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A STIMULATOR OF KIDNEY MITOCHONDRIAL OXYGEN UPTAKE

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In the course of another investigation it was noticed that results obtained with unwashed kidney mitochondria differed from those obtained with washed mitochondria. These differences were found to be due to a material present in the supernatant after the mitochondria had been removed. Washing the mitochondria eliminated this material, which was subsequently shown to be a potent stimulator of kidney mitochondrial oxygen uptake. Attention was centered on a study of the properties of this stimulatory material because of the profound significance of such activity in all work with mitochondria.

METHODS

Mitochondria were prepared by making a 20% kidney homogenate in 0.44 M sucrose. The homogenate was centrifuged at 600 x *g* for 10 minutes. The resulting supernatant was centrifuged at 58,000 x *g* for 12 minutes yielding a mitochondrial pellet which was washed by resuspension in 0.44 sucrose and recentrifugation at 58,000 x *g* for 12 minutes. This entire procedure was a modification of the methods of Schneider and Hogeboom (1,2).

Oxygen uptake was measured manometrically on a constant volume Warburg apparatus at a temperature of 37°. The gas phase was air. The medium used consisted of the following: tris-hydroxymethyl-aminomethane buffer (Tris), 80 μmoles; KCl, 200 μmoles; $MgCl_2$, 40 μmoles; adenosine triphosphate (ATP), 6 μmoles; cytochrome c, 0.08 μmoles; citrate 20 μmoles; and potassium phosphate buffer, 4 μmoles, pH = 7.4.

The stimulatory material was prepared from kidneys homogenized in 0.25 M sucrose. This homogenate was centrifuged at 105,000 x *g* for one hour. The resulting supernatant contained the stimulatory material. It was very stable; only repeated freezing and thawing diminished its effects. The supernatant could be prepared in large amounts, lyophilized and stored at 0° until used. Later work showed that the supernatant could be prepared from kidneys homogenized in water rather than sucrose. This eliminated sucrose from the lyophilized material.

RESULTS AND DISCUSSION

Ashing the material destroyed its ability to stimulate oxygen uptake, which indicated that the stimulator was probably not inorganic. Dialysis of the material for five hours against water resulted in a loss of about 40% of the activity.

Placing the material in a boiling water bath for 10 minutes resulted in a decreased stimulatory activity. Activity was found, however, in the solution and with the precipitated protein. If the denatured protein was centrifuged out of the solution, only about ¼ of the original activity remained in solution. This remaining activity could be removed by dialysis (Figure 1). Ultrasonic ocsillations at maximum frequency for 35 minutes obtained with a Ratheon Magnetostriction Oscillator, model S-102A, were without effect on the stimulatory activity of the material.

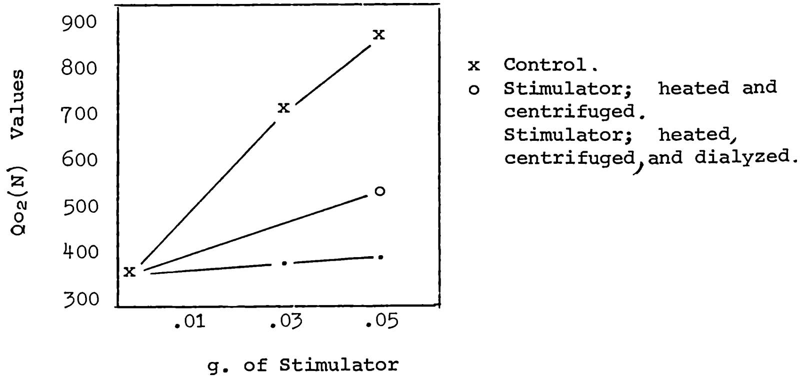

FIGURE I—Effect of heat and dialysis on the stimulator.

The stimulatory material itself supported little oxygen uptake if mitochondria were not present (Table I). This would suggest that the stimulatory material is not an enzyme system capable of citrate oxidation but rather a substrate or co-enzyme. It could be an

TABLE I
EFFECT OF STIMULATOR WHEN NO MITOCHONDRIA ARE PRESENT

	Citrate[1] Stimulator	No Citrate[2] Stimulator	Tris buffer Stimulator	0.25 M Sucrose Stimulator
μlO_2	56	61	30	39

Stimulatory material was added to each flask as a solution of 0.025 g./.5 ml. of water. Readings were taken for one hour. The 0.2 M Tris buffer was adjusted to pH 7.4.

[1] Complete media except for mitochondria.

[2] Complete media except for citrate and mitochondria.

enzyme system which is lacking some co-factor which the mitochondria can supply. It might also be some secondary system producing an activator, or tying up an inhibitor. There are many possibilities.

In Table II the oxygen uptake and disappearance of citrate are compared. As the amount of added stimulatory material was increased the citrate utilization increased.

TABLE II
EFFECT OF THE STIMULATOR

	Citrate Control	Stimulator 0.025 g.	Stimulator 0.05 g.
Q_{O_2}(N)	372	970	1320
μ moles of citrate oxidized	1.9	3.1	9.1

The effect of placing the stimulatory material in a boiling water bath for 10 minutes is shown in Table III. It is apparent that part of the stimulatory activity was destroyed by heat. When citrate was present heating reduced the effect of the stimulator by approximately 43%. In the case where citrate was not present heating reduced the effect of the material by approximately 51%. Of greatest interest is the observation that the stimulator supports a large oxygen uptake by washed mitochondria in the absence of added substrate. The latter observation suggests that there is a second mode of oxygen consumption in addition to the stimulated oxygen consumption accompanied by citrate utilization.

TABLE III
EFFECT OF HEAT ON THE STIMULATOR

Oxidation in the Presence of Citrate

	Control	Stimulator 0.025g.	Heated Stimulator 0.025 g.
Q_{O_2}(N)	350	925	678

Oxidation in the Absence of Citrate

	Control	Stimulator 0.025 g.	Heated Stimulator 0.025 g.
Q_{O_2}(N)	24	750	375

Lipid extraction was attempted using ethyl ether. The ether soluble residue, after removal of the ether, was taken up in a 10% ethanol-sucrose (0.25 M) solution. The ethanol-sucrose solution alone had previously been found to have no effect on mitochondrial oxidation (3). When the extracted material was added to Warburg flasks, no stimulatory activity was found either in the presence or absence of citrate. Butanol extraction was also attempted yielding the same results. Neither of the two types of stimulatory activity then appear to be lipid.

CONCLUSION

Some properties of a stimulator of oxygen uptake by washed kidney mitochondria have been investigated. This material, found in the supernatant of kidney homogenates after high speed centrifugation, is stable and after lyophilization can be stored for long periods of time at 0°. Ashing the material demonstrated that it is probably organic. Dialysis and heat lability experiments demonstrated that the material is most likely protein bound. The material does not appear to be lipid. It is now evident that the material contains two types of oxygen uptake stimulating activity; one which is related to citrate disappearance, and another which causes an increased oxygen consumption in the absence of added substrate.

Since this material will be found in unwashed mitochondria and will be absent from preparations of washed mitochondria it is important that the nature of the stimulatory activity be determined in order to accurately assess the results obtained in mitochondrial studies.

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FACTORS AFFECTING THE DISTRIBUTION OF CERTAIN SPECIES OF COMPOSITAE ON AN EASTERN NORTH DAKOTA PRAIRIE

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The prairies of North Dakota have not been the subject of any intensive vegetational study. Sarvis (1920) investigated the composition and density of native vegetation in the vicinity of Mandan. Hanson and Whitman (1935) made a study of major grassland types in western North Dakota. These studies used an extensive approach, sampling a large number of stands, and also concentrated upon the grasses because of their importance as forage producers.

This investigation concerns the family Compositae. Dix and Butler (1960) found the Wisconsin prairie which they investigated to be dominated by four plant families: Compositae, Gramineae, Leguminoseae, and Asclepiadaceae, with 30%, 15%, 4% and 4% of the total species, respectively. Studies by Whitford (1958), Curtis and Green (1949), and Steiger (1930) found the family Compositae to show very similar high percentages. Dix and Butler (1960) suggest that this order of families might be characteristic of the entire prairie region. A floral list of the Oakville Prairie and a nearby section shows Compositae with 22% of the total species and Gramineae with 24%.

It is because of this evident importance of the family Compositae on the prairie that this group of plants was chosen for our investigation. The purpose of this project was to (1) determine the most abundant of Compositae, and (2) to investigate some of the factors which might influence the distribution of these species.

THE STUDY AREA

This investigation was carried out at the University of North Dakota Biology Area, Oakville Prairie, NW¼, Section 9, T15IN R52W. This quarter section is located in Oakville Township in east central Grand Forks County, North Dakota.

The prairie area is composed of lake sediments overlying glacial till with the exception of some doubtful mounds which are possibly old fixed dunes (Laird 1944). One of these mounds is located slightly east of center and the second in the southeast corner of the quarter section. The remainder of the quarter section is relatively flat except for a rise at the southwest corner which is probably an extension of the Ojata beach of Glacial Lake Agassiz.

The distinctly alkaline soil of the Oakville Prairie is classified as solonchak (Sandowal *et al.*, 1964). Such soils have high concentrations of soluble salts in relation to those of other soils. These salts frequently collect on the surface under arid or semiarid conditions, forming a saline crust. This crust may be seen at Oakville after periods of evaporation and no rainfall. Alkaline soils occur in subhumid or semiarid climates in areas of poor drainage; these criteria fit the low land of Oakville Prairie.

METHODS

Thirty-eight sampling plots were randomly located in the study area (Figure 1). Within each five meter square plot were located 16 quadrats, each 50 centimeters square. Density of each species was obtained by counting the number of plants within each quadrat. Density per square meter was obtained by dividing the total density of a species in the plot by four.

Within each plot soil samples were taken at a depth of 10 inches. Five samples from each plot were pooled, air dried and stored for later analyses.

On August 6-7 measurements of total soil moisture were made from 9-12 A.M. One sample was taken in each plot, immediately

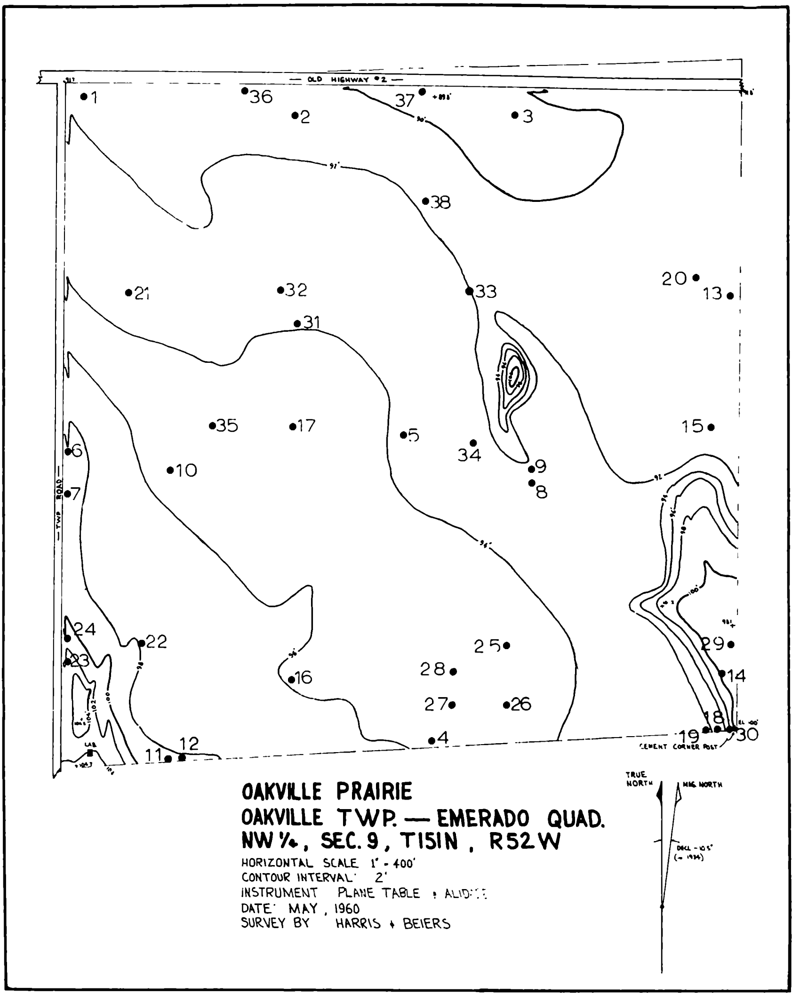

FIGURE 1—Locations of sampling plots.

placed in a covered aluminum can to prevent dessication, and weighed within 15 minutes. After field weighing the samples were taken to the laboratory and oven dried at 105°C for 24 hours. The samples were then reweighed and total moisture was calculated as percent of dry weight.

