop display apparatus.

RESULTS

Two kinds of measurements are reported here. In the first case, the resistance along the easy direction was measured as a function of the percent of saturation of the film. Two plots showing this result are shown in Figure one at the top. Those points marked with circles show the resistance for the case in which the film was originally demagnetized by applying an alternating field in the difficult direction. Those points marked with horizontal dashes show the resistance for the case in which the film was originally demagnetized by applying the demagnetizing field parallel to the easy direction of the film. As can be seen, the method of demagnetization greatly effected the resistance and in addition, the change in resistance did not appear to be systematic in either case. The notable difference in the magnetic state of the film when the film is demagnetized by the two methods is that in the former case more possibility exists for the formation of walls and domains in the difficult direction as the film is progressively driven to saturation. When one realizes that while we speak of the magnetic behavior of the entire film, we in reality only examined the resistance of the small central portion of the film, and while the overall magnetic state of the film effected the current pattern in the film, the main effect on the resistance measured was due to the magnetic state of the film in the space between the two voltage probes.

In order to establish the effect on the resistance of the film as a function of the direction of the domains in the film, a second experiment was performed in which sufficient field was applied to the film to saturate it in any direction in the plane of the film (in this case, 24 Oersteds). The resistance of the film was measured as a function of the direction of the applied field which should also be the direction of the resulting single domain. This result is shown in the bottom graph of Figure one. The change in the resistance was relatively small when compared to the change observed as a function of magnetization and within the limit of error, almost a constant.

The overall change in resistance which was observed as a function of the magnetization was of the order of magnitude of the measured magnetoresistance of bulk permalloy, but the change was not systematic. The explanation of this must lie in the fact that we are examining the behavior of a very small number of do-

mains. The small variation of the resistance as a function of angle implies that these variations in resistance must be due in a large part to the resistance caused by domain walls in the vicinity of the voltage probes. It has been predicted¹ on the basis of a phenomenological model, that the magnetoresistance of a domain in polycrystalline materials should vary as the square of the cosine of the angle between the direction of the magnetization of the domain and the axis along which the resistance is measured. Such does not seem to be the case in this instance.

The resistivity was computed on the basis of the measured dimensions of the film, the current through the film and the voltage drop between the voltage contacts. The computed value was of the order of 2×10^{-5} Ohm—centimeter, which agrees quite well with the resistivity of bulk permalloy. The thickness of the film was measured using the hysteresis loop display apparatus which had previously been calibrated with a standard.³

CONCLUSIONS

One concludes that the resistivity of thin films of permalloy is the same order of magnitude as the resistivity of bulk material. One further concludes that a magnetoresistance effect is observed and that the magnitude of this effect is of the order of that in bulk material; however, because few domains are involved in the measurement, behavior of the film as a function of its magnetic state is very erratic. Finally, one concludes that the variation of the magnetoresistance of a single domain with angle cannot explain the observed phenomena. One is tentatively inclined to conclude that the major cause of the magnetoresistance effect is some type of wall phenomena.

Further investigation in this area is planned, including examination of the domain structure using the Kerr effect, measurement of the film thickness using interferometer techniques, and measurement of the magnetoresistance in directions other than the easy direction of the film.

The authors wish to thank their colleagues for the help they have given and the University of North Dakota for its cooperation in this research. Credit is also due the National Science Foundation who in part supported this work.

BIBLIOGRAPHY

1. R. M. Bozorth, **Ferromagnetism**, D. Van Nostrand Company (1951)
2. E. N. Mitchell, **Proc. No. Dak. Acad. of Sc.** (1960)
3. E. N. Mitchell and D. C. Boknecht, **Proc. No. Dak. Acad. of Sc.**, (1960)
4. H. C. Van Elst, **Physica**, 25, 708 (1959)

MEASUREMENT OF THE SATURATION MAGNETIZATION OF THIN IRON NICKEL FILMS

E. N. Mitchell and T. J. Cebulla

(A Progress Report)

University of North Dakota, Grand Forks, North Dakota

Department of Physics

INTRODUCTION

Since the advent of the study of thin ferromagnetic films, investigators have been concerned with the saturation magnetization of these materials and its dependence on thickness. In particular, earlier investigators¹ found that the saturation magnetization deviated from that of bulk material for films less than about 100 Angstroms thick. On the other hand, Neugebauer² reported on the basis of his investigations of nickel that films no thicker than twenty Angstroms showed the same saturation magnetization as bulk material. He speculated that the main cause of the difference in results may have been due to the fact that his films were prepared in a lower vacuum and that the necessary torque measurements on the films were made without exposing the films to the atmosphere. In an effort to get some independent measurements on alloys of iron and nickel, investigation has been started in this laboratory and is reported here. It is planned to measure the saturation magnetization of a single film as a function of its thickness. The thickness is to be varied by using a non-preferential etch to make the film thinner and to make successive torque measurements on the film as its thickness is reduced.

The results reported here are the consequence of investigations performed on a single permalloy film of one thickness. The film exhibited the usual magnetic anisotropy in the plane of the film which was the consequence of the application of a 24 Oersted steady magnetic field in the plane of the film during its deposition. The substrate (microscope slide glass) was prepared by washing in a detergent, rinsing in distilled water, and baking at 300° C. in a vacuum. The metal was evaporated from a heated tungsten filament which was wrapped with iron and nickel wire whose average composition was 83% nickel and 17% iron. The vacuum during deposition was the order of 5×10^{-6} mm. Hg.

From a measurement of the torque on the film in a known field and an absolute measurement of the film volume, it was possible to compute an absolute value for the saturation magnetization of the film.

APPARATUS

In order to measure the volume of the film, it was necessary to measure the thickness and diameter of the film. The diameter of

the film was measured using a calibrated ocular and found to have an average value of .82 cm. The thickness of the film was measured using a multiple-beam interferometer³ which was constructed in our laboratory. A schematic diagram of the operation of this instrument is shown at the right in Figure one. Light from the mercury arc illuminates a hole in a disk which serves as a point source. The light is made parallel by the two lenses shown in the diagram. A monochromator, a reflecting prism, a stop, and a half aluminized mirror are interposed between the two lenses. The parallel light from the top lens passes through a second half-aluminized mirror and is reflected from the half-aluminized coating which was evaporated onto the substrate and the iron nickel film shown at the top. The multiple reflection of the light waves between these two surfaces interfered constructively and produced an interference pattern whose image was projected through the top lens, was reflected again by the half-aluminized mirror, and projected onto the photographic film in the camera. This interference was essentially Fabry-Perot in nature. The light waves interfered many times causing the interference fringes to be very sharp and relatively widely spaced. The interference pattern appeared in the form of straight parallel lines with an offset in them at the point represented by the step between the iron nickel film and glass. The ratio of the distance between the parallel lines to the amount of the offset was in direct proportion to the ratio of the wave length of the light to the thickness of the iron nickel film. Using this technique, the average thickness of this film was found to be 1118 Angstroms.

In order to find an absolute value for the saturation magnetization, it was necessary to measure the torque on the film in a known field which was applied at a specified angle to the surface of the film. This measurement was performed in the torque magnetometer which is pictured schematically at the left in Figure one. The instrument consisted of a support member for the film which was suspended on a phosphor bronze fiber between the poles of the electromagnet. A damping vane was suspended in a viscous fluid at the bottom. The lower of the two pointers was used to determine the direction of the film, and the top pointer was used to determine the twist in the fiber. In operation, the film was oriented at a specified angle (45 degrees in this case) to the known magnetic field, and the fiber twisted until the film could be maintained in this position. This operation was performed for several known fields in the interval from 50 to 5000 Oersteds. The torque constant for the fiber was measured by suspending a brass bob of known moment of inertia from the fiber and measuring the period of the resulting torsion pendulum.

It was shown by Neugebauer² that if one plotted the ratio of the torque to the applied magnetic field as a function of the applied magnetic field, the extrapolation of this curve to zero field yielded

a quantity which was directly proportional to the saturation magnetization. If the extrapolated ratio was designated $(L/H)_0$, the saturation magnetization designated B_s , and the volume of the film designated V then, for the case in which the film was oriented at 45° to the applied magnetic field,

$$B_s = \left(\frac{L}{H} \right) \frac{8\pi}{\sqrt{2}V}$$

CONCLUSION

Using 1.12×10^{-5} centimeters for the thickness of the film, .82 centimeters for the diameter of the film and 2.74×10^{-3} gram-centimeters² per Oersted-seconds² for $(L/H)_0$, the saturation magnetization of the film was found to be 7,680 gauss. This is to be compared with a saturation magnetization for bulk material of 9,500 gauss. No accurate error determination has been made on this experimental value, but it is estimated that it could be in error by at most ten per cent. The cause for the difference between the two values must either be due to the fact that this film does not exhibit bulk properties, or that the film is of a different composition from the melt. As-

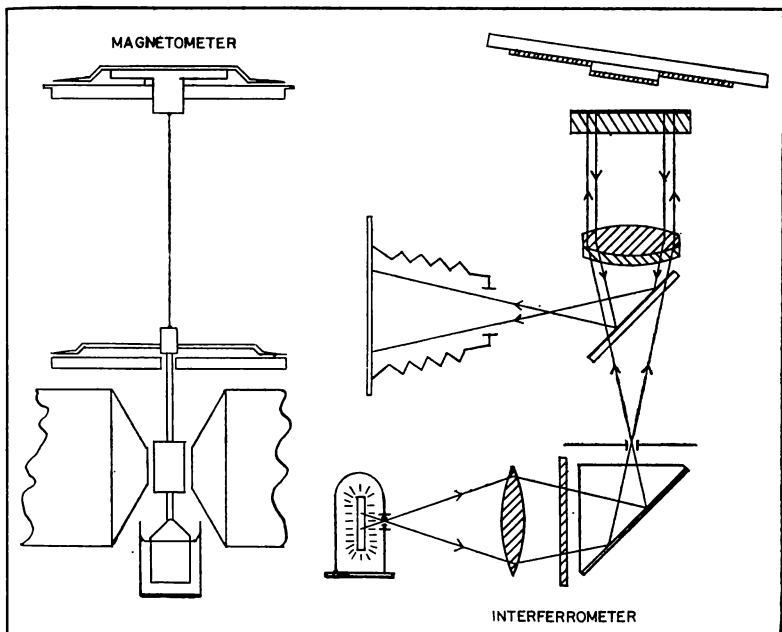


FIGURE ONE

The schematic at the left above shows the components of the torque magnetometer. The schematic at the right illustrates the operation of the multiple beam interferometer.

suming the film exhibits bulk properties, it would have to contain greater than 85% nickel to agree within the maximum error with data for bulk material. Such a deviation is not unreasonable.