Salinity was measured as conductivity per square centimeter. A saturated soil paste was filtered, and the liquid extract was placed in a conductivity bridge circuit. Water retaining capacity was determined by a modification of Hilgard method, using Gooch perforated-bottom crucibles. Measurements of elevation were made by scaling the paced locations of the plots onto a contour map. These figures are approximate due to possible inaccuracy of pacing.

STATISTICAL PROCEDURE

The data, including species present, species density, and total density, were recorded on special data cards. These plot data cards were inspected, and six plots were chosen which seemed the most dissimilar to each other and to the remaining plots. Then the similarity coefficients of each of these plots to those remaining were calculated by the formula:

$$K = \frac{2W}{a + b} X\ 100$$

K is the coefficient of similarity between two plots (A and B), a and b are the sums of frequencies for all species in plots A and B, respectively, and W is the sum of frequencies shared by the two plots. Therefore, if two plots have the same species composition, K = 100, and K = 0 when the plots have no species in common.

Table I shows the matrix which was obtained. Similarity coefficients of the selected plots were totaled and the plot with the smallest total was selected as one end of an ordination axis. Plot 29 was chosen rather than plot three because the basis of the low degree of similarity in plot three is the lack of a single species of Compositae. An ordination involving plot three would be based on differences of total plot density rather than differences in species and species density.

To establish the opposite end of the ordination axis, a plot was chosen which was the least similar to plot 29. As the matix (Table I) shows, there are 19 plots which show no similarity to plot 29. After inspection of these plots, number 21 was arbitrarily chosen giving the second of two dissimilar end plots, A and B, of the ordination axis.

In order to locate the remaining plots on the axis, the procedure of Beals (1960) was used. This is a modification of the original method described by Bray and Curtis (1957). These methods arrange stands according to their relative dissimilarity, therefore inverse values of the similarity coefficients were used. Table II gives these inverse or dissimilarity values.

Bray and Curtis (1957) located stands along the axis by striking arcs which represented dissimilarity from the two ends (A and B). These arcs intersect below and above the axis and the intersection of the arcs is projected onto the axis. By observing the triangles formed and using the Pythagorean theorem, Beals (1960) derived a formula which simplifies the location of plots.

A y-axis was now constructed in order to separate stands which, although located close together in the x ordination, were actually dissimilar. Again the method of Beals (1960) was used. The first

TABLE I

SIMILARITY COEFFICIENTS

Plot No.	1	3	11	19	21	26	29	34
1		00	04	01	82	01	00	01
2	22	00	06	19	18	49	00	46
3	00		00	00	00	00	00	00
4	02	00	45	56	20	03	04	36
5	24	00	12	68	17	19	00	28
6	02	00	49	01	05	01	33	13
7	02	00	73	03	06	02	29	58
8	55	00	19	52	62	02	00	12
9	51	00	53	16	61	01	17	31
10	42	00	24	25	50	18	00	30
11	04	00		08	14	01	24	43
12	03	00	68	07	12	01	14	60
13	20	00	12	30	33	68	00	30
14	01	00	16	01	01	00	40	00
15	02	00	11	55	18	54	00	29
16	01	00	14	41	13	40	00	27
17	04	00	21	37	23	56	00	43
18	03	00	27	03	04	04	54	08
19	01	00	08		14	14	00	17
20	11	00	19	39	30	37	01	44
21	82	00	19	14		01	00	03
22	06	00	73	06	14	01	10	28
23	40	00	12	01	30	01	15	01
24	05	00	62	02	06	01	31	41
25	03	00	28	18	05	56	01	68
26	01	00	01	14	01		00	33
27	00	00	11	41	19	50	00	38
28	02	00	50	06	04	08	26	44
29	00	00	24	00	00	00		00
30	04	00	17	05	04	04	30	07
31	42	00	05	19	34	25	00	33
32	56	00	09	56	71	07	01	08
33	09	00	12	91	22	18	00	24
34	01	00	43	17	03	33	00	
35	44	00	04	17	36	43	00	40
36	03	00	42	53	21	09	10	33
37	05	00	40	60	21	09	17	28
38	67	00	05	13	55	16	00	21

reference stand of the y-axis was selected on the basis of the highest e value on the axis. The e is the distance from the intersection of dissimilarity arcs to the axis. A high e value indicates a poor fit along the axis. This e value is calculated by the formula $e^2 = Da^2 - x^2$. The other end stand is the one most dissimilar to the first end within a distance from the latter along the x-axis of less than 10% of the total length of the x-axis. After these two ends (A and B) were found, the other plots were located along the axis as they were for the x-axis.

To choose those species of Compositae which were most abundant on Oakville Prairie, the plot data cards were inspected. From the total number of species, six were selected. Those six species which

TABLE II

DISSIMILARITY COEFFICIENTS

1	96	18	99	100
2	94	82	51	100
3	100	100	100	100
4	55	80	97	96
5	88	83	81	100
6	51	95	99	67
7	27	94	98	71
8	81	38	98	100
9	47	39	99	83
10	76	50	82	100
11		86	99	76
12	32	88	99	86
13	88	67	32	100
14	84	99	100	60
15	89	82	46	100
16	86	87	60	100
17	79	77	44	100
18	73	96	96	46
19	92	86	86	100
20	81	70	63	99
21	81		99	100
22	27	86	99	90
23	88	70	99	85
24	38	94	99	69
25	72	95	44	99
26	99	99		100
27	89	81	50	100
28	50	96	92	74
29	76	100	100	
30	83	96	96	70
31	95	66	75	100
32	91	29	93	99
33	88	78	82	100
34	57	97	67	100
35	96	64	57	100
36	58	79	91	90
37	60	79	91	83
38	95	45	84	100

occurred in the largest number of plots were *Grindelia squarrosa, Ambrosia artemisiifolia, Sonchus* sp., *Aster ericoides, Solidago altissima, and Helianthus maximiliani.*

TABLE III

CONDUCTANCE OF EXTRACT

(mmhos per sq. cm)

0 2 4 8 16

Salinity classification (Jackson 1958)	Nonsaline	Very slightly saline	Moderately saline	Strongly saline	Very Strongly saline
Total no. of plots	4	2	1	16	15

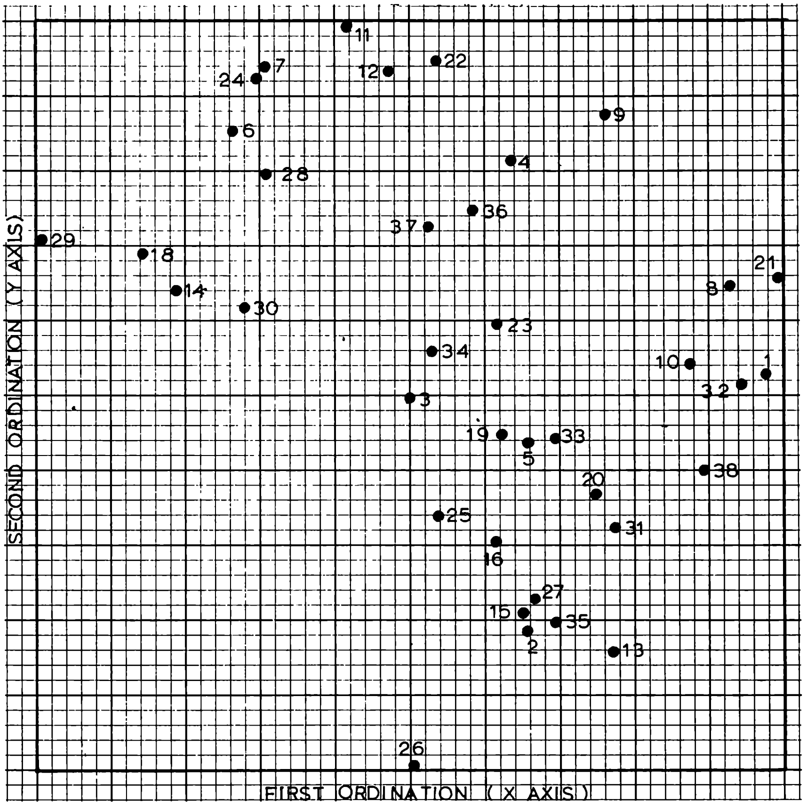

FIGURE 2—Locations of plots on two dimensional graph.

TABLE IV

PHYSICAL FACTORS

Plot No.	Total moisture % dry wt.	Water retaining Capacity % dry wt.	Salinity mmhos per sq. cm. Conductivity	Elevation ft.
1	47	83	26	91
2	38	83	25	91
3	36	108	28	89
4	38	99	17	95
5	72	96	23	94
6	38	94	10	98
7	43	95	13	96
8	57	106	19	92
9	53	100	17	92
10	38	103	35	95
11	50	101	15	98
12	45	107	14	97
13	30	106	16	91
14	19	79	2	100
15	30	82	15	91
16	28	91	20	96
17	18	103	22	92
18	17	72	4	99
19	30	90	15	94
20	22	98	12	91
21	54	113	15	93
22	49	110	11	94
23	14	61	2	102
24	26	78	3	100
25	41	116	15	97
26	42	111	25	95
27	44	106	29	95
28	29	106	6	99
29	32	74	1	99
30	6	67	2	100
31	44	104	22	94
32	46	75	13	93
33	55	112	15	95
34	45	122	14	90
35	43	112	19	95
36	38	81	10	91
37	40	101	11	92
38	42	78	18	91