On the other hand, it is entirely possible that the film, due to voids and impurities, does not exhibit bulk properties. This matter is under further investigation at present. An experiment is in progress in which the saturation magnetization will be remeasured and then examined as a function of thickness. One refinement which should improve the measurement is the addition of a Hall effect gaussmeter to replace the ballistic measurements of field which were made in this case.

The authors wish to thank their colleagues for the assistance they have rendered and to thank the University of North Dakota for its cooperation in this venture. Credit is also due the National Science Foundation who in part supported this work.

BIBLIOGRAPHY

1. E. C. Crittenden, Jr. and R. W. Hoffman, **Rev. Mod. Phys.** **25**, 310 (1953)
2. C. A. Neugebauer, **Structure and Properties of Thin Films**, 358, (1959), John Wiley and Sons.
3. S. Tolansky, **Multiple-Beam Interferometry** (1948) Oxford Press.

DIETARY CHOLESTEROL ON THE CHOLESTEROL CONTENT OF RABBIT AND RAT TISSUES. ETHER AS A CARRYING AGENT FOR DIETARY CHOLESTEROL

Jay W. Constantine

Department of Zoology and Physiology

North Dakota Agricultural College, Fargo, North Dakota

Young male albino rabbits and rats of the New Zealand and Wistar strains, respectively, were each divided into three experimental groups based upon addition of a supplement to the regular diet: A) normal—no supplement, D) 1% cholesterol in 10% ether, and E) 10% ether. For the latter two groups the indicated supplements were thoroughly mixed with the stock rations, and the ether was immediately evaporated. Rabbits were maintained on the indicated regimen for 9 to 21 weeks, and rats for 8 to 9 weeks. All animals were starved 19 to 21 hours prior to sacrifice. Cholesterol was determined by the Sperry-Schönheimer method. For a given tissue all data are presented as the percent difference between free and esterified cholesterol, respectively. Statistically significant (Probability < 0.05) values are underlined. Groups E-A. Rabbits: aorta 14, -89; plasma 6, 53. Rats: aorta 9, -57; plasma -67, 8. Groups D-E. Rabbits: aorta 533, 20,900; plasma 2,002, 1251. Rats: aorta -4, -33; plasma -18, 0.

EXTRACTION OF THE ANTIPYRIDOXINE FACTOR IN FLAX COTYLEDONS.¹

*H. J. Klosterman, R. B. Olsgaard, W. C. Lockhart and
J. W. Magill*

*Departments of Agricultural Biochemistry and Poultry Husbandry
North Dakota Agricultural College, Fargo, North Dakota*

INTRODUCTION

The poor chick growth resulting from feeding raw linseed meal has been reported to be the result of a combination of factors (2). When fed high levels of linseed meal, chicks showed typical symptoms of a B vitamin deficiency which were alleviated by the addition of pyridoxine to the ration. Improved growth rates were obtained at the same time. It appeared that poor growth resulted from either the presence in the linseed meal of a specific pyridoxine antagonist or from the inavailability of pyridoxine in the linseed meal. Since pyridoxine is rarely a limiting factor in chick rations based on common feedstuffs, it seemed unlikely that a simple vitamin B₆ deficiency could result from the incorporation of as little as 5 percent linseed meal into a standard ration. It was more likely that linseed meal contained a substance which acted as a vitamin B₆ antagonist and counteracted the vitamin B₆ naturally present in the feedstuffs. It has been shown (4) that the growth depressing effect of linseed meal was concentrated in the cotyledon portion rather than the hull. This report is concerned with further studies of the nature of the chick growth inhibitor in linseed cotyledons and the preparation of highly active concentrates of the inhibitory substance.

MATERIALS AND METHODS

Unsexed chicks of either Indian River or Leghorn breed were housed in standard chick batteries and fed a standard basal ration (5) until feeding experiments commenced. When linseed cotyledons or protein-rich fractions were tested, the amounts of corn meal and soybean meal were adjusted to maintain a uniform protein level (19%). The birds were from one to two weeks old at the start of a test and duplicate pens were used in most cases. The number of birds per pen varied with the availability of the test ration. It was noted that the Indian River breed was more sensitive to the inhibitor than was the Leghorn. As a consequence, gains noted from one trial to the next were not exactly comparable. However, only one breed of chicks was used in each experiment to permit comparisons between treatments. Chicks were fed ad libitum.

Cotyledons were prepared by the grinding-flotation method of Clagett et al. (5). The cotyledons contained 60-64% crude protein.

In the study dealing with nitrogen metabolism, the excreta were stored in 95% alcohol until the analysis could be performed. The fecal samples were homogenized with 95% ethanol in a Waring Blendor and centrifuged. The residue was washed with ether and dried by vacuum. The dried samples were sieved through a 20 mesh screen to remove feathers and grit. The nitrogen percentage was determined on the residue and the supernatant by the macro Kjeldahl method.

Uric acid was estimated by the Bose method (1) which consists of fecal extraction with a hot lithium carbonate solution followed by iodometric titration of the extract.

Linamarin was synthesized by the method of Fischer (3) and was incorporated into the check diet at the 0.23% level.

Linseed globulins were obtained by dispersion of 100 g. finely ground, oil-free cotyledons in 800 ml. of N/28 sodium hydroxide solution. After stirring at room temperature for fifteen minutes, the suspension was centrifuged in a basket centrifuge and the residue washed with water, acetone and dried. The residue contained 3.6% nitrogen.

The globulins were precipitated by adjusting the centrifugate to pH 4.2 by the addition of acetic acid. The precipitated proteins were collected by centrifugation, washed with alcohol, acetone and dried by suction. The dried protein contained 15.5% nitrogen. On the average, 100 g. cotyledons yielded 40 g. globulin, 25 g. residue and 35 g. water-soluble material.

Aqueous ethanol extracts of oil-free, ball mill ground cotyledons were prepared by grinding a suspension of the ground cotyledons for one hour in an Eppenbach colloid mill. One liter of solvent was used per 100 g. of solids and the temperature of the solvent was kept below 30°C. by circulating tap water through the colloid mill. The insoluble material was removed by filtration and the extract concentrated to a small volume under reduced pressure. The concentrate was taken to dryness by lyophilization.

Aqueous extracts were prepared by shaking ball mill ground cotyledons in ice water. After centrifugation, the extracts were dried by lyophilization.

Ion exchange resins were used to effect concentration of the inhibitor. Commercial Amberlite IR-120x8, 16-50 mesh, was utilized to absorb the inhibitor. The resin bed was supported by a glass column, 2.5 x 40 cm. The resin was prepared for adsorption by cycling several times from the sodium to the hydrogen form with 1 N sodium hydroxide and 1 N hydrochloric acid.

The alcohol extracts were taken up in water after the removal of the alcohol and were allowed to run over the resin bed at a flow rate of 2-3 milliliters per minute. After washing with water, the column was treated with 1 N ammonium hydroxide to elute the inhibitor. The ammonia and water were removed from the eluate

It was somewhat difficult to evaluate the nutritional status of birds fed linseed cotyledons. It was apparent that a vitamin B₆ deficiency had been induced. By the time the birds showed severe symptoms—weight loss, excitability, loss of equilibrium—they had greatly reduced their feed consumption and were probably suffering from starvation as well.

Examination of the excreta showed that uric acid was being produced. Apparently the small amount of feed the birds consumed was being digested for a large portion of the nitrogen was uric acid nitrogen.

TABLE III
Nitrogen Analysis of Excreta

Fraction	Mg. N/g. Feces (dry weight basis)	
	Linseed Fed	Control Fed
(a) Total nitrogen	66	43
(b) Alcohol soluble nitrogen	12.8	3.7
(c) Alcohol insoluble nitrogen	53.2	39.7
(d) Uric acid nitrogen	34.3	18.3
Difference (d — c = undigested protein)	18.9	21.4

The alcohol-insoluble, non-uric acid nitrogen represents undigested protein and was about the same in both cases. The high uric acid nitrogen probably arose from a poor retention of absorbed amino acid nitrogen. It is likely that the birds were in a negative nitrogen balance. No further studies of the physiology of the birds were undertaken. Emphasis was placed on obtaining the inhibitor in concentrated or pure form.

Previous experiments had shown that the cotyledons were more inhibitory than hull fractions. Since the cotyledons are high in protein, it was conceivable that the toxicity could derive from an allergenic protein such as is found in several oilseed meals. The globulin fraction was prepared and incorporated into chick diets at the 8% level. The residue resulting from the protein extraction was incorporated at the 5% level. These levels are equivalent to a 20% cotyledon fraction. (One hundred grams of cotyledons yields 40 g. globulins and 25 g. residue). The birds were fed the test diets for seven days. The results are shown in Table IV.

TABLE IV
**Growth Response produced by Linseed Globulins
and Undispersed Residue.**

Ration	Gain, 7 days (grams)	Feed Efficiency (g. feed/g. gain)
Control	51.4	2.91
8% Linseed globulin	53.5	4.20
5% Non-dispersed residue	63.3	3.19
20% Cotyledon	10.0	2.80

Both the globulin and residue supported good growth but with poorer feed efficiencies. Apparently these two diets were very palatable, but were lacking in protein quality. Neither contained the inhibitor found in the cotyledons. The inhibitor either remained in the water soluble portion or was destroyed during the protein isolation. Since the isolation had been performed under mild conditions, it seemed likely that a water-soluble factor was involved.