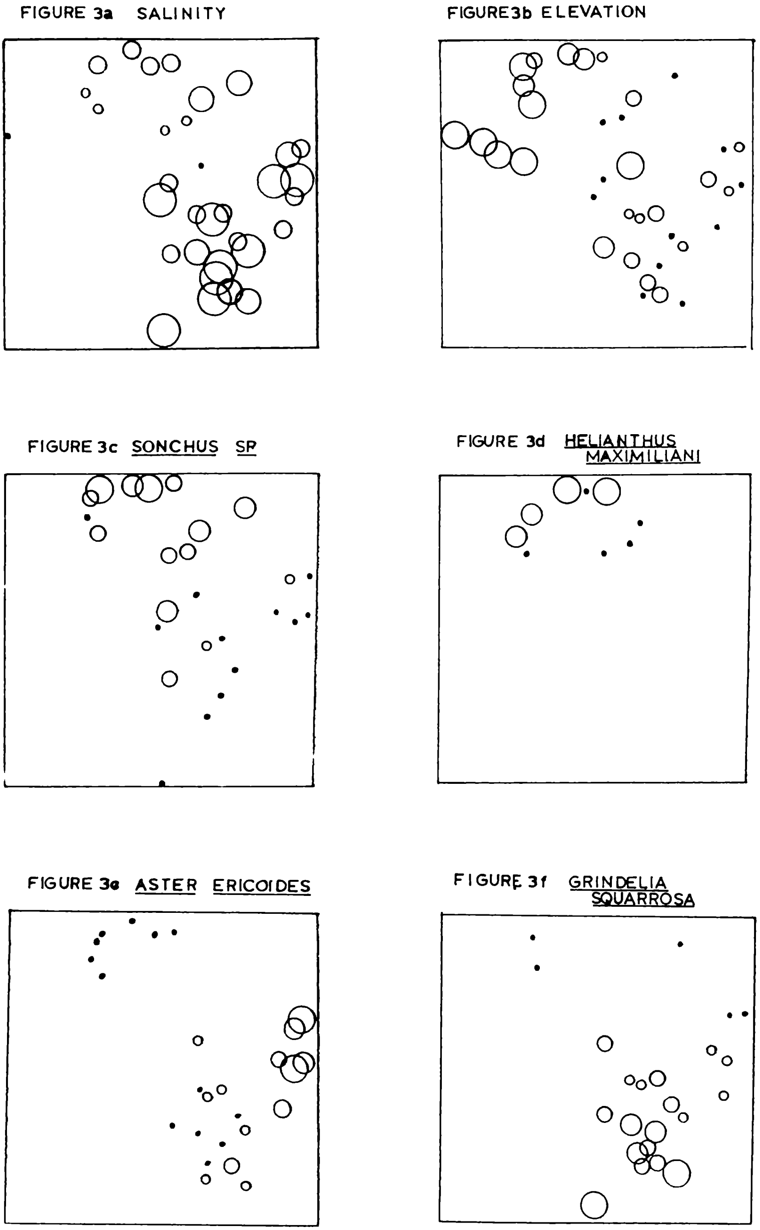

FIGURE 3a SALINITY

FIGURE 3b ELEVATION

FIGURE 3c SONCHUS SP

FIGURE 3d HELIANTHUS MAXIMILIANI

FIGURE 3e ASTER ERICOIDES

FIGURE 3f GRINDELIA SQUARROSA

FIGURE 3—Environmental data and individual species plotted on the two dimensional graph.

RESULTS AND DISCUSSION

Table III shows a salinity scale and the resulting number of plots found at each salinity level. The strongly and very strongly saline plots were all located on the low and relatively flat areas of the prairie, while the nonsaline and very slightly saline plots were located on the high "dune" or beach areas.

Water retaining capacity, total moisture, salinity, and elevations of the plots are given in Table IV. The information regarding factors of the physical environment was plotted against both the x and y ordinations. Correlation-coefficients are given in Table V.

Plotting individual species against ordination axis x gave results which did not seem to show informative relationships to that single ordination. Exceptions were those species in the end plots, the abundance or lack of abundance of which determined the ordination. It was decided that ordination axis x placed plots close to each other which were in fact quite dissimilar. For this reason a second ordination, axis y, was constructed.

TABLE V

CORRELATION COEFFICIENTS

Physical factors	Ordination x-axis	Ordination y-axis
Water-retaining cap.	.14	.12
Total moisture	.46*	—.08
Salinity	.89*	.49*
Elevation	—.59*	—.38*

*p $(r = 0) < .05$

Ordination axes x and y were combined to form a two dimensional graph and the plots were located on this graph as in Figure 2. Figures 3a and 3b show environmental data plotted on the two dimensional graph. When graphs of individual species as in figures 3c, 3b, 3e and 3f were compared with the graphs of environmental data, the following trends were observed:

1. *Sonchus arvensis* tended to occur at its greatest abundance in moderately elevated areas of strong salinity and relatively high soil moisture.
2. *Aster ericoides* reached its highest aboundance at low elevation with very strong salinity and high total moisture.
3. *Solidago altissima* occurred from high to lower elevations with corresponding low to higher salinity and total moisture.
4. *Helianthus maximiliani* seemed to prefer those higher elevations with high moisture content.
5. *Ambrosia artemisiifolia* tended to occur in areas of low elevation with high salinity and high total moisture.

6. *Grindelia squarrosa* occurred in areas of low elevation with very strong salinity and high total moisture content. This species illustrated well the fact that there were certainly more factors involved in species distribution than those which this project investigated. Referring to the graph figure 3f, *Grindelia* does not occur abundantly at right center, although the conditions of moisture, elevation, and salinity are the same there. Figure 3e shows that *Aster ericoides* occurs at its greatest aboundance in the low *Grindelia* area, but abundantly in areas where *Grindelia* is abundant. This corresponds with the idea that a plant community is a complex which is dependent upon both environmental and biotic factors. Dix and Butler (1960) suggested that competition between species was the primary biotic agent which determined the local distribution of species. This importance of the biota has been shown by a number of other investigators, whose studies have shown that the biota can actually modify the physical environment (Steiger 1930, McIntosh 1957).

Therefore, it is felt that vegetational patterns cannot always be accounted for by simply plotting species against a certain number of physical factors. Rather the patterns are due to a complex of factors, both physical and biotic, more than were considered in this investigation.

ACKNOWLEDGMENT

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A STUDY OF TEMPERATURE EFFECTS ON α-CHYMOTRYPSIN CATALYZED HYDROLYSIS

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INTRODUCTION

The effect of temperature on the rate of enzyme reactions is a matter of considerable interest and importance, which has been studied for many years. The subject is a fairly complicated one, and it is only recently that a reasonable degree of understanding has been achieved.

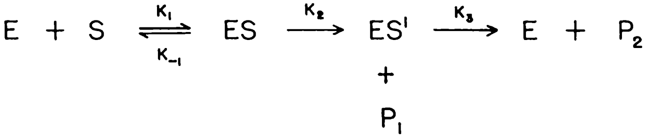
It has been known for sometime that when an enzyme-catalyzed reaction is studied over a range of temperature the over-all rate follows a bell shape curve. The temperature at which the rate is a maximum is known as the optimum temperature and was, at one time, thought to be characteristic of the enzyme system.

The correct explanation of this type of behavior, first given by Tammann (1), is that raising the temperature affects two independent processes, the catalyzed reaction itself and the thermal inactivation of the enzyme. At lower temperatures, up to 30°C, inactivation is very slow and has no appreciable effect on the rate of the catalyzed reaction, the over-all rate therefore increases with rise in temperature in the same manner as for simple chemical recations. At high temperatures inactivation becomes more and more important, so that the concentration of active enzyme falls during the course of reaction.

Under the condition that the enzyme inactivation is unimportant (at low temperatures) the present investigations were carried out using two synthetic substrates with α-chymotrypsin.

THEORETICAL

For chymotrypsin, the kinetic theory of catalyzed hydrolysis is based on a three-step mechanism (2),



$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} \underset{\underset{P_1}{+}}{ES^1} \xrightarrow{K_3} E + P_2$$

In this scheme the substrate (S) reacts with the enzyme (E) to produce substrate-enzyme complex (ES). The decomposition of the complex results in the formation of the acyl-enzyme derivative (ES_1) with the concomitant liberation of the first product (P_1), which is usually the alcoholic component. The hydrolysis of (ES_1) regenerates the enzyme with the release of the second product (P_2), which is usually the acidic component.

The mathematical treatment of the above scheme has been carried out (3) so that the equations are applicable to the experimental conditions of either excess substrate or excess enzyme. Under the first condition, the transient phase and steady-state equations were obtained subject to various assumptions based on the magnitude of the rate constants and concentrations. Under the second condition only the transient phase equations were derived, since the steady state kinetics are non-existent.

In this work, the acetylation rate constant k_2 was determined from steady-state kinetics by using the excess substrate method. These rate constants were found at several temperatures, and from the Arrhenius law (4),

$$k = A(\mathrm{Exp}) - E/RT \qquad (1)$$

the activation energy was calculated. The entropy of activation was obtained from the theory of absolute reaction rates, that may be expressed for reactions in solutions (4) as

$$k = e(\bar{k}T/h)\ (\mathrm{Exp})\Delta S^{*\prime}/R(\mathrm{Exp}) - E/RT \qquad (2)$$

EXPERIMENTAL

I. Apparatus.—The instrumental techniques employed involved spectrophotometry and automatic titration. The acetylation reactions were studied by means of spectrophotometric method. A Beckman DU spectrophotometer was used, and the reactions were recorded with a Leed and Northrup strip-chart recorder. The deacetylation reactions were studied by means of automatic titration. The automatic titrator consisted of a Radiometer TTTI control unit with magnetic relay and a titration assembly constructed in this laboratory. This assembly was constructed on an aluminum base which supported a one ml tuberculin syringe to hole titrant, a motor-controlled feed mechanism to deliver titrant, and an auxillary six-digit counter. The reading on the counter, whose last digit represents 1/8,000 of an inch travel for the syringe plunger, was sensitive to 5.34×10^{-5}ml. The temperature was controlled by the constant temperature water bath to within 0.01 of a degree.

II. Substrates.—The substrate 2,4-dinitrophenyl trimethylacetate was used to investigate the acetylation kinetics because the trimethylacetyl-chymotrypsin derivative does not decompose appreciably. On the other hand, *p*-nitrophenyl acetate was used to investigate the deacetylation kinetics.

III. Solvent and Buffer.—A mixed solvent of 10% (v/v) acetonitrile in water was used for all the reactions. In the case of spectrophotometric methods, phosphate buffers were used to maintain the desired pH value.

RESULTS AND DISCUSSION

The acetylation rate constant, k_2, and the deacetylation rate constant, k_3, were studied over a temperature range from 10°C to 30°C, results are tabulated in Table I. As mentioned earlier, k_2 is for 2,4-dinitrophenyl trimethylacetate and the k_3 for *p*-nitrophenyl acetate with α-chymotrypsin.

TABLE I

VARIATION OF RATE CONSTANT WITH TEMPERATURE

Temp. (°C)	10	20	30
$k_2(\text{sec}^{-1})$	0.0833	0.1667	0.3333
$k_3(\text{sec}^{-1})$	0.00267	0.00666	0.0200

From this data, and according to the equation 1, the energies of activation may be calculated. For k_2, this energy corresponds to bringing the substrate-enzyme complex, ES, to the activated state, and was found to be 11.7 Kcal/mole. The energy of activation for the acetylated enzyme, ES^1, was 17.1 Kcal/mole, as calculated from the data for k_3.

According to equation 2 one can calculate the entropy of activation, and from thermodynamics the enthalpy and the free energy of activation may be estimated. These results are tabulated in Table II.