About one-third of finely ground linseed cotyledons is soluble in cold water. The globular proteins remain undispersed and an extract consisting of albumins, minerals and crystalloids is obtained. The possibility of enzymatic destruction could not be avoided in a water extraction procedure, but the maintenance of near-freezing conditions and short extraction periods (15 minutes) reduced the likelihood of enzymatic or chemical destruction. The lyophilized water extract was fed at a 20% cotyledon equivalent. The results are shown in Table V.

TABLE V
Effect of Water Extract on Rate of Gain.
 Avg. Accumulative Wt. Gain (grams)

Ration	—days—						
	1	2	3	4	5	6	7
Control	9	16	22	30	38	46	56
Water extract at							
20% cotyledon equiv.	3	—2	—3	—3	—6	+7*	+20*
20% Cotyledons	0	—4	—6	—8	—9	+2*	+7*

*Pyridoxine added: 40 ppm.

The loss of weight shown by the birds fed the water extract indicated that the inhibitor was in the water soluble portion. Furthermore, the birds fed the water extract for five days made remarkable gains when fed supplemental pyridoxine.

As noted above, water extracts contained about one-third of the cotyledons. Extracts which are nearly as potent may be prepared by extraction with 70% aqueous ethanol (Fig. 1) with the advantage that only about 5% of the cotyledon is extracted. On the other hand, acetone and 95% ethanol were ineffective as solvents.

The solubility properties of the inhibitor closely parallel those of linamarin, the cyanogenetic glucoside of flaxseed. Linamarin is the B-D-glucoside of acetone cyanohydrin and is toxic to many animals. The addition of 0.23% linamarin resulted in no growth inhibition. This is about four times as much as naturally occurs in flaxseed. The same result was obtained with acetone cyanohydrin.

Further examination of the 70% alcoholic extract revealed that the inhibitor was dialyzable.

Some of the growth responses to various diets are shown graphically in Figure 1.

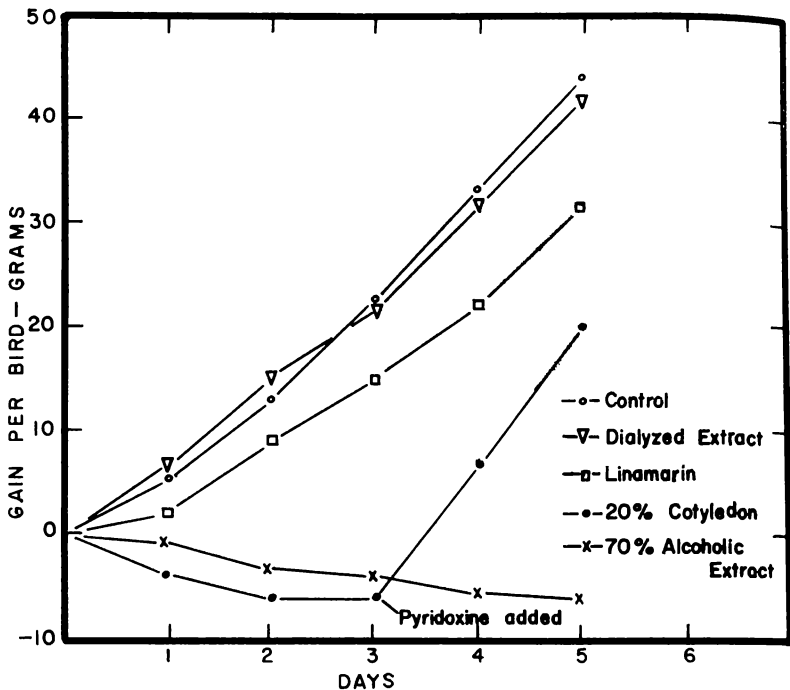


FIGURE 1

Growth Response of Chicks to Various Treatments

Further fractionation was achieved by adsorption of the inhibitor onto the cation exchange resin Amberlite IR-120. When the resin was used in the hydrogen form, the inhibitor could not be recovered in either the effluent or ammonia eluate. However, when the resin was partially neutralized by preconditioning with 2 M pH 3 phosphate buffer, the inhibitor was partially adsorbed by the resin and was eluted by 1 M ammonia solution. Similar results were obtained by first converting the resin to the sodium form and regenerating with 1 M acetic acid. In both cases the resin was only partially in the hydrogen form.

When buffered resins were used, the inhibitor was found in both the column effluent and eluate. Increasing the resin to cotyledon ratio did not alter the situation. Even though a portion of the inhibitor was lost, the large amount of neutral substances that were extracted were separated from the inhibitor which was eluted from the resin.

The growth response suggested that the inhibitor was about equally divided between the effluent and eluate.

TABLE VI

Growth Response from Resin Effluent and Eluate.

Ration	Five-day Gain (grams)
Control	30
Effluent of buffered column	3
Eluate of buffered column	2

DISCUSSION

The inhibition of chick growth by linseed cotyledons is definitely associated with a pyridoxine antagonist. The growth inhibition may be counteracted by supplementation with pyridoxine suggesting that the antagonist functions as a competitive inhibitor of the naturally-occurring vitamin B₆. The failure of previous workers to obtain complete reversal suggests that they may have encountered a palatability factor or they were dealing with an additional factor which does not occur in the cotyledon portion of the seed.

Since the growth inhibitor is extracted from the cotyledons by 70% aqueous alcohol and is dialyzable, it is definitely not protein in nature, but is associated with crystalloidal material.

The inhibitor is polar in nature since it is readily extracted by water and aqueous alcohol. Its partial retention by cation exchange resins suggests that it probably contains an amine or other form of reduced nitrogen as a functional group. The basicity is not strong as it is not completely retained by the buffered cation exchange resin. This partial retention suggests that there may be more than one chemical species present which function as pyridoxine antagonists individually or synergistically.

The site of metabolic inhibition is not known. It seems likely that the antivitamin could either be inhibiting the transport of the vitamin across the intestinal mucosa or competitively inhibiting specific B₆ dependent enzymes at the cellular level. The latter hypothesis would seem more probable since vitamin B₆ is intimately associated with many enzymes which catalyze the transformations of amino acids in the living organism. It is possible that the inhibitor could be analogous in structure to the vitamin and attach to the active site on the enzyme surface. The competitive inhibition could then be explained on the basis that the antivitamin lacks the structural features required for catalysis and resultant transformation of the amino acids.

Partial success in retaining the inhibitor on cation exchange resins has encouraged further investigation in this area. Studies are under way to determine the conditions that are necessary for obtaining the inhibitor in pure state.

SUMMARY

Further studies on the chick growth inhibition factor in linseed have been carried out. Experimental data have been cited which

show that the effect of the growth inhibitor may be counteracted by administering pyridoxine orally or subcutaneously. Physicochemical properties of the inhibitor preclude a proteinaceous structure.

Solubility data indicates that the inhibitor is a polar molecule. This is further substantiated by the reversible adsorption of the antivitamin to a cation exchange resin.

The site and mechanism of inhibition and some metabolic anomalies associated with inhibition are also discussed.

ACKNOWLEDGMENT

Some of the early experiments were carried out with the assistance of Donald Christianson.

BIBLIOGRAPHY

1. Bose, S., "An Iodometric Estimation of Uric Acid in Poultry Excreta", *Poultry Sci.*, **23**, 130-4 (1944).
2. Clagett, C. O., D. D. Christianson, R. L. Bryant and W. Lockhart, "Further Studies on Chick Growth Inhibition with Linseed Meal", *Proc. N. Dak. Acad. Sci.*, **X**, 67-71 (1956).
3. Fischer, E., and G. Anger, "Synthese des Linamarins und Glykolnitril-cellosids", *Ber.*, **52**, 854-868 (1919).
4. Schlamb, K. F., C. O. Clagett, and R. L. Bryant, "Comparison of the Chick Growth Inhibition of Unheated Linseed Hull and Cotyledon Fractions", *Proc. N. Dak. Acad. Sci.*, **IX**, 58-9 (1955).
5. Schlamb, K. F., C. O. Clagett, and R. L. Bryant, "Comparison of the Chick Growth Inhibition of Unheated Linseed Hull and Cotyledon Fractions", *Poultry Sci.*, **34**, 1404-7 (1955).

OSMOTIC TRANSFER OF ERYTHROCYTE WATER

Edwin G. Olmstead, M.D.

Assistant Professor of Medicine

University of North Dakota, Grand Forks, North Dakota

(This study was supported by a grant from the North Dakota Heart Association.)

Washed rabbit erythrocytes were placed in buffered NaCl solutions of varying pH and of tonicity equidistant from intracellular tonicity (-0.558°C). After incubation for $\frac{1}{2}$ hour the cell suspension was centrifuged briefly and freezing point depression (Δ') measured on the supernatant. Change in the Δ' reflects a change in the water content of the extracellular solution—water being removed by erythrocytes from hypotonic solutions (W_1) and water passing from erythrocytes into hypertonic solutions (W_E).