TABLE II

THERMODYNAMIC DATA FOR ACYLATION AND DEACYLATION OF CHYMOTRYPSIN

Temp. (°C)	ΔS^*(cal/mol-deg)	$\Delta H^‡$(cal/mole)	ΔF^*(cal/mole)
	Acylation State		
10	−23.05	11,133	17,642
20	−23.1	11,118	17,886
30	−23.00	11,098	18,067
	Deacylation State		
10	−9.9	16,533	19,333
20	−9.9	16,518	19,814
30	−10.9	16,498	19,798

In considering the magnitudes of the entropies of activation listed in Table II, two types of effects, structural and solvent, must be taken into account. By structural effects is meant the possibility that the enzyme actually undergoes some reversible change in conformation during the progress of reaction. The theory (5) that enzyme becomes partly unfolded during complex formation and refolded in subsequent reactions leads to the conclusion that the entropy of activation should be positive for complexing and negative for the subsequent reactions such as acylation and deacylation. However, this structural effect does not describe the entire behavior of enzyme action. Frequently, experimental results are obtained which do not fit. By studying the reaction in solvents of various dielectric values, it is possible to separate the entropy into the non-electrostatic and electrostatic, which approximately correspond to the structural and solvent effects, respectively. In the few cases studied for enzyme systems, these structural effects have been small.

By the solvent effect is meant the interaction between the solvent and the reaction species. In the case of the present results, the large negative entropies for acylations suggests that the substrate-enzyme complex becomes quite polar before releasing the acylate-ion. For example, an increase of polarity will bind more solvent bringing order to the system and hence a negative entropy of activation. The fact that the entropy of activation does not vary greatly with temperature is also proved here.

Since the entropies for deacylation are not nearly as negative as those for acylation, it appears that the solvent effects are not so pronounced. This indicates that the acetyl-chymotrypsin derivative does not become very polar before releasing the acetate ion.

From the data in Table II and the fact that $\Delta F^* = \Delta H^\ddagger - T\Delta S$, it is apparent that the rate of enzyme catalyzed hydrolysis is controlled by deacylation and not the acylation step because of the larger energy of activation and not because of entropy effects.

ACKNOWLEDGMENT

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INVESTIGATION OF THE *IN VIVO* AND *IN VITRO* SYNTHESIS OF LIPIDS BY FLAX RUST UREDOSPORES, *MELAMPSORA LINI (PERS.) LEV.*

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ABSTRACT

Whole uredospores, germinating on a pH 7.0 phosphate buffered solution containing C^{14}-acetate, were found to be moderately active in the synthesis of lipids. Gas chromatography has shown that the incorporation of C^{14}-acetate into fatty acids was greatest in stearic acid and lesser amounts in oleic acid, palmitic acid, *cis*-9,10-epoxyoctadecanoic acid, linoleic acid and linolenic acid in that order. Germinating uredospores have been shown to actively incorporate P^{32}-phosophate as well as C^{14}-acetate into uredospore phospholipids. The major phospholipid has been tentatively identified by thin-layer chromatography as a lecithin.

Cell-free preparations from germinated and ungerminated spores were fractionated into soluble and particulate fractions by centrifugation at 10,000 x g for 30 minutes. Ungerminated uredospore particulate fractions were shown to be the most active in the synthesis of lipids from acetate. Cofactor studies for lipid synthesis by uredospore cell-free systems have shown a requirement for adenosine triphosphate (ATP), reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and coenzyme A.

EFFECT OF BILATERAL NEPHRECTOMY AND PARATHYROIDECTOMY ON PLASMA MAGNESIUM LEVELS IN THE RAT

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INTRODUCTION

The transient rise in plasma citrate and calcium levels which occurs in the rat following bilateral nephrectomy has been shown to be dependent upon normal parathyroid function (1) and adequate vitamin D in the diet (2). If the elevated plasma calcium level following nephrectomy is caused by the accumulation of citrate in the circulation, the plasma magnesium level should also rise since citrate complexes either ion (3). The work reported here was done to determine the behavior of plasma magnesium following nephrectomy under various conditions of parathyroid function and vitamin D status.

METHODS

Male albino rats of the Sprague-Dawley strain were fed Purina rat chow or a vitamin D-deficient semi-synthetic diet (2) *ad libitum.* All bleedings from the heart or the aorta were performed under ether anesthesia. Bilateral nephrectomy and parathyroidectomy were performed under ether anesthesia using standard surgical techniques and electrocautery for destruction of the parathyroid tissue.

Rats receiving vitamin D were given 75 I. U. of the vitamin dissolved in Wesson Oil (0.1 ml = 50 I. U.) orally, 3 times weekly. Rats receiving parathyroid extract were injected subcutaneously with 10 I. U./100 gm body weight twice daily for 4 days following parathyroidectomy.

Plasma magnesium determinations were made using the sensitive and accurate spectrophotometric method of Levine and Cummings (4). Eriochrome black T was used as the indicator because it involves a true solution of a colored magnesium complex for which the optical density is directly proportional to the color intensity. The method was found to give a linear relationship between concentration and absorbancy between 5 to 40 μg. Plasma citrate analyses were made by the method of Ettinger *et al.* (5). The method gave a linear relationship between concentration and absorbancy between 2 to 40 μg of citric acid and was accurate to ± 5%.

RESULTS AND DISCUSSION

Bilateral nephrectomy of stock animals receiving the Purina diet resulted in a rise of the plasma magnesium level to 162% of the control value in 4 hours as shown in Table I. Animals were bled in four hours since Elliott and Freeman (1) had found that the elevation of calcium levels following bilateral nephrectomy reached

TABLE I

EFFECT OF BILATERAL NEPHRECTOMY ON PLASMA MAGNESIUM LEVEL

Conditions	No. of rats	Plasma magnesium mg/100 ml	Plasma citrate mg/100 ml
Control: 1st. bleeding	12	1.95 ± 0.15	—
Control: 2nd. bleeding	4	1.95 ± 0.09	3.66 ± 0.77
Nephrectomy	7	3.15 ± 0.24	24.83 ± 5.66

Three ml of blood was removed by heart pucture from large male rats (approx. 300 gm) from a stock colony fed Purina rat chow. Two hours later 8 of the rats were nephrectomized, and 4 hours following nephrectomy all were bled through the aorta.

a maximum in that length of time. The citrate level rose simultaneously to 678% of the control value suggesting that there may be a causal relationship between the two phenomena. The elevation of magnesium was greater on percentage and molar bases than that of calcium reported by Elliott and Freeman (1). It is likely that magnesium is more readily available from the soft tissues than is calcium which is undoubtedly derived from the skeleton, at least in part.

When rats were parathyroidectomized a slight but significant ($P<0.001$) decrease in plasma magnesium levels was found as shown in Table II. This decrease suggests that magnesium levels, like calcium levels, are under the control of the parathyroid glands. The elevation of plasma magnesium and citrate following bilateral nephrectomy of the parathyroidectomized rat is surprising in view of the observation of Elliott and Freeman (1) that plasma calcium and citrate do not rise following the operations. These findings by Elliott and Freeman have never been confirmed in our laboratory despite many efforts to do so. The results suggests that the parathyroid function is not the only factor determining plasma citrate levels.

TABLE II

EFFECT OF PARATHYROIDECTOMY AND BILATERAL NEPHRECTOMY ON PLASMA MAGNESIUM LEVEL

Conditions	No. of rats	Plasma magnesium mg/100 ml	Plasma citrate mg/100 ml
Control	8	2.15 ± 0.17	—
Ptect.	8	1.74 ± 0.07	—
Ptect. control	2	1.80 (ave.)	3.85 (ave.)
Ptect. and neph.	6	2.66 ± 0.34	18.51 ± 5.43

Three ml of blood was removed by heart puncture from large male rats (approx. 300 gm) from a stock colony fed Purina rat chow. Parathyroidectomy was performed immediately after heart puncture. Four days later another heart puncture was performed with the same amount of blood removed. With the exception of two control rats, all were nephrectomized, and all rats were bled through the aorta 4 hours later.

The injection of parathyroid extract into parathyroidectomized rats had no significant effect on the plasma magnesium levels (Table III) despite the fact that effect of the operation in decreasing the levels was observed again ($P < 0.005$). Yet a significant rise in plasma magnesium was observed following nephrectomy although the rise in plasma citrate was slight. This failure of the plasma citrate to rise following nephrectomy is contrary to the expected results since the hormone actually eliminated the response which was obtained with the parathyroidectomized rats. These results are again in direct contrast with previous reports (1) in which citrate rose following nephrectomy of the parathyroidectomized rat if the extract was administered.

The vitamin D status of the rat had no significant effect on the plasma magnesium elevation following nephrectomy (Table IV). An elevation of 127% was observed with rats which had received the vitamin as compared with a rise of 133% with rats which were vitamin D-deficient. Vitamin D did affect, however, the plasma citrate rise following nephrectomy as previously reported (2).

TABLE III

EFFECT OF PARATHYROID EXTRACT ON PLASMA MAGNESIUM LEVELS OF PARATHYROIDECTOMIZED RATS

Conditions	No. of rats	Plasma magnesium mg/100 ml	Plasma citrate mg/100 ml
Control	8	2.07 ± 0.10	—
Ptect. (Extract injected twice daily)	8	1.82 ± 0.19	—
4 hours later			
Ptect. control	4	1.87 ± 0.14	2.62 ± 0.65
Ptect. and neph.	4	2.66 ± 0.27	3.70 ± 1.61

Three ml of blood was removed by heart puncture from large male rats (approx. 300 gm) from a stock colony fed Purina rat chow. Parathyroidectomy was performed immediately after heart puncture. Four days later another heart puncture was performed with the same amount of blood removed. During this 4 day period all rats received parathyroid extract (10 I.U./100 gm wt) twice daily. Four of the 8 rats were then nephrectomized and 4 hours later both the control and nephrectomized rats were bled through the aorta.

Plasma magnesium elevation following nephrectomy also appeared to be dependent upon the age and size of the rat. In Table IV where younger rats were used the rise in plasma magnesium levels

TABLE IV

EFFECT OF VITAMIN D ON MAGNESIUM ELEVATION IN RAT BLOOD PLASMA FOLLOWING NEPHRECTOMY

Conditions	No. of rats	Plasma magnesium mg/100 ml	Plasma citrate mg/100 ml
Control: —D	12	1.25 ± 0.10	—
Neph: —D	11	1.67 ± 0.31	8.9 + 3.1
Control: +D	12	1.33 ± 0.31	—
Neph: +D	12	1.68 ± 0.23	24.9 ± 2.6

Three ml of blood was removed by heart puncture from large male rats (approx. 250 gm) which had received a vitamin D— deficient semisynthetic Ca and P containing diet. The +D rats had received 75 I.U. of the vitamin orally, 3 times weekly. Two days later rats were nephrectomized and bled 4 hours later through the aorta.

following nephrectomy was less than that observed with older animals as shown in the other tables. It is also possible that the use of the semi-synthetic diet may have influenced the results.

SUMMARY

A magnesium elevation in rat blood plasma has been shown to occur 4 hours following bilateral nephrectomy. Neither parathyroid function nor vitmin D had an effect on this elevation.

Although parathyroidectomy produced a significant decrease in plasma magnesium levels, subcutaneous injection of parathyroid extract into parathyroidectomized rats for 4 days following the operation failed to restore the plasma magnesium levels. It appears that parathyroid function exerts little control over plasma magnesium levels.