Effect of ph and Temperature on Osmotic Exchange of Erythrocyte H₂O

ph	number tested	Mean W _E		Mean W _I		W _E /W _I	W _E /W _I
		ml/100 ml RBC	S. E.	ml/100 ml RBC	S. E.		
6.5	20	8.04	0.17	16.00	0.39	0.502	126.0
7.1	20	7.75	0.16	14.09	0.27	0.550	109.2
7.4	20	6.98	0.12	14.55	0.39	0.480	101.2
7.7	20	6.62	0.65	15.98	0.54	0.414	105.8
8.0	20	4.84	0.19	17.28	0.40	0.250	83.6
temp							
+4°C	20	8.47	0.18	13.11	0.28	0.645	111.0
+38°C	20	6.98	0.12	14.55	0.39	0.480	101.6
+45°C	20	6.75	0.16	16.66	0.32	0.405	112.4

S.E. = Standard error

The table indicates that W_E is always less than W_I and is inversely proportional to the ph of the cell suspension in the range of 6.5 — 8.0. W_E is significantly increased (from standard temperature 38°C, ph 7.4) by lowering the temperature of the cell suspension to 4°C (p<0.01). W_I is significantly increased (p<0.01) by raising or lowering the ph or raising the temperature of the cell suspension. W_E X W_I ‡ k and W_E and W_I are affected differently by change in ph and temperature. It is concluded that W_E and W_I are probably under different physico-chemical control.

MALIC DEHYDROGENASE AND DIAPHORASE FROM GERMINATED FLAX RUST UREDOSPORES

Kenneth W. Dockter and D. Stuart Frear

Department of Agricultural Biochemistry

North Dakota Agricultural College, Fargo, North Dakota

INTRODUCTION

The host-pathogen relationship between flax (*Linum usitatissimum* L.) and flax rust (*Melampsora lini* (Pers.) Lév.) has been established on a gene to gene basis by Flor¹. Genes are now generally thought to control the synthesis of individual enzymes². Therefore, if a gene in both the pathogen and the host is required for virulence or avirulence and susceptibility or resistance, a study of gene controlled differences in the enzymes concerned with the metabolism of the resistant and susceptible host varieties and the virulent and avirulent rust races will aid in the complete understanding of the association between the pathogen and the host.

Basic information concerning the metabolism of the flax rust fungus is practically non-existent. Such pertinent information should

be considered part of the basic foundation upon which further insight into the host-parasite relationship may be achieved. This is a preliminary investigation of enzymes concerned with the metabolism of the flax rust parasite. Malic dehydrogenase and diaphorase enzymes were chosen for an initial investigation because they both appeared to be quite active and stable in crude homogenates of germinated flax rust uredospores.

The term diaphorase was originally applied to a solubilized flavoprotein which catalyzed the transfer of electrons from reduced diphosphopyridine nucleotide (DPNH) by way of flavin-adenine dinucleotide (FAD) to an artificial electron acceptor¹⁴. Until recently, the physiological role of diaphorase was not known.

Massey^{10, 11} and Searls and Sanadi¹³ have recently shown that diaphorase exhibits potent lipoyl dehydrogenase activity. This may be the physiological function of the enzyme rather than the function of a DPNH oxidase in the electron-transport mechanism that has been previously ascribed to it by Mahler and Elowe⁸. Lipoyl dehydrogenase plays a role in keto acid oxidation, in which the function of the enzyme is to oxidize a reduced lipoic acid derivative with accompanying reduction of diphosphopyridine nucleotide⁶ (DPN).

Malic dehydrogenase is a soluble enzyme which catalyzes the oxidation of L-malic acid to oxalacetic acid with accompanying reduction of DPN.

MATERIALS AND METHODS

Race no. 1 flax rust uredospores were harvested from infected Bison flax plants grown in the greenhouse.

The DPNH and reduced triphosphopyridine nucleotide (TPNH) of 90% purity as well as the *p*-chloromercuriphenyl sulfonic acid (PCMS) were obtained from Sigma Chemical Company.

Enzyme activity measurements were made using a Beckman Model DK-2 Ratio Recording Spectrophotometer equipped with a time drive attachment and a temperature regulated cell holder maintained at $25 \pm 1^\circ\text{C}$. Silica cells of 1.0 cm. light path were used in all spectrophotometric measurements.

All steps in the isolation of diaphorase and malic dehydrogenase from the germinated uredospores were carried out at $0-4^\circ\text{C}$. Centrifugation steps were carried out in the refrigerated Spinco Model L Ultracentrifuge using a no. 30 rotor.

DIAPHORASE ASSAY: The procedure used was essentially that of Mahler et al.⁹ and involved the reduction of 2, 6-dichlorophenolindophenol (DCIP) in the presence of the enzyme and DPNH. The reaction mixture was routinely composed of the following: 0.10 ml. of appropriately diluted enzyme, 0.50 ml. of 0.2 M potassium phosphate buffer, pH 6.0 (100 μM), 0.1 ml. of 1.2×10^{-3} M DCIP (0.12 μM), 0.3 ml. of 0.01 M potassium cyanide (3 μM), 0.10 ml. of 1×10^{-3} M DPNH (0.1 μM) and demineralized water to 3.0 ml. final volume.

A blank cuvette containing everything but the enzyme was run simultaneously to correct for non-enzymatic dye reduction caused by the DPNH. The reaction was initiated by the addition of the enzyme. The decrease in optical density at 600 mu was measured continuously for four minutes. The decrease in optical density from 1 to 2 minutes after the start of the reaction was used to calculate the enzyme activity. The rate of indophenol dye reduction was directly proportional to protein concentration under these conditions of measurement. A unit of enzyme activity is defined as that which causes a decrease in optical density at 600 mu of 0.001 per minute under the above conditions. Specific activity is defined as units of enzymatic activity per milligram of protein. Protein was determined by the biuret reaction using the procedure of Gornall *et al.*⁵ and standardized with crystalline bovine serum albumin.

MALIC DEHYDROGENASE ASSAY: The procedure used was essentially that of Mehler *et al.*¹². The enzyme activity determination is based on the measurement of the rate of oxidation of DPNH at 340 mu in the presence of the enzyme and excess oxalacetate. The reaction mixture contained 0.4 ml. of 0.2 M TRIS (2-amino-2-hydroxymethyl-1, 3-propanediol) buffer, pH 7.5 (80 uM), 0.1 ml. of 7.6×10^{-3} M freshly prepared oxalacetate (0.76 uM), 0.2 ml. of 1×10^{-3} M DPNH (0.2uM) and demineralized water to a final volume of 3.0 ml. The reaction was started by the addition of appropriately diluted enzyme. Readings of the optical density were made against a blank containing all components except DPNH for a period of four minutes. The decrease in optical density at 340 mu between 30 and 90 seconds after the start of the reaction was used to calculate the enzyme activity. Under these conditions the rate of DPNH oxidation was directly proportional to enzyme concentration. One unit of enzyme is defined as that amount which causes a decrease in optical density of 0.01 per minute under the above conditions. Specific activity is expressed as units of enzymatic activity per milligram of protein. Protein was determined as described for diaphorase.

EXPERIMENTAL RESULTS

PURIFICATION OF DIAPHORASE AND MALIC DEHYDROGENASE: Fresh uredospores, usually 1 gram, were germinated by dusting on 50 ml. portions of demineralized water in 6-in. petri dishes (approximately 60-70 mg. per dish). Germination was usually better than 90% and was allowed to proceed for 12 hr. at 16°C. The resulting germ tube mats were then harvested at room temperature by vacuum filtration, washed and pressed dry with a filter paper pad. This spore mat was then homogenized in the cold for 10 minutes in a Potter-Elvehjem tissue homogenizer driven at 1,000 rpm. with 10 ml. of isolating medium. The isolating medium contained 0.05 M potassium phosphate buffer pH 7.0 and 0.001 M ethylenediamine tetraacetate (EDTA). The remaining unruptured

whole uredospores (usually a few non-germinated uredospores) and large uredospore fragments were removed from the crude, deep orange colored homogenate (Fraction 1) by centrifugation at 500 x g for 5 minutes. The resulting pellet of whole spores and spore fragments was resuspended and washed twice with 5 ml. portions of the isolating medium. The wash supernatants were combined with the original whole spore-free supernatant to make up the crude, orange colored extract (Fraction 2). Small, deep orange colored particles (probably mitochondria) were then removed from the crude extract by further centrifugation at 10,000 x g for 30 minutes. The resulting opaque supernatant was called the low speed supernatant (Fraction 3). This fraction was then centrifuged again at 75,000 x g for 2 hr. and the resulting clear, pale yellow supernatant termed the high speed supernatant (Fraction 4). Ten milliliters of the high speed supernatant was then mixed with an equal volume of aged calcium phosphate gel (24 mg./ml.) and allowed to stand at 0° C. for 15-30 minutes with intermittent stirring. The gel was removed by centrifugation at 3,000 x g for 10 minutes. The gel was resuspended and washed twice with 10 ml. portions of 0.02 M potassium phosphate buffer pH 7.0. Diaphorase and malic dehydrogenase were eluted from the gel with two 5 ml. washes of 0.2 M potassium phosphate buffer pH 7.0. The combined almost colorless supernatant eluates were termed gel eluate (Fraction 5). Tables I and II represent a typical purification experiment where Fraction 5 represents approximately a 10-fold purification with a recovery of over 20% of the activity of both enzymes. A number of similar preparations were obtained by this procedure in which the degree of purification varied from 5-10 fold. Dialysis of Fractions 3, 4 or 5 did not alter the activity to any great extent.

TABLE I

Summary of Purification of Flax Rust Uredospore Diaphorase

Fraction	Total protein mg.	Total units $-\Delta E_{600}$ per minute	Specific activity, units per mg. protein	Per cent recovery
1. Crude homogenate	356.0	56,300	158	100
2. Crude extract	255.0	43,200	169	77
3. Low speed supernatant	55.4	31,800	574	56
4. High speed supernatant	41.9	28,500	681	51
5. Calcium phosphate gel eluate	11.4	13,100	1,150	23

TABLE II

Summary of Purification of Flax Rust Uredospore Malic Dehydrogenase.