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REDUCTIONS OF CYCLIC β-AMIDO-α, β-UNSATURATED KETONES WITH HYDRIDE

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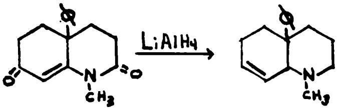
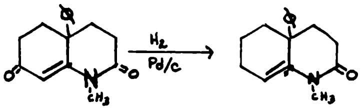
INTRODUCTION

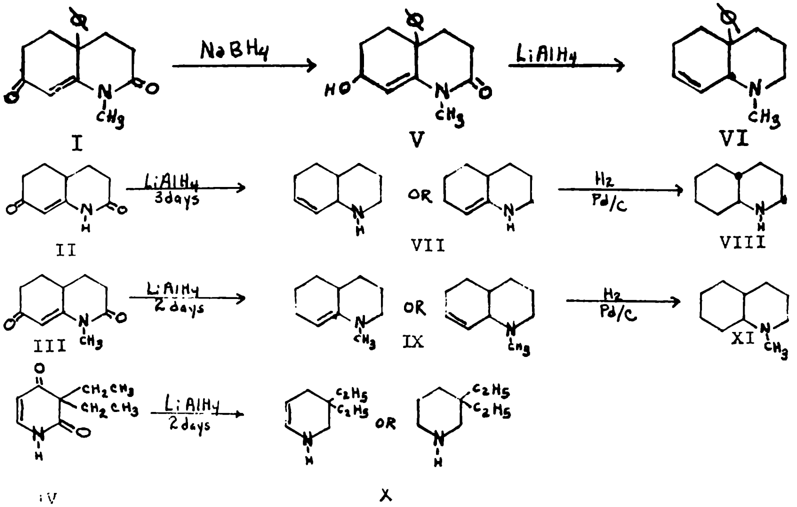
In 1961, Koelsch and Ostercamp reported selective reduction of the compound, 1-methyl-4α-phenyl-Δ^8-octahydro-2,7-quinolinedione (I). The reduction products are dependent on the type of reagent utilized (1). With lithium aluminum hydride as the reductive reagent, both oxygens were completely reduced and the unsaturated bond shifted to the Δ^7 position. In the presence of hydogen and palladium-carbon catalyst, only the ketonic oxygen underwent hydrogenolysis.

The present work is concerned with the extension of the above results. Several other cyclic compounds having the identical β-amido-α, β-unsaturated ketonic structure have been investigated, including (I), with the use of a third reducing agent, sodium borohydride.





The following is an outline of the synthesis of compounds which have been derived from these original compounds: (II), Δ^8-octahydro-2, 7-quinolinedione; (III), 1-methyl-Δ^8-octahydro-2, 7-quinolinedione; (IV), 3, 3-diethyl-2, 4-dioxotetrahydropyridine.



EXPERIMENTAL

Lithium aluminum hydride reduction of all the model compounds was carried out in essentially one general procedure. To a stirred suspension of lithium aluminum hydride in anhydrous ether was added the finely powdered model compound in small portions over a period of fifteen minutes. The hydride and starting reactant were mixed in a molar ratio of 1:0.2. The ethereal mixture was then refluxed for the desired length of time. The reaction mixture was hydrolyzed with water (4 ml), sodium hydroxide (6 ml; 10%), and water (10ml). (The quantities indicated are for every 0.1 mole of lithium aluminum hydride added at the beginning.) Usually additional ether had to be added to insure proper stirring during hydrolysis. The white oxides and hydroxides thus formed were filtered off, washed thoroughly with ether, and then discarded. Fractional distillation of the ethereal filtrate under reduced pressure produced the final product.

Sodium borohydride reduction was effected in methanol. A fresh solution of hydride in methanol was added dropwise to a stirred suspension of the model compound in methanol. Again a molar ratio of hydride to compound, 1:0.2, was found to be adequate. Work-up of the reaction mixture involved acidifying with glacial acetic acid to a pH of 6, and then distilling under reduced pressure. The desired product crystallized out of solution as the distillation proceeded.

RESULTS

Synthesis of 1-methyl-7-hydroxy-4α-phenyl-Δ^1-octahydro-2-quinolone, (V).—Both the infrared spectrum and elemental analysis of compound V supported the proposed structure. Compound V was recrystallized twice from ethyl acetate and gave a m.p. of 142-144°C (52% yield). The infrared spectrum obtained for this compound gave an OH band at 3320 cm^{-1}, and phenyl bands at 1500 cm^{-1}, 770 cm^{-1}, and 702 cm^{-1}.

Anal. Calcd. for $C_{16}HH_{19}NO_2$ (257.32): C, 74.68; H, 7.44; N, 5.44. Found: C, 74.85; H, 7.43; N, 5.55.

1-Methyl-4α-phenyl-Δ^7-octahydroquinoline, (VI).—Confirmatory evidence for VI was obtained when compared with the compound previously characterized by Koelsch and Ostercamp. The I.R. spectrum of the perchlorate derivative of VI demonstrated that the product was identical with that previously prepared, 4α-phenyl-Δ^7-octahydroquinoline (b.p. 172-173°C/13mm, m.p. 57-59°C) (2). The picrate (m.p. 181-183°C), hydroiodide (m.p. 251.5-254.5°C) and perchlorate (m.p. 248-252°C) derivatives were all prepared and recrystallized from 95% ethanol, water, and absolute ethanol, respectively. Compound VI was obtained in 43.8% yield. Reported m.p.s are: picrate, 182-184°C; hydroiodide, 256-258°C; and perchlorate, 253-255°C.

Δ^7 or Δ^8-Octahydroquinoline.—Reaction of I with lithium aluminum hydride over a period of three days produced a major liquid fraction, VII, (b. p. 75-80°C/10mm) upon fractional distillation of the reaction mixture under reduced pressure. Elemental analyses of the hydrochloride (m. p. 264-266°C) and hydroiodide (m. p. 249°C) derivatives plus the I.R. spectrum of VII indicated that it was Δ^7- or Δ^8-octahydroquinoline (58.2% yield). Samples have been submitted for NMR spectra, but the results, which should provide the information required to make this distinction, have not yet been received.

Anal. Calcd. for $C_9H_{16}NCl$ (173.69): C, 62.23; H, 9.29; N, 8.07. Found: C, 62.31; H, 9.53; N, 8.41.

Anal. Calcd. for $C_9H_{16}NI$ (265.04): C, 40.78; H, 6.05; N, 5.29. Found: C, 40.74; H, 6.21; N, 5.18.

Trans-decahydroquinoline.—Subsequent reduction of VII with hydrogen in the presence of palladium-carbon catalyst yielded *trans*-decahydroquinoline (b.p. 74-76°C/11.5 mm, m.p. 46.2-48.5°C) (VIII) in 45.4% yield, according to the physical constants reported by S. Fujise (3). The reaction took up 2.0 pounds of hydrogen (calc'd. 2.9 lbs.) for 4.860 g of starting compound, VII. The hydrochloride derivative (m.p. 275-277°C) of VIII was prepared and the N-benzoyl derivative attempted. A commercially prepared sample of VIII was obtained (Eastman, 6480) and the hydrochloride derivative (m.p. 286-287.5°C) was prepared. A mixed melting point of the known and experimental hydrochloride derivatives was 279.7-282°C. Further investigation of the structure of VIII is under current investigation.

1-Methyl-Δ^7 or Δ^9-octahydroquinoline.—Lithium aluminum hydride reduction of III over a two day period gave a major liquid fraction upon fractional distillation of the reaction mixture (b.p. 83-88°C/10mm, n^{256} 1.5000) (IX), under reduced pressure. Both the methiodide (m.p. 226-228°C) and pictrate (m.p. 159.2-160.2°C) derivatives were prepared, and their elemental analyses indicated the structure to be 1-methyl-Δ^7 or Δ^8-octahydroquinoline (64.2% yield). The I.R. spectrum for the pure liquid IX gave further evidence for the existence of the C = C linkage with bands at 3080 cm^{-1} and 1655 cm^{-1}. Precise location of the C=C linkage should be possible from NMR studies, but results have not been received at this time.

Anal. Calcd. for $C_{11}H_{20}NI$ (293.19): C, 45.06; H, 6.88, N, 4.78. Found: C, 45.06; H, 6.77; N, 4.61.

Anal. Calcd. for $C_{16}H_{20}O_7N_4$ (380.35): C, 50.52; H, 5.30; N, 14.73. Found: C, 50.15; H, 5.32; N, 14.46.

Cis or *Trans*-1- methyl-decahydroquinoline. — Reduced pressure distillation following catalytic reduction of IX in methanol gave a liquid fraction (b.p. 80-85°C/11mm, n^{20} 1.4841) for which a picrate derivative (m.p. 177.2-180°C) was prepared. Ehrenstein and Bunge reported the synthesis of both *Cis* and *trans*-1-methyldecahydroquinoline having picrate derivatives with melting points of 199-200°C and 173°C, respectively (4).

The respective index of refraction for each isomer has also been reported at n^{20} 1.4851 (*cis*) and n^{20} 1.4820 (*trans*) (5). It appears at this time that the compound XI above, obtained in 37.4% yield, is largely a mixture of both *cis* and *trans*-1-methyldecahydroquinoline, with the *cis* form in predominance. Further investigation of this reduction product is currently under study.

3,3-Diethylpiperidine.—Reaction of IV with lithium aluminum hydride over a two day period yielded a major liquid fraction (b.p. 74-76°C/13mm) upon reduced pressure distillation of the reaction mixture for which phenylisocyanate (m.p. 1165-118.5°C) and α-naphthylisocyanate (m.p. 126-218°C) derivatives were prepared (6). The I.R. spectrum for the reduction product, X, showed that both the ketonic and amide oxygens had been lost and weak vinyl C-H and C = C bands were observed at 3000 cm^{-1} and 1640 cm^{-1}, respectively. An N-H band was also observed at 3250 cm^{-1}. Elemental analyses of the two derivatives correspondingly bore this discrepancy, and hence there remains the task of concluding the presence or absence of the C=C linkage. Further investigation is pending on this reduction product.

Anal. Calcd. for $C_{16}H_{24}ON_2$ (260.43) (no double bond): C, 73.80; H, 9.29; N, 10.76. Found: C, 75.07; H, 8.95; N, 10.41. Calcd. for $C_{16}H_{22}ON_2$ (258.35) (double bond): C, 74.38; H, 8.59; N, 10.84.

Anal. Calcd. for $C_{20}H_{26}ON_2$ (310.43) (no double bond): C, 77.38; H, 8.44; N, 9.03. Found: C, 79.26; H, 8.20; N, 8.50. Calcd. for $C_{20}H_{24}ON_2$ 308.41) (double bond): C, 77.88; H, 7.84; N, 9.09.

SUMMARY

Treatment of 1-methyl-4α-phenyl-Δ^8-octahydro-2,7-quinoline-dione (I) with excess sodium borohydride resulted in the formation of 1-methyl-7-hydroxy-4α-phenyl-Δ^8-octahydro-2-quinoline (V). Proof of structure was provided by infrared specra studies and elemental analysis, in addition to subsequent reduction of V to 1-methyl-4α-phenyl-Δ^7-octahydroquinoline (VI). Confirmatory evidence for VI followed from comparison with the known compound.

Lithium aluminum hydride reduction of Δ^8-octahydro-2,7-quinolinedione produced Δ^7- or Δ^8-octahydroquinoline (VII). The precise location of the C = C is still in question. The cited structures were supported by infrared spectra studies and elemental analyses of the hydrochloride and hydroidodide derivatives. NMR samples have been submitted and the results should enable one to locate the site of unsaturation. Further evidence resulted from subsequent catalytic reduction of VII over palladium-carbon catalyst which produced *trans*-decahydroquinoline (VIII). Mixed melting points of a commercially obtained sample of *trans*-decahydroquinoline were in relatively good agreement in support of the structure cited. Further study is underway.

Reaction of 1-methyl-Δ^8-octahydro-2,7-quinolinedione gave 1-methyl-Δ^7 or Δ^8-octahydroquinoline (IX). Again infrared spectra and elemental analyses of the picrate and methiodide derivatives supported the existence of a carbon to carbon double bond, although the precise location is not known at this time. Subsequent reduction of IX under catalytic conditions over palladium-carbon catalyst was believed to have produced a mixture of *cis* and *trans*-1-methyl-decahydroquinoline (XI). Proof of structure is hoped to follow from an independent synthesis of *trans*-XI from a commercially obtained sample of *trans*-decahydroquinoline.

Reduction of 3,3-diethyl-2,4-dioxotetrhydropyridine resulted in a mixture of products, although melting points of the phenylisocyanate and α-naphthylisocyanate derivatives of the major fraction were quite sharp. The proposed reduction products are pending further investigation.

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THE KINETICS OF RIBONUCLEASE WITH CYTIDINE-2',3'-CYCLIC MONOPHOSPHORIC ACID

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During the past few years, much attention has been focused on the enzyme ribonuclease. Of all the known enzymes, its structure is the only one which has been fully characterized. In spite of this, few studies on its kinetics have been made, and these investigators are not in complete agreement (2,6,8,9). The hydrolysis of a ribonucleic acid by ribonuclease is very complicated, and a mathematical analysis of the kinetics would be difficult. However, cyclic pyrimidine ribose nucleotides (2', 3'-monohydrogen phosphate esters of nucleosides) are hydrolyzed by ribonuclease to give 3'-riboside phosphates. This corresponds to the last step in the breakdown of a ribonucleic acid. Therefore, this ester is better suited for the role of the substrate in a preliminary kinetic study because of the comparative simplicity of the reaction.

The work to be described concerns our efforts to determine the reaction mechanism of the enzymatic hydrolysis of the sodium salt of cytidine-2', 3'-cyclic monophosphoric acid with bovine ribonuclease in the pH range 5.5 to 7.5.

EXPERIMENTAL

The reactions were carried out using a Radiometer TTT lc automatic titrator. The titrator added NaOH to neutralize the acid produced during the hydrolysis of the substrate. All runs were made at 30° C, and nitrogen was flushed over the system to eliminate the interference from carbon dioxide from the atmosphere.

Ribonuclease 5x crystallized, Lot R23B-55, activity 55 Kunitz units; cytidilic acid (mixed 2' and 3' mixed isomers), Lot C122B-276; and cytidine-2', 3'-monophosphoric acid, Ba salt, Lot 103B-1510 were all purchased from the Sigma Chemical Company. However, the substrate had to be converted to the sodium salt by the method of Gundlach, Stein, and Moore (5).

RESULTS

The system was studied over the pH range 5.5 to 7.5 by varying the substrate concentration in excess of the enzyme concentration, which was held constant. The results were plotted on a Lineweaver-Burk plot of 1/rate vs 1/substrate concentration as shown in figure 1. It can be seen that the lines curve in the high substrate region, especially at low pH. This created a problem in that a straight line would not fit the data. We replotted the results using two methods; the first uses only the points at high substrate concentrations, and the second uses only the points at low substrate concentrations. In

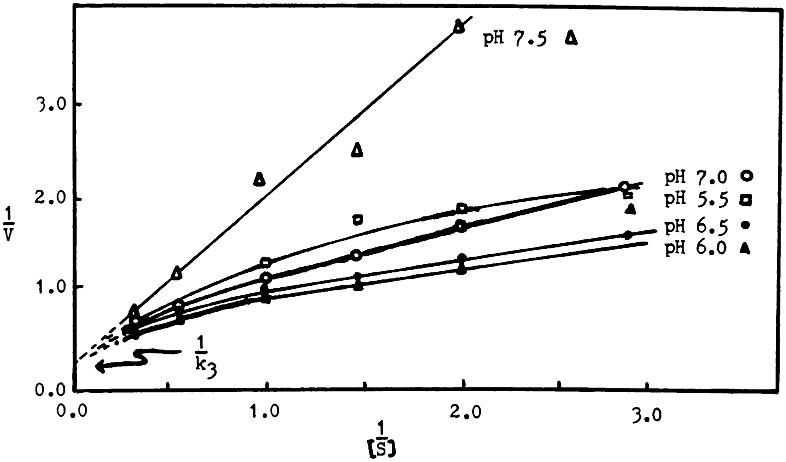

FIGURE 1—Lineweaver-Burk plot of the results.

both methods, the true rate constants, k_3, which is the specific rate constant for the rate-controlled step of the reaction, were estimated from the intercepts on the rate axis, and the Michaelis constants, K_m, were estimated from the slope of the plots. These values are shown in Table I. In the first method, the k_3's and K_m's appear to be pH independent whereas in the second method, they appear to be pH dependent.

TABLE I

RATE AND MICHAELIS CONSTANTS AT VARIOUS pH VALUES

	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5
First method:	High Substrate Concentrations				
k_3, sec^{-1}	4.55	4.55	4.55	4.55	4.55
K_m, x 10^{-3}	2.18	2.29	2.39	2.34	2.11
Second method:	Low Substrate Concentrations				
k_3, sec^{-1}	0.80	1.35	1.61	1.61	4.55
K_m, x 10^{-3}	0.54	0.34	0.58	0.84	7.82

In the course of a typical reaction, the rate decreased abruptly with time. From this, it was evident that there may be product inhibition. This has also been reported by other workers (3). With this in mind, the kinetics were reinvestigated in a different manner.

This time the concentrations of both the substrate and enzyme were held constant while the pH was varied between 5.5 and 7.5. A high substrate concentration was used in order to saturate the enzyme. Then the same series of reactions were repeated except that the enzyme was incubated for 10-15 minutes prior to use with a fixed quantity of the product (cytidilic acid). In order to determine whether or not the extent of product inhibition varies with pH, a plot of log $\frac{V_o}{V_i}$ vs pH was made. This plot is shown in figure 2, where V_o is the rate when no product was added to the enzyme prior to use, and V_i is the corresponding rate when a fixed quantity of product was edded prior to use. However, there is no break in the plot over the pH range studied.

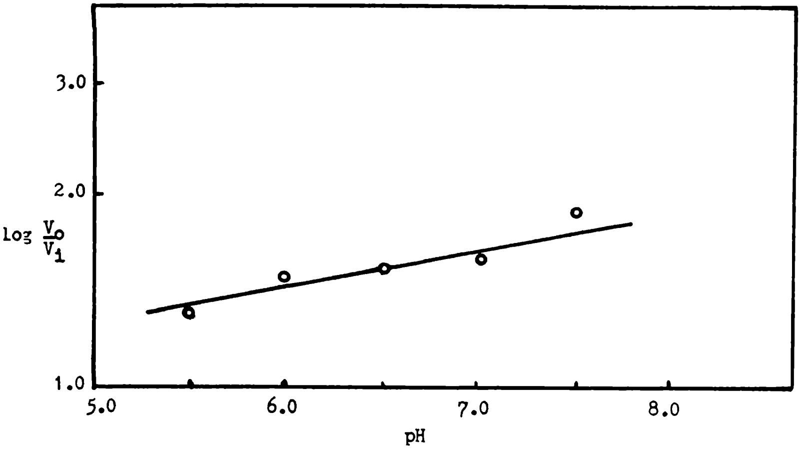

FIGURE 2—Ratio of the rates of hydrolysis of 3.5 x 10^{-3} M. cyclic ester with inhibited and non-inhibited 7.88 x 10^{-7} M. ribonuclease.

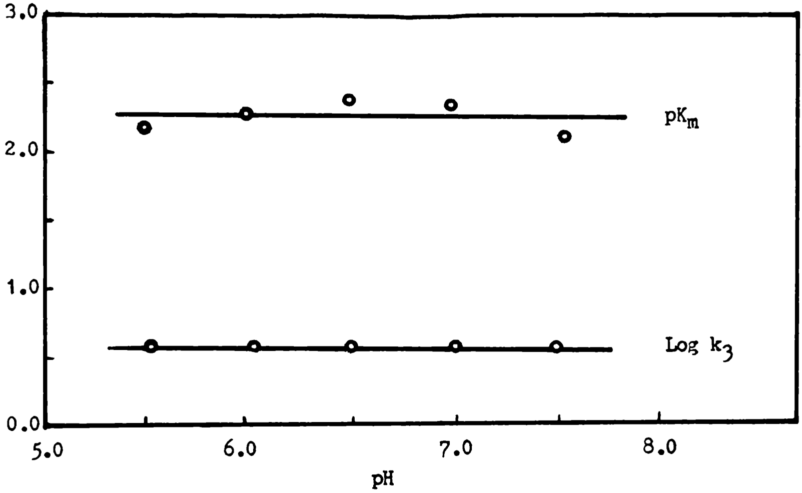

FIGURE 3—Plot of pK_m vs pH and log k_3 vs pH with first method (high S_o) of Lineweaver-Burk plots.

DISCUSSION

We are not sure what causes the deviation from linearity in the Lineweaver-Burk plots in figure 1. Since ribonucleic acids are known to hydrolyze spontaneously, one explanation for the curvature which occurs could be the spontaneous hydrolysis of the substrate. The correct procedure for plotting the results is still unknown to us. It appears that two methods exist. In the first method, which uses the results at high substrate concentration, the system appears to be pH independent, and in the second method, for low substrate concentration, the system appears to have a pH optimum. This difficulty is yet to be resolved.

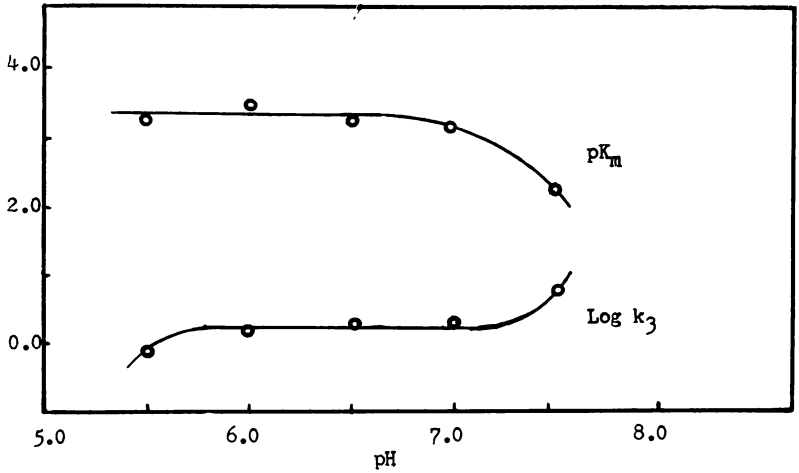

FIGURE 4—Plot of pK_m vs pH and log k_3 vs pH with second method (low S_o) of Lineweaver-Burk plots.

The first method used in plotting the results shows the rate of the reaction to be pH independent in the region studied. The plots of log k_3 vs pH and pK_m vs pH as shown in figure 3 produce straight horizontal lines. This indicates that any functional groups operating in the enzyme must be pH independent in the pH range 5.5 to 7.5.

The second method used in plotting the results shows the rate of the reaction to be pH dependent. The plots of log k_3 vs pH and pK_m vs pH produce curves as shown in figure 4. This indicates that the functional groups in the enzyme have pK's which govern their activity at various pH values. Qualitatively, there are two pK's related to k_3 at about 6 and 7, while for K_m there is only one at about 7.