Fraction	Total protein mg.	Total units $-\Delta E_{340}$ per minute	Specific activity, units per mg. protein	per cent recovery
1. Crude homogenate	356.0	32,500	91.3	100
2. Crude extract	255.0	31,200	122.2	96
3. Low speed supernatant	55.4	27,000	489.0	83
4. High speed supernatant	41.9	24,000	573.0	74
5. Calcium phosphate gel eluate	11.4	9,450	829.0	29

STABILITY OF ENZYME: Both the diaphorase and the malic dehydrogenase of germinating flax rust uredospores appear to be quite stable, soluble enzymes. Preparations can be kept at 0° C. for over a week or frozen at -17° C. and thawed several times with little loss of activity. Preparations can also be dialyzed at 4°C. for 12 hours or more with virtually no loss of activity.

pH OPTIMUM: Potassium phosphate proved to be the most effective buffer for diaphorase with a pH optimum at about 6.4. Pyrophosphate and phthalate buffers were not as effective as phosphate but also gave optimal activity at pH 6.4.

With malic dehydrogenase, TRIS and glycylglycine buffers were more effective with a pH optimum at about pH 8.2. Phosphate buffer was not as effective as TRIS and glycylglycine, and gave optimal activity at pH 7.4.

PYRIDINE NUCLEOTIDE SPECIFICITY: Malic dehydrogenase was found to be specific for DPNH. Diaphorase was not specific for DPNH and also oxidized TPNH. TPNH oxidation activity, however, was only about 40% as rapid as DPNH and was not followed during purification of the enzyme.

EFFECT OF p-CHLOROMERCURIPHENYL SULFONIC ACID ON MALIC DEHYDROGENASE AND DIAPHORASE: In inhibition studies with PCMS, the enzyme was incubated with the inhibitor for 15 minutes at 0°C. prior to assay. No attempts were made to reverse the inhibition with cysteine or glutathione.

Both enzymes were inhibited by PCMS. Malic dehydrogenase was 90% inhibited by 5×10^{-4} M PCMS. Diaphorase, however, was only 70% inhibited by 8×10^{-3} M PCMS.

REVERSAL OF MALIC DEHYDROGENASE CATALYZED REACTION: The equilibrium position of the reaction catalyzed by malic dehydrogenase markedly favors the reduction of oxalacetate to L-

malate at pH 7.0. At higher pH's, and with large amounts of DPN and recrystallized L-malate, it is possible to reverse the reaction. It was possible to show the reversal of this reaction at pH 10.0 using the procedure of Wolfe and Neilands¹⁰.

DISCUSSION

Both of the enzymes isolated and partially purified in these experiments were found to be soluble, quite active and fairly stable. The whole-spore and spore fragment pellet as well as the pellets from the low and high speed centrifugation all had low diaphorase and malic dehydrogenase specific activities. This activity, however, was probably primarily due to incomplete washing of the particulate matter.

The fact that dialysis does not significantly alter the activities of diaphorase and malic dehydrogenase indicates the absence of any naturally occurring inhibitors and also indicates that any necessary activators or cofactors are probably quite tightly bound.

Ethylenediamine tetraacetate was included in the isolating medium to remove inhibitory trace metals since lipoyl dehydrogenase activity attributed to diaphorase has been reported to be inhibited by trace metals by Veegar and Massey¹⁵. Several attempts were made to find lipoyl dehydrogenase activity in the gel eluate fraction, but none were successful using the procedure of Massey¹⁰. In this regard, King and Howard⁷ have recently isolated a soluble DPNH dehydrogenase from heart particles which was able to use DCIP as an electron acceptor, but was practically inactive toward lipolic acid.

Using the procedure of Clum and Nason³, no DPNH or TPNH oxidase activity was found in any of the fractions assayed. These enzymes, therefore, would not interfere with the malic dehydrogenase assay. TPNH and DPNH cytochrome C reductase preparations have been reported by Mahler *et al.*⁹ to also exhibit diaphorase activity. Fractions 4 and 5, however, when assayed for DPNH and TPNH cytochrome C reductase according to the procedure of Clum and Nason³, showed no activity.

Inhibition of both diaphorase and malic dehydrogenase by PCMS indicates the necessity of SH-groups for enzyme activity.

The fact that diaphorase activity was exhibited with both DPNH and TPNH may indicate the presence of specific DPNH and TPNH diaphorases. Both DPNH and TPNH diaphorases have been recently reported to be present in plants by Clum and Nason³ and Avron and Jagendorf¹. The activity of the diaphorase with TPNH might be explained by the presence of a transhydrogenase in addition to the diaphorase. If this were the case, however, one would also expect TPNH as well as DPNH malic dehydrogenase activity to be present in these fractions. There was no TPNH malic dehydrogenase activity found in any of the fractions prepared and assayed.

SUMMARY

1. Diaphorase and malic dehydrogenase were purified approximately 10-fold from germinating flax rust uredospores.
2. The pH optimum of diaphorase was 6.4 using phosphate buffer while the pH optimum for malic dehydrogenase was 8.2 using TRIS or glycylglycine buffers.
3. Both diaphorase and malic dehydrogenase were found to be quite stable, soluble enzymes.
4. The reaction catalyzed by malic dehydrogenase was reversed.
5. Both enzymes are inhibited by *p*-chloromercuriphenyl sulfonic acid.
6. Diaphorase was active with both DPNH and TPNH while malic dehydrogenase was DPNH specific.

ACKNOWLEDGMENT

This work was supported in part by a research grant (G-9081) from the National Science Foundation.

BIBLIOGRAPHY

1. Avron, A. M., and Jagendorf, A. T., "A TPNH Diaphorase from Chloroplasts", *Arch. Biochem. Biophys.*, **65**, 475-490 (1956).
2. Beadle, G. W., "Biochemical Genetics", *Chem. Revs.*, **37**, 15-96 (1945).
3. Clum, H. D., and Nason, A., "Triphosphopyridine Nucleotide Diaphorase from Wheat Germ", *Plant Physiol.*, **33**, 334-359 (1958).
4. Flor, H. H., "Host-Parasite Interaction in Flax Rust—Its Genetics and Other Implications", *Phytopath.*, **45**, 680-685 (1955).
5. Gornall, A. G., Bardawill, C. S., and David, M. M., "Determination of Serum Proteins by means of the Biuret Reaction", *Jour. Biol. Chem.*, **177**, 751-766 (1949).
6. Gunsalus, I. C., and Smith, R. A., "Oxidation and Energy Coupling in Keto Acid Metabolism", *Proc. International Symposium on Enzyme Chemistry, Tokyo and Kyoto*, 77-86 (1957).
7. King, T. E., and Howard, R. L., "A Soluble DPNH Dehydrogenase from Heart-Muscle Particles", *Biochem. Acta*, **37**, 557-559 (1960).
8. Mahler, H. R., and Elowe, D. G., "Studies on Metalloflavo-proteins. II. The Role of Iron in Diphosphopyridine Nucleotide Cytochrome C Reductase", *Jour. Biol. Chem.*, **210**, 165-179 (1954).
9. Mahler, H. R., Sarkar, N. K., Vernon, L. P., and Alberty, R. A., "Studies on Diphosphopyridine Nucleotide—Cytochrome C Reductase", *Jour. Biol. Chem.*, **199**, 585-597 (1952).
10. Massey, V., "The Identity of Diaphorase and Lipoyl Dehydrogenase", *Biochim. Biophys. Acta*, **37**, 314-322 (1960).
11. Massey, V., "The Identity of Diaphorase and Lipoyl Dehydrogenase", *Biochim. Biophys. Acta*, **30**, 205-206 (1958).
12. Mehler, A. H., Kornberg, A., Grisolia, S., and Ochoa, S., "The

- Enzymatic Mechanism of Oxidation-Reduction between Malate or Isocitrate and Pyruvate", Jour. Biol. Chem., **174**, 961-877 (1948).
13. Searls, R. L., and Sanadi, D. R., "Dihydrothioctyl Dehydrogenase—A Flavoprotein", Proc. Nat'l. Acad. Sci., **45**, 697-701 (1959).
 14. Straub, F. B., "Isolation and Properties of a Flavoprotein from Heart Muscle Tissue", Biochem. J., **33**, 787-792 (1939).
 15. Veeger, C., and Massey, V., "Inhibition of Lipoyl Dehydrogenase by Trace Metals", Biochem. Biophys. Acta, **37**, 181-183 (1969).
 16. Wolfe, R. G., and Neilands, J. B., "Some Molecular and Kinetic Properties of Heart Malic Dehydrogenase", Jour. Biol. Chem., **221**, 61-69 (1956).
-

THE USE OF PERCHLORIC ACID AS A SOLE OXIDIZING REAGENT FOR THE ORGANIC MATTER AND CHROMIUM OXIDE

Donald W. Bolin and William Lockhart

North Dakota Agricultural College, Fargo, North Dakota

One of the objectives of this paper is to present a procedure for the oxidation of organic matter using perchloric acid and water with sodium molybdate as a catalyst. In this procedure, perchloric acid is used as the sole oxidizing reagent. In general, most procedures for oxidation call for the predigestion of the organic material with sulfuric acid or nitric acid. Perchloric acid is then added to complete the oxidation of the organic matter. It is assumed that the use of nitric or sulfuric acid reduces the chance of an explosion. Some of the disadvantages resulting from the use of nitric acid is the foaming or frothing of the samples and a large amount of nitrogen dioxide fumes evolved during the process of digestion. The additions of the sulfuric acid adds sulfate ions to the solution which are objectionable for many analysis. These sulfates must be removed from the solution before analyses can be completed.

The following quotation is taken from Merck's Index, 6th Ed. "Perchloric Acid. HClO_4 ; mol. wt. 100.47 Cl 35.29%, H 1.01%.

The anhydrous acid is a colorless, volatile, very hygroscopic liquid. d_{20}^{20} 1.768; b_{11} 19°. Decomposes when distilled at atmospheric pressure, sometimes with explosive violence. m. -112°. Combines vigorously with water with evolution of heat, undergoes spontaneous and explosive decomposition, hence it is marketed only in mixture with water containing 60-70% HClO_4 , density 1.5 and 1.6 respectively. The aq. acid is very caustic and may deflagrate in contact with oxidizable substances."