In previous studies, other workers have found the k_3 to be pH dependent at low substrate concentrations (6) and pH independent at high substrate concentrations (9). Our results are similar in this respect, but in published work (8) the former has been assumed to be the actual case. However, an attempt should be made to explain both cases theoretically.

The product possesses a phosphate group with a pK of about 6.0. Therefore, since a plot of the ratio of the rates of hydrolysis with product inhibited and noninhibited ribonuclease is linear in the range of pH 6.0 as shown in figure 2, it is not likely that this group is involved in binding to the enzyme. Probably the group which attaches to the enzyme in both the product and substate is the $-NH_2$ group in the cytidine part of the molecule with a pK of 4.2.

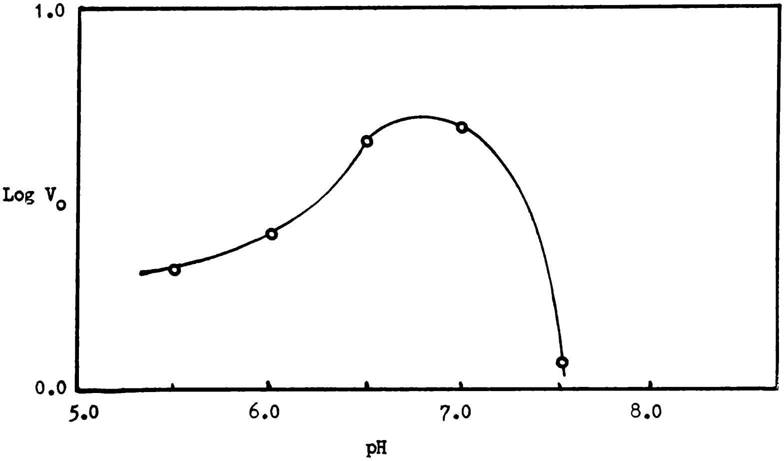

FIGURE 5—Rate of hydrolysis of 3.5 x 10^{-3} M cyclic ester by 7.88 x 10^{-7} M ribonuclease.

In figure 5, a plot of the rate of the reaction with respect to pH is shown. A high substrate concentration was used to swamp out the K_m so that the k_3 would be directly related to the rate. However, this plot is not completely valid since the substrate concentration is actually about the same order of magnitude as the K_m (see Table I). A fairly sharp maximum can be seen in the plot. From this, it appears as if there are two ionizable sites operating in the enzyme. One seems to have a pK about 6.5 (imidazole) and the other about 7.0 (amino) which explains a pH optimum of 6.5 to 7.0. It should be noted that similar results are obtained when the pH dependence of k_3 and K_m is considered using slopes and intercepts at low substrate concentrations as given in figure 1.

Other workers have proposed that at least three residues are necessary for the activity of ribonuclease. These are cystine, lysine (3), and histidine (4) residues. It appears that cystine is necessary as a structural entity, whereas the histidine and lysine function as part of the active site. The latter findings are verified here, since histidine has an imidazole group with a pK in the 6.5 range, and lysine in a peptide linkage could have an amino group with a pK between 7.5 and 7.0.

ACKNOWLEDGMENT

We wish to thank the North Dakota Academy of Science for their research grant in support of this work.

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A STUDY OF THE CORRELATION BETWEEN JAMESTOWN, NORTH DAKOTA EARTH CURRENTS AND ZURICH SUNSPOT NUMBERS

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INTRODUCTION

Variable electric currents in the earth's crust were first observed when telegraphy along extended lines was introduced. In the circuit formed by grounding the lines at both ends, irregular currents of much higher intensity than the artificial currents used for telegraphic communication occasionally appeared and obliterated the signals. Burbank, in a bibliography of early literature, ascribes to Barlow the first observations "on spontaneous electric currents observed in the wires of the electric telegraph" made in 1847 on English telegraph lines (1).

There are several sources and citations which link such currents in the earth's crust with magnetic and auroral activity. These are characteristically under titles like one by Clement on "The great northern light in the night before the 29th of August, 1859, and the confusion of telegraphy in North America and Europe"; "aurora was seen in latitudes as low as 14° (north) on the Atlantic, and on some telegraphic lines in France the greatest effects observed were equivalent to 800 volts over a distance of 600 km." On the occasion of the great magnetic storm of 16 April 1936, "natural potential differences of several hundred volts also occurred in Norwegian cables" (2).

Various citations in Chapman and Bartels indicate that there may be relationships between sunspot activity and magnetic disturances. Their charts of average yearly sunspot activity and average yearly magnetic activity indicate that there is a tendency for years of high sunspot activity to have also high magnetic activity (3). Using these yearly averages, they obtain correlation coefficients of +0.869 and +0.884 (4). There are, however, observed instances of large magnetic storms recorded at times when there was no unusual solar activity within a reasonable period, and in some cases the magnetic storm occurred when the sun was spotless for many days in a row (5). "The connection between the cause of magnetic storms and the aurora borealis is further emphasized by the tendency of the latter to be more widespread and intense at times of magnetic disturbances" (6).

"The simultaneous occurrence of magnetic and earth current disturbances is the best established fact about earth currents" (7). Hessler and Wescott obtained correlation coefficients between the two of over +0.93 in some randomly selected months of 1957-58 for data obtained at College, Alaska (8). Since earth current storms occur at the same time as magnetic storms and aurorae, they share several characteristics such as world-wide appearance, relations to sunspots, 27-day recurrence tendency, higher frequency at the equinoxes, and so on (9). Observations of earth current storms at Jamestown support a connection with aurorae.

Previous work by Happ at Jamestown done over a one year period indicated a reasonable value of positive correlation between earth current activity and sunspot activity during equinoctial periods (10). It is the intent of this study to refine and extend the work by Happ.

EARTH CURRENT RECORDING SYSTEM

Earth currents are not measured directly but are determined from the potential gradient and resistivity using Ohm's law. The earth current data at Jamestown are obtained by measuring the potential between copper rods driven into the ground 500 feet apart. The rods are along a north-south line. Since the system was set up with the intention of measuring the short period fluctuations, long period changes are not considered. Hence, the need to compensate for contact potential differences, changes in resistivity of the soil, and other long period variations is not necessary. Rather, a zero offset voltage network at the input end of the recorder is used. This offset requires adjustment from time to time to keep the pen on the scale, as long period factors such as resistivity and temperature of the soil vary. Construction of the original equipment is described by Van Beek (11). He also conducted several tests, concluding that the short term variations were indeed due to earth current storms and that there were no effects attributable to interference such as currents induced by power lines. An additional test for thunderstorm interference has been run, and none has been found. The recorder used was a Brown Electronik Class 15 self-balancing recording potentiometer with a balancing time of 10 seconds. The chart scale is 200 divisions and the sensitivity of the system was 710 microvolts per division.

PROCEDURE

The correlations were carried out over the period January 1961 to June 1963. The charts were divided into three-hour periods, beginning at midnight of each day. Each period was coded as to year, day, and three-hour period. Earth current activity values were derived from the charts by selecting the 5-minute interval within each three-hour period that had the largest variation (thereby discounting long period changes). If part of any three-hour period was missing, due to equipment or power failure, the maximum was selected from that part that was available. Thus, 8 three-hour maximums were obtained for most days. The arithmetical mean of these three-hour maximums was taken as the earth current activity value for that day. This scaling process is similar to the K scaling process commonly used for magnetic index determination, and also to the earth current scaling process used by Hessler and Wescott. Such a scaling process weights the amplitudes of the more rapid short period fluctuations heavily.

Zurich sunspot numbers, as published in *Sky and Telescope* were used as the daily values of sunspot activity (12).

The correlation method used was rank-difference (13). The application of this method calls only for orderly ranking of the observations.

Sunspot and earth current daily average pairs were grouped into 27.275 day periods. This 27.275 day time interval is the mean synodic rotation period of the sun relative to the earth (14), and will hereafter be refered to as a "month". These monthly periods were rounded off so that about three out of four were 27 days long, while one out of four was 28 days long.

RESULTS

Four correlation coefficients were derived using different methods of data grouping and are detailed below.

I. *Daily Correlation.*—Data pairs were chosen by taking the daily earth current activity value and the sunspot number for the same day. Coefficients were computed for each of the 34 monthly periods. The coefficients appear in figure 1 where they are plotted against the period number of the month they represent. The arithmetical mean of these 34 coefficients was -0.035. The presence of a reasonable high positive coefficient for only one of the five equinoxes of the January 1961-June 1963 correlation period indicates that previous results due to Happ do not hold for all equinoxes.

II. Two Day Lag Correlation.—Estimates for the sun-earth travel time of particles believed to be responsible for magnetic storms range from 26 hours to 2.5 days (15). With this in mind it was decided to carry out a correlation using a procedure similar to the above (I), except that each daily sunspot activity value would be paired with the earth current activity value of 2 days later. In this manner, correlation coefficients were obtained for 32 monthly periods. These coefficients appear in figure 2 plotted against the month number to which they belong. The arithmetic mean of these coefficients over the entire 2½ year period was +0.039.

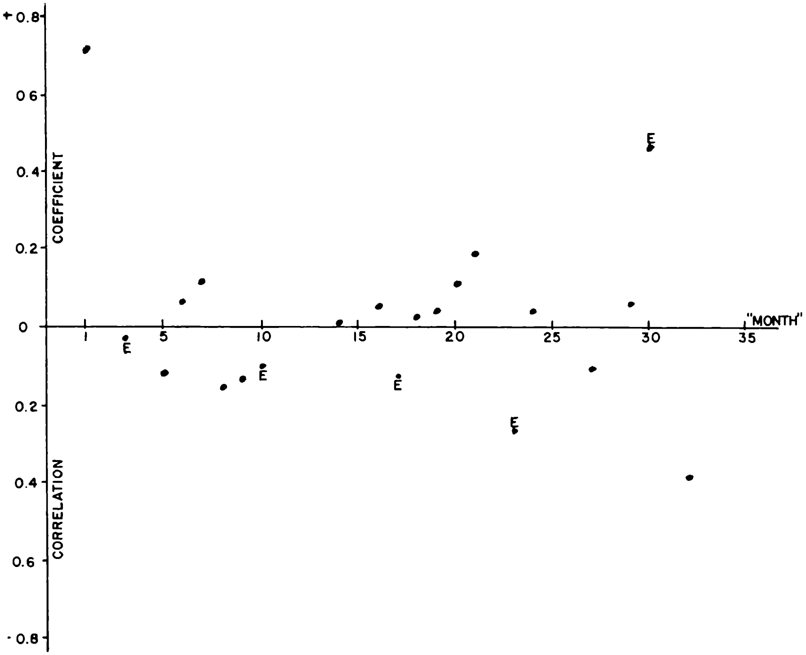

FIGURE 1—Zero day lag.

III. *Monthly Mean Correlation.*—We have cited some yearly sunspot-magnetic activity correlation coefficients of around +.0.88 which were made on the basis of 60 years of data. Since sunspot-earth current means do not seem to be related, a correlation between the monthly arithmetic means of Jamestown earth current activity and monthly arithmetic means of Zurich sunspot numbers was computed. Figure 3 shows a scatter diagram plotted from values for these two means. The correlation coefficient between these monthly means is —0.185.