From this quotation it would seem that perchloric acid is a dangerous and explosive acid to use. In this laboratory several thousand feeds and feces samples have been oxidized with perchloric

acid with no mishap. A safe, short, simple procedure for the use of perchloric acid as an oxidizing agent is described below.

Preparation of the oxidizing reagent: Dissolve 5 grams of sodium molybdate in one liter of water, add 1 liter of perchloric acid (70-72%) to this solution and mix thoroughly.

Oxidation of organic matter: Transfer not more than 1 gram of feed or feces to a 100 ml Kjeldahl flask; add 15 ml of the digestion reagent to the Kjeldahl flask, in such manner that it will wash down any material adhering to the neck of the flask. Make certain the acid has come in contact with all the sample and that no dry spots exist. Heat the contents in the flask with a micro burner until the excess water is driven off and the oxidation of the organic matter with perchloric acid begins. With some samples it is often desirable to turn off the heat and let the oxidation proceed under its own generation of heat. Swirl the flask to wash down any material on the sides of the flask or wash down the sides of the flask with more of the oxidizing reagent or with the concentrated perchloric acid (70-72%). Reheat the flask until oxidation has been completed. Cool and add water. The solution is now ready for filtering. Remove the white, insoluble silica and the filtrate is suitable for sulfates, phosphorus, calcium, etc. analyses.

Oxidation of chromium oxide: If the chromic oxide is present, the above solution will show a greenish precipitate immediately after complete oxidation of the organic matter. Prolonged oxidation of the sample with perchloric acid will completely oxidize the chromium oxide (Cr_2O_3) to chromium trioxide (CrO_3) which is a reddish yellow soluble compound. The sample is then to definite volume.

Color is made use of colorimetrically in the determination of chromic oxide. From the chromic oxide content of both feces and feed the apparent digestibility of the dry matter in a ration can be calculated. The digestibility of any nutrient can be found by analyzing the feed and feces for that particular nutrient and relating it to chromic oxide content of the feces and feed.

An alternate procedure is used in our laboratory for the determination of chromic oxide. This alternate procedure has an advantage in that it requires less chromic oxide in the ration, which is an important factor in the cost of adding chromic oxide to the ration for the larger farm animal.

A five to ten gram sample in an aluminium dish is ashed overnight in an electrically controlled muffle set at a low dull red heat. If ashed at proper temperature the bottom of the aluminium foil dish remains smooth and polished. Always put the samples in a cool muffle because if the muffle is too hot the sample will catch fire and melt the aluminum dish. Transfer the ash to a Kjeldahl flask and add 15 ml of the oxidizing reagent and proceed with the oxidation of the chromic oxide as previously described. Never add

the concentrated perchloric acid (70-72%) directly to the ash. When chlorides are present chromyl chloride is formed, which will result in a loss of chromium. Also, never add the concentrated acid directly to the sample of feces or feed. An explosion is very likely to result if the concentrated acid is added. Always add the diluted perchloric acid with the molybdate present as a catalyst.

The above described chromic oxide method was compared with conventional method. In the conventional method, the weight of feed consumed and feces excreted are determined on the oven dry basis. The digestibility can be calculated from these two values.

Chicks were used in two different trials, the results of these two feeding trials are shown in the table below, for the chromic oxide method and the conventional method.

A comparison of nitrogen retention by the conventional method and chromic oxide method.

Trial I			Trial II		
Bird No.	Conv. method	Chromic oxide method	Bird No.	Conv. method	Chromic oxide method
1	49.1	47.0	5	43.2	44.0
2	51.2	47.3	6	38.0	41.0
3	51.0	48.0	6	39.1	42.8
4	52.7	52.5	8	47.0	47.8

Although, there are rather wide differences of nitrogen retained among birds in some instances, the comparison of the methods on a per bird basis shows that the chromic oxide method is equivalent or superior to the conventional method with considerable less work in making the determination.

SUMMARY

Perchloric acid can be used alone in the oxidation of organic matter. No predigestion with nitric acid is needed. Transfer a 1 gram sample to 100 ml Kjeldahl flask. Add 15 ml of a solution containing 1-1 HClO₄ (70-72%) and water and 0.25 percent sodium molybdate. Heat the contents on a microburner, evaporate the water, the oxidation proceeds vigorously but smoothly. The entire operation requires less than five minutes.

Chromic oxide in feces and feeds can be oxidized in a similar manner.

THE REACTION TO PARTICULATE MATTER AND THE HISTOCHEMISTRY OF CERTAIN RETICULO-ENDOTHELIAL CELLS OF THE RAT'S SPLEEN**

*Theodore Snook, Joel Linford and Robert J. Bache**

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Studies were made to determine the significance of clusters of R E cells at the white pulp margins of the rat's spleen. If these

are R E cells they should: (1) give Marshall's metalophil reaction; (2), be phagocytic; (3), contain the enzymes acid phosphatase and nonspecific esterase.

In a previous communication they were termed "marginal metalophils" because they were blackened by Marshall's silver nitrate technique.

Chlorazol black E, india ink, and saccharated oxide of iron were injected into a tail vein to test for phagocytosis. In fifteen minutes, injected granules were found almost exclusively among the cells of the marginal zone. In twenty-four hours it had largely dispersed into the surrounding red pulp. Little or none was found in the marginal metalophils. Twenty-four hours following an initial injection of india ink, a second injection of saccharated oxide of iron was given. In ten minutes these cells gave a positive test for iron.

The marginal metalophils normally contain acid phosphatase but give only a weak reaction for nonspecific esterase. When the cells are stimulated by india ink, typhoid vaccine antigen, or surgery, the esterase content rises.

These reactions show that in the normal rat the marginal metalophils are relatively undifferentiated reticular cells. They change into active phagocytic reticular cells when the need arises.

*Undergraduate Research Participants.

**This investigation supported by grant from American Medical Association.

GRANULAR STABILIZATION OF ION EXCHANGE RESINS FROM LIGNITE COAL

R. E. Dunbar, F. L. Minnear and James M. Olson

School of Chemical Technology

North Dakota Agricultural College, Fargo, North Dakota

A recognition of the principle of ion exchange had its beginning when Thompson (9), noted that soil would absorb ammonium sulfate but that only calcium sulfate could be removed by washing. This phenomenon was explained by Way (9), as an ion exchange with the complex silicates in the soil. From this simple beginning the technology of ion exchange has grown until today it is a highly diversified tool of the chemist.

Coals have been investigated to a limited extent to determine their feasibility for use as the base for an ion exchange resin (4). The presence of carboxyl and hydroxyl groups give coal a certain amount of natural exchange capacity. Large amounts of coal are available at relatively low cost and it is for these reasons that it has been the subject of much interest. The exact chemical structure of coal is not yet completely known. However, Fuchs (3), Wheeler (5),

Howard (6), Ward (15), and Kinney (8), have shown that it is made up primarily of complex aromatic ring systems linked together through nonaromatic bridges containing oxygen atoms. Attached to these rings are various functional groups which give the coal its natural ion exchange capacity. The hydroxyl and carboxyl groups are the most prevalent while methoxyl and carbonyl groups are also present in lesser amounts.

Treatment of coal with sulfuric acid will cause the addition of sulfonic groups which markedly increases the cation exchange capacity of the coal. Several changes in both the chemical and physical properties of the coal occur during these sulfonations. Kinney and Gray (7), have shown that treatment of bituminous coal with sulfuric acid in the range of 25° to 150° causes the loss of about 13 per cent of the carbon and over 50 per cent of the hydrogen. Part of the loss of carbon is attributed to oxidation to carbon dioxide. A low hydrogen-carbon ratio of 0.4 in the sulfonated coal is interpreted as indicating a poly-aromatic or more probably a quinoid type structure (14). Kinney and Gray (7), also found that the amount of sulfur introduced during the sulfonation process remains fairly constant regardless of conditions of the treatment. For example, a sample of raw coal analyzing 2.5 per cent total sulfur upon sulfonation will analyze 5 to 9 per cent sulfur. A likely structure for the addition type reaction, according to Kinney and Gray (7), would involve the presence of unsaturated rings, which, upon treatment with the acid, could undergo oxidation to aromatic or quinoid structures. Any of the phenolics when sulfonated would probably be oxidized to quinoid structures also. The reflectivity of the coal is altered during sulfonation, being increased in humus and bituminous coals (11). This is thought to be due to the gelling of the coal particles as a result of the reaction with hot concentrated sulfuric acid (12). Swelling also occurs during sulfonation and may be an indication that the sulfonation reaction is complete although this conclusion has not yet been fully related to structural changes (13).

A previous study by Seitz and others (10), has shown that lignite exhibits some ion-change properties, and that these could be improved by chemical treatment. They found that drying the lignite at 100-110° followed by sulfonation for 8 hours at 90-100° gave an ion-exchange resin of high capacity, but a product that disintegrated badly on prolonged use or handling. It seemed desirable, therefore, to study the degree to which this breakdown occurred and to explore possible ways and means of preventing the same.

EXPERIMENTAL

The sulfonation techniques and general procedures, with the exceptions noted herein, were the same as those previously used by Seitz (10). The lignite coal was again obtained from the Baukol-Noonan Mine at Noonan, North Dakota, and its proximate and

ultimate analysis has been previously reported (10). The equilibrium method of exhausting the ion exchangers was used throughout. In this method a known weight of the resin was placed in a 500 ml. Erlenmeyer flask. A 100 ml. portion of standard hard water was then added to the flask and the same was agitated gently on a mechanical shaker for 24 hours. The flask was then allowed to stand until the particles had settled out, when a 10 ml. aliquot was then withdrawn from the solution and titrated by the versene method (1). The equilibrium reaction was maintained at room temperature which varied between 23° and 27°. Regeneration of the lignite samples was accomplished by pouring a sufficient amount of saturated sodium chloride solution to cover the lignite sample, shaking on a mechanical shaker for 15 minutes, and allowing the same to settle for approximately 1 hour. The sodium chloride solution was then decanted off and the resin washed with distilled water until the chloride ion was absent as indicated by a silver nitrate test. Batches of the most satisfactory sulfonated and phosphonated lignites, as recommended by Seitz (10), were prepared, and the same were subjected to repeated exhaustion and regeneration cycles, in order to compare their sustained properties. This procedure was supplemented by treatment of the lignite in a variety of ways, both before and after sulfonation, in an attempt to keep the particles from disintegrating. All lignite used in this investigation was 12 to 15 mesh size. All dried lignite was treated in a convection oven at 100° to 110° for 1 hour. The coal was acid treated by placing a weighed amount of dried lignite in a standard tapered flask, equipped with stirrer and reflux condenser, and adding an excess of 85 per cent acid to completely cover the lignite. The reaction was continued for 8 hours at 90° to 100°. After the reaction was completed, distilled water was cautiously added to dilute the unreacted acid, the solution decanted, and the resin washed with distilled water to a pH between 4 and 6. The same general method was used in both the sulfonation and phosphonation treatment of the lignite. Analysis of a sample of lignite treated in this manner with sulfuric acid showed a sulfur content of 3.29 per cent (as received), 3.71 per cent (moisture free), and 3.92 per cent (moisture + ash free), as compared to 0.47, 0.71, and 0.79 per cent respectively on the comparable original lignites. This represents an increase of over 390 per cent in sulfur content.

Several methods were attempted to stabilize lignite against disintegration but most procedures introduced more complications than they eliminated. Among such was the treatment of dried lignite with styrene and benzoyl peroxide followed by sulfonation. Similar treatment with a phenol-formaldehyde foaming resin gave a very non-homogeneous product. The use of Pliolite Latex containing 55.9 per cent solids gave a non-porous product.

The most satisfactory stabilizing and supporting additive was

prepared from soluble sodium silicate or water glass. The basic procedure was adapted from *Inorganic Syntheses*, Volume II (2). A 25 g. sample of sulfonated lignite was treated with a 50 ml. portion of diluted sodium silicate solution. The wet mass was then spread evenly on a glass plate to a depth of 0.25 in. and 35 ml. 6M sulfuric acid was poured slowly over the entire sample. This product was then allowed to stand overnight at room temperature, removed, and washed eight times with distilled water. This procedure worked well and was used for all applications of sodium silicate to the lignite coal. Different concentrations of commercial sodium silicate solution to water, namely 3:1, 1:1, and 1:3; were used to determine which, if any, was superior to the others in preventing structural breakdown of the lignite.

A consistent and uniform method was adopted for determining the percentage of disintegration while each of the treated lignites was subjected to repeated exhaustion and regeneration. After use, the lignite was washed quantitatively into an evaporating dish and dried in a convection oven. Sieving the residue on a No. 40 sieve gave a weighable amount of fine particles passing through the sieve. A 125 g. portion of lignite, which had been dried 1 hour and sulfonated for 8 hours, was shaken on a No. 40 sieve to remove any small particles of coal. The larger particles were divided into 25 g. samples and each was placed in a 500 ml. Erlenmeyer flask. One hundred ml. distilled water was placed on each sample and they were all placed on the mechanical shaker simultaneously. One sample was removed every 24 hours, the water removed by evaporation, and the dry residue sieved. The percentage of disintegration was determined by dividing the weight of fine particles passing through a No. 40 sieve by the total amount of resin originally present. The results, as summarized in Table III, show some slight inconsistencies due to slight variations in different samples.

The results were divided into three main sections consisting of (I), a comparison of sulfonated and phosphonated lignite through six exhaustion-regeneration cycles; (II), treatment of lignite with various concentrations of sodium silicate; and (III), determination of particle disintegration. The results are summarized in Tables I, II, and III.

DISCUSSION OF RESULTS

The results of the first phase of this investigation, i.e., the comparison of sulfonated lignite and phosphonated lignite shows that the sulfonated product had better ion exchange properties over a period of six exhaustion-regeneration cycles.

Various methods of treating the lignite were used to see if, not only particle stability might be improved, but also the exchange capacity enhanced. The results are summarized in Table II. Mixing sodium silicate with lignite and treating with 6M sulfuric acid to

form silica gel around the lignite particles, gave better exchange results than any other chemical method tried. Experiments with various concentrations showed that the 1:1 and the 1:3 sodium silicate-water ratio gave comparable and slightly better results. It must be concluded, however, that while treatment with sodium silicate yields ion exchangers with high capacities, there is little difference between these products and the sulfonated lignite having no silicate treatment.

An examination of Table III reveals that a large amount of disintegration is apparent in all the lignite samples treated with sodium silicate. This is due both to mechanical breakdown and solubility of the sodium silicate in the hard water and sodium chloride solutions used in the exhaustion-regeneration cycles. It was further noted that the actual disintegration of the sulfonated lignite was relatively small as compared to the conclusions of Seitz (10). This newly discovered difference can now be attributed to the fact that fuming sulfuric acid was used in the earlier sulfonations, while 85 per cent sulfuric acid only was used in the present investigation.

A recommended procedure for making a satisfactory ion exchange resin from lignite coal now evolves. Approximately 200 g. of 12-25 mesh lignite is dried for 1 hour at 100-110°, and the coal is then sulfonated with 400 ml. of 85 per cent sulfuric acid at 90-100° for 8 hours. One liter of distilled water is cautiously added, the mixture allowed to settle, the excess acid decanted, and the resin washed with distilled water to a pH of 6. Screening this product on a No. 40 sieve yields a product of high ion exchange capacity and low disintegration tendency.

SUMMARY

As a result of the above investigation, the following conclusions appear to be justified. Sulfonated lignite showed better ion exchange capacity than phosphonated lignite when treated under comparable conditions. Sulfonated lignite treated with sodium silicate gave resins with slightly higher ion exchange capacities than sulfonated lignite having no sodium silicate treatment. The amount of particle disintegration in sulfonated lignite is less than in sulfonated lignite treated with sodium silicate. Treatment of lignite with diluted solutions of sodium silicate gave generally better results both with respect to ion exchange capacities and particle disintegration than did treatment with an undiluted solution of sodium silicate.

LITERATURE CITED

1. Bersworth Chemical Co., Framingham, Mass., "The Versenes for Exactng Control of Cations in Solution, Technical Bulletin No. 2," Sec. 3, p. 5, 1952.
2. W. C. Fernelius, "Inorganic Syntheses, Vol. II," McGraw-Hill Book Co. Inc., New York, N. Y., pp. 95-7, 1946.
3. W. Fuchs and A. C. Sandhoff, "Theory of Coal Pyrolysis," Ind. Eng. Chem., **34**, 567-71 (1942).

4. I. L. Gordon, "Chemical Desalination of Water," *Teplo-Silovoe Khoz.* 1941, No. 2, 29-32; *Khim. Referat. Zhur.* **4**, No. 9, 114 (1941).
5. F. Heathcoat and R. V. Wheeler, "Studies in the Composition of Coal. The Constitution of the Ulmins," *J. Chem. Soc.*, **1932**, 2839-47.
6. H. C. Howard, "Evidence for the Cyclic Structure of Bituminous Coals," *Ind. Eng. Chem.*, **44**, 1083 (1952).
7. C. R. Kinney and V. E. Gray, Paper presented before Division of Gas and Fuel Chemistry, American Chemical Society, Boston, Mass., April, 1959.
8. C. R. Kinney and D. L. Love, "Quinone Character of Oxidation Products of a Bituminous Coal," *Anal. Chem.*, **29**, 1641-5 (1957).
9. F. S. Nachod and J. Schubert, "Ion Exchange Technology," Academic Press, Inc., New York, N. Y., p. 3, 1956.
10. D. S. Seitz, F. L. Minnear, and R. E. Dunbar, "Ion Exchange Resins from Lignite Coal," *Proc. N. Dakota Acad. Sci.*, **13**, 76-82 (1959).
11. H. Stack and M. Teichmuller, "The Chemistry and Petrography of Ion Exchangers from Brown Coal and Coal, III. Increased Reflectivity of Coals on Activation as Exchangers in Comparison with the Increase during Natural Coalification." *Brennstoff-Chem.*, **34**, 337-8, (1953).
12. M. Teichmuller, "The Chemistry and Petrography of Ion Exchangers from Brown Coal and Coal. II. The Petrographic Investigation of Ion-Exchangers and the Coals from which They are Derived," *Brennstoff-Chem.*, **34**, 333-6 (1953).
13. D. W. van Krevelen, "Physical Characteristics and Chemical Structure in Bituminous Coal," *Brennstoff-Chem.*, **34**, 167-82 (1953).
14. D. W. van Krevelen and J. Schuyer, "Coal Science," Elsevier Publishing Co., New York, N. Y., pp. 145-7, 1957.
15. S. G. Ward, "Coal-Its Constitution and Utilization as a Chemical Raw Material," *J. Inst. Fuel*, **21**, 80-8 (1947).

TABLE I

Ion Exchange Capacities of Lignite, Sulfonated and Phosphonated. (Six Cycles).

Type of Lignite	Wt. g.	Mg. Ca ion Retained/g. Lignite						Ave.
		1	2	3	4	5	6	
A. Sulfonated (moist)	17	13.5	13.4	13.2	12.9	12.9	12.9	13.13
B. Phosphonated (dry)	16	9.76	8.46	7.73	8.30	7.50	8.89	8.44
C. Phosphonated (moist)	16	11.4	11.8	10.6	10.7	9.50	9.96	10.66

A. Lignite dried, sulfonated 8 hours, and used moist.

B. Lignite dried, phosphonated 8 hours, and used after drying.

C. Lignite dried, phosphonated 8 hours, and used moist.

TABLE II
Ion Exchange Capacities of Lignite Subjected to Various Treatments.
(Three Cycles). (All 25 g. Portions).

Type of Lignite	Mg. Ca ion Retained/g. Lignite			
	1	2	3	Ave.
D. As received-Dried	5.56	5.48	3.99	5.01
E. Sulfonated (Control)	8.10	9.20	9.06	8.79
F. Sulfonated- Na_2SiO_3 (Concd.)	9.32	9.22	9.10	9.21
G. Sulfonated- Na_2SiO_3 (Dil. 3:1)	9.32	9.22	9.10	9.21
H. Sulfonated- Na_2SiO_3 (Dil. 1:1)	9.32	9.28	9.14	9.25
I. Sulfonated- Na_2SiO_3 (Dil. 1:3)	9.32	9.28	9.06	9.22
J. Raw- Na_2SiO_3 (Concd.)	7.48	5.40	4.83	5.90
K. Raw- Na_2SiO_3 (Dil. 1:3)	7.44	5.36	5.03	5.94
L. Na_2SiO_3 (Concd.)-Sulfonated	6.56	8.26	7.93	7.58
M. Na_2SiO_3 (Dil. 1:3)-Sulfonated	6.80	8.81	8.40	8.70

D. Lignite, as received, dried 1 hour.

E. Lignite dried, sulfonated 8 hours, and used as control.

F. Lignite dried, sulfonated 8 hours, and treated with undiluted sodium silicate.

G. Lignite dried, sulfonated 8 hours, and treated with sodium silicate solution-water (3:1).

H. Lignite dried, sulfonated 8 hours, and treated with sodium silicate solution-water (1:1).

I. Lignite dried, sulfonated 8 hours, and treated with sodium silicate solution-water (1:3).

J. Lignite, as received, treated only with undiluted sodium silicate.

K. Lignite, as received, treated only with sodium silicate solution-water (1:3).

L. Lignite dried, treated with undiluted sodium silicate, followed by sulfonation for 8 hours.

M. Lignite dried, treated with sodium silicate solution-water (1:3), followed by sulfonation for 8 hours.

TABLE III
Disintegration of Lignite Treated in Various Ways.

Type of lignite	Per Cent Disintegration After		
	1 day	2 days	3 days
N. Lignite as received, Dried 1 hour	1.41	5.88	10.0
O. Lignite as received-Concd. Na_2SiO_3	30.6	28.7	25.7
P. Lignite as received-Dil. Na_2SiO_3	17.1	12.7	12.8
Q. Dried Lignite-Sulfonated 8 hours	0.78	1.11	1.11
R. Dried Lignite-Sulfonated, Concd. Na_2SiO_3	19.3	16.6	16.9
S. Dried Lignite-Sulfonated-Dil. Na_2SiO_3	11.8	10.2	9.0
T. Dried Lignite-Concd. Na_2SiO_3 , Sulfonated	12.0	15.5	12.7
U. Dried Lignite-Dil. Na_2SiO_3 , Sulfonated	27.7	28.6	32.1

POPULATION FLUCTUATIONS AND CYCLOMORPHOSIS IN TWO SPECIES OF ROTIFERS DURING THE ICE-FREE PERIOD OF THE YEAR

G. W. Comita and Jane Ann Munro

Department of Zoology and Physiology

North Dakota Agricultural College, Fargo, North Dakota

The population fluctuations and the cyclomorphosis of two species of rotifers were studied from samples taken from a small lake in the Detroit Lakes Region of Minnesota between 22 April to 2 November 1955. The samples were obtained with a modified Clarke-Bumpus plankton sampler. Triplicate samples were taken every two and three days. The populations were counted and ten specimens were measured for each day of sampling for both species. The changes in length of the anterior and posterior spines, and the whole body were studied.

After mid-April, the first rotifer populations emerged and increased as temperature and photosynthesis increased; thus, photosynthesis seemed to provide an abundance of food for all populations present. An emerging large population of **Diaptomus** nauplii were noted in the catches in increasing numbers through the latter half of May. When this occurred, the number of rotifers decreased abruptly, suggesting that the rotifers are sensitive to the presence of the nauplii, probably due to competition for food.

K. cochlearis showed a sharper and earlier decrease than did **K. quadrata** and both populations fluctuated during June. This fluctuation was correlated with invasion of the lake by an insect larva, **Chaoborus punctipennis**, which is predaceous on both rotifers as well as on the **Diaptomus** nauplii. Competition for food between the nauplii and the rotifers probably brought about the early rapid decline. The predation by **Chaoborus** is probably responsible for the fluctuations in both the nauplius, as well as the rotifer populations, being most apparent between mid-May and early in August. After early August the **Chaoborus** population emerged from the lake, the **Diaptomus** nauplii again became abundant and the **K. cochlearis** population which had already begun its recovery, decreased again, probably because of the competition for food. The nauplii became steadily fewer in numbers during September and October and as this happened, **cochlearis** recovered, but **quadrata** did not.

Cyclomorphosis was evident in both rotifers. The most pronounced change occurred in the length of the posterior spine.

K. cochlearis declined steadily in length until mid-August. After a temporary recovery a decrease in size was noted during September. Length at the end of the season was approximately equal to that noted in the early spring.

K. quadrata began the season as short forms, increased in size

during late May, then short forms were noted in mid-August, when other herbivores were present in large numbers.

These data indicate that quantity food was most likely the main factor influencing rotifer size.

URINARY EXCRETION OF ALKALINE PHOSPHATASE FOLLOWING RENAL ISCHEMIA IN RATS

John Lunseth

University of North Dakota, Grand Forks, North Dakota

Bilateral temporary ligation of the renal vessels in rats of both sexes for 15, 30, 45 or 60 minutes resulted in a significant rise in total UAP excretion only during the first 12 hours after surgery. During this period the AP excretion was greatest in the groups with 30 and 45 minutes of renal ischemia.

There was a considerable drop in excretion in the animals with 60 minutes of renal ischemia to below the level found in animals with 15 minutes renal ischemia. Preliminary experiments indicate that the cause for this drop was the appearance of a heat stable, dialyzable AP inhibitor which produced a non-competitive type of inhibition.

Histological evidence of tubular necrosis was not apparent following 15 minutes and 30 minutes of ischemia. However, it became marked after 45 minutes and 60 minutes of ischemia. Depletion of histochemically visible renal AP correlated with the AP excretion only up to 30 minutes of ischemia.

MICROBIOLOGICAL ASSAY OF YEAST AND CHICK INHIBITORS FROM FLAXSEED¹

J. L. Parsons, J. W. Magill, and H. J. Klosterman

*Departments of Bacteriology and Agricultural Biochemistry
North Dakota Agricultural College, Fargo, North Dakota*

ABSTRACT

The inhibitory effect of linseed meal on the growth rates of chicks has been known for many years. In 1948, Kratzer showed that pyridoxine counteracted this inhibition. Research is currently underway on the isolation, characterization, and mode of action of this antivitamin B₆ inhibitor.

A rapid assay was needed to facilitate the isolation of the chick inhibitor. Since vitamin B₆ reverses the chick inhibition, it was natural to select, for a microbiological assay, an organism that is used in the biological assay of vitamin B₆. The yeast, *Saccharomyces*

carlsbergensis ATCC 4228, was chosen because it uses all of the various forms of vitamin B₆ and because it grows readily on the surface of agar medium. Early in the work on the microbiological assay, it was discovered that assay of extracts of flax meal on a vitamin B₆ assay medium, containing low levels of the B vitamins, gave good inhibition of the yeast. With this as a lead nine of the water soluble vitamins were used singly and in all possible dual combinations. Biotin with pyridoxine in the assay medium consistently gave the best inhibitory response with various inhibitor preparations. Calcium pantothenate and inositol, used singly with pyridoxine, resulted in good inhibition on some inhibitor preparations and not others. Thiamin reversed the yeast inhibition whereas pyridoxine did not. The other vitamins showed little effect. It appeared that the yeast inhibitor interfered with thiamin rather than pyridoxine.

Glucose levels in the assay medium could be reduced from 10% to a 0.5% level and still result in good inhibition. Medium with no sugar gave no inhibition even though background growth was satisfactory. Casein levels could be reduced from 0.8% to 0.4%, but lower levels resulted in only faint inhibition, whereas levels increased to 2.4% still gave good inhibition. The sugar requirement in the medium for the inhibitory response and reversal of inhibition by thiamin would relate the mechanism of action of the inhibitor to the carbohydrate metabolism. However, the nitrogen metabolism must also be involved since reduction of casein also eliminated the yeast inhibition.

Seventy per cent ethanol extracts of flax cotyledons were passed over IR-120 and IRA-400 resins. Eluates from the resins inhibited the growth of both chicks and yeast. However, when the inhibitor preparations from IRA-400 resin were chromatographed on Dowex-1, the yeast and chick inhibitors were separated. Fractions that contained the yeast inhibitors did not inhibit chicks, and fractions that inhibited chick growth caused only slight yeast inhibition.

Paper chromatographic methods have demonstrated at least five separate yeast inhibitors, only two of which are adsorbed onto Dowex-1 resin. Several ninhydrin positive compounds are present in the samples that contain multiple yeast inhibitors, but paper chromatography of these samples followed by ninhydrin treatment and bioassay showed that the yeast inhibitors do not correspond to amino acids and are not destroyed by the ninhydrin reagent.

Work is in progress on the development of a microbiological assay for the chick inhibitor and on the isolation and characterization of both the yeast and chick inhibitors.

ACKNOWLEDGEMENTS

Some of the early work on the microbiological assay, by Jack Moreland, a participant in the National Science Foundation Research Participation Program, is gratefully acknowledged. We also wish to express our appreciation to Mr. W. J. Lockhart and the Poultry Husbandry Department for assistance and use of facilities in the chick assay work.

Published with the approval of the Director as Journal Article No. 4. This investigation is supported in part by: a PHS Research Grant (A-3024) from the National Institutes of Health, Public Health Service, and the National Science Foundation.