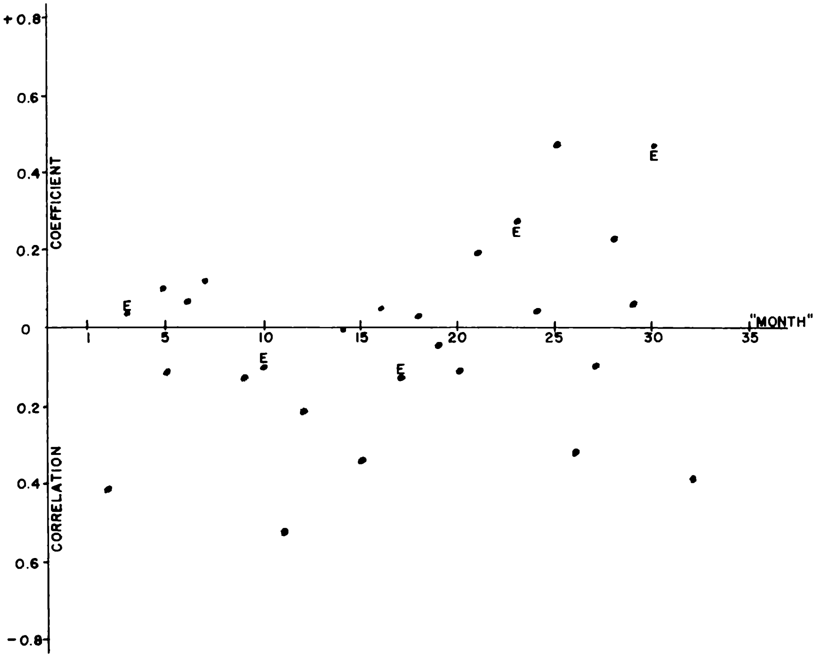

FIGURE 2.—Two day lag.

IV. *Day Number Mean Correlation.*—Because of the recurrence of sunspots over 27 day periods there is the possibility of a 27 day periodicity in the data. To check if such a periodicity was correlated with a similar periodicity in earth currents, data were grouped by day numbers within the month. The 28th day of periods with 28 days was dropped. The arithmetic mean was determined for each day number earth current activity group and for each day number sunspot activity group. These data were correlated by matching each earth current mean with the corresponding sunspot mean for the same day number. Figure 4 is a scatter diagram from the 27 data value pairs. The correlation coefficient by this procedure is +0.045.

CONCLUSIONS

I. On a daily correlation basis the monthly correlation coefficient range is −0.729 to +0.567, but the pattern appears to be random. The arithmetic mean of these monthly coefficients is −0.035. Therefore, sunspots and earth currents appear, for this case, to be unrelated.

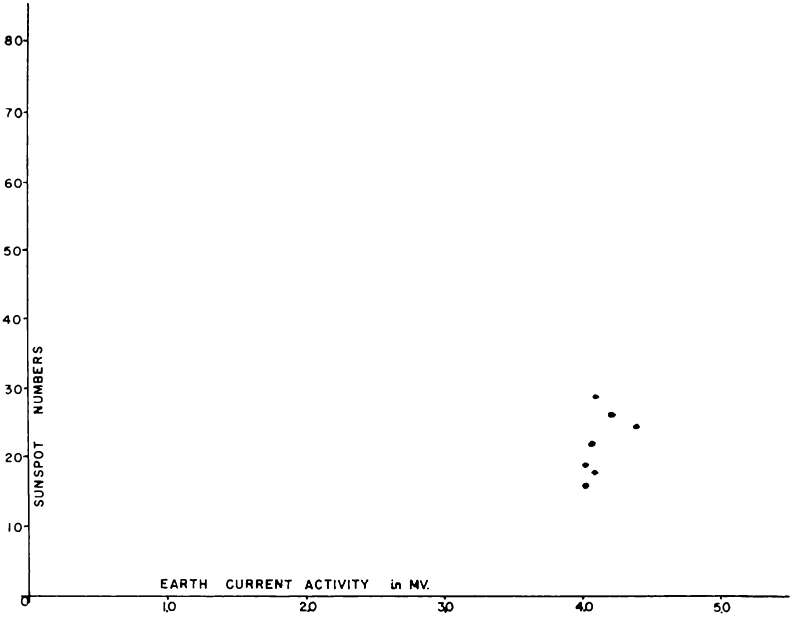

FIGURE 3.—Sunspots vs. earth currents (monthly).

Furthermore, these data do not support a pattern of high correlation coefficients during equinoctial months.

II. On a two day lag correlation basis, the monthly correlation coefficient range is —0.503 to +0.710,, but the pattern appears to be random. The arithmetic mean of these monthly coefficients is +0.039. Therefore, sunspots and earth currents appear to be unrelated for this case.

III. On a monthly mean correlation basis, the coefficient computed is —0.185. The absolute value of this coefficient is too small to indicate any significant degree of relationship.

IV. On a day number mean correlation basis, the coefficient computed is +0.045. The low absolute value of this coefficient indicates that related periodicity between the two phenomena is not supported.

The small absolute values of correlation coefficients obtained in this study of daily and monthly means are in contrast to the large positive values obtained when yearly means of earth current activity and sunspot activity are compared. Therefore, we conclude that sunspots and earth current activity are not directly related to each other but are probably both secondary effects resulting from some more primary cause.

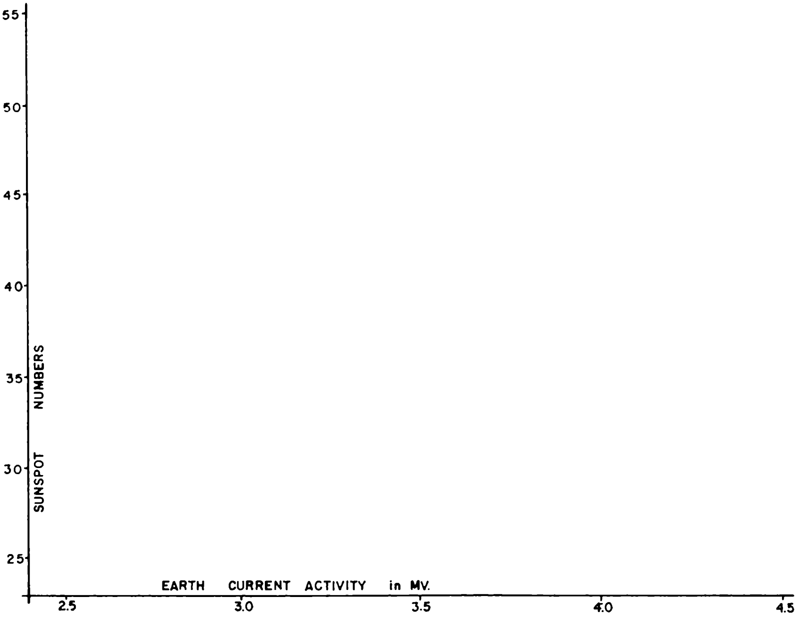

FIGURE 4.—Sunspots vs. earth currents (mean day numbers).

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THE INFLUENCE OF CHLORPROMAZINE ON BLOOD BARBITURATE LEVELS IN THE DOG AND SLEEPING TIME IN THE RAT[1]

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INTRODUCTION

Holck (3) reported many years ago that when rats were given comparable doses of pentobarbital, the females slept much longer than the males. More recently Wallis (5) reported that pentobarbital depression in man was potentiated by chlorpromazine. In the present study our attention was focused upon the possibility that chlorpromazine (Thorazine), a phenothiazine tranquilizer, might affect pentobarbital depression of male and female rats to different degrees. We were also interested in ascertaining whether the blood levels of barbiturate following a single dose of pentobarbital might be modified by concomitant chlorpromazine administration.

METHODS AND MATERIAL

I. *General procedure—rats.* — Adult Sprague-Dawley male rats weighing approximately 400 grams and female rats weighing approximately 250 grams were used for this experiment. The rats were maintained on standard laboratory rations. At the time of an experimental procedure, each rat was weighed accurately and the drug dosage calculated. The test dose of pentobarbital used was 25 mg/kg. Chlorpromazine was given 10 mg/kg or 25 mg/kg. All injections were

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[1] Guy and Bertha Ireland Research Laboratory, School of Medicine. This work was supported in part by a grant (AMA—ERF 88).

[2] Summer medical fellow, 1963.

made I. P. The pentobarbital and chlorpromazine (Thorazine) were given as 10 mg/cc solutions. At least 1 week elapsed between experiments using the same rats. In these experiments, the duration of sleep was taken as the interval between injection of pentobarbital and the time the rat assumed an upright position.

II. *General procedure—dogs.* — Five adult mongrel dogs weighing approximately 9 kg were used. Each dog was given a single I. V. injection of pentobarbital 25 mg/kg and blood samples were taken at regular intervals over 5-6 hour period. When chlorpromazine was given it was administered 40 minutes before the pentobarbital. Rectal temperatures were recorded for chlorpromazine injected animals.

III. *Method for analyzing barbiturate in dog blood.* — Approximately 2 mls of blood was drawn from a vein at each sampling time. The blood was placed in a paraffin dish and 2 ml accurately pipetted into 50 mls of chloroform in a 100 ml graduated cylinder. The resulting material was shaken for five minutes and the chloroform fraction filtered into a separatory funnel. Seven mls of sodium hydroxide (0.45 N) was then added and the resulting material shaken for five minutes. The chloroform fraction was discarded. From the aqueous fraction, two 3 ml aliquots were pipetted. To one aliquot, ½ ml of ammonium chloride (16%) was added, and to the other, ½ ml of sodium hydroxide (0.45 N). The differential spectra of the paired solutions were read in a Beckman model DU spectrophotometer at the wave-length of 260 μ. Readings were compared to a standard curve of pentobarbital concentration prepared in the same manner using known amounts of barbiturate. The procedure used was reported by Williams and Zak (6).

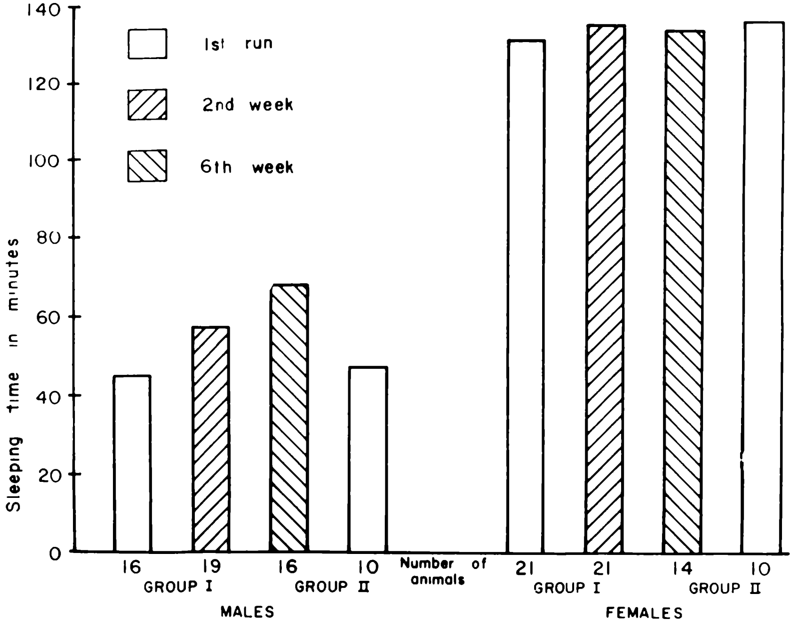
RESULTS

I. Rat Study.

A. *Sex differences.*—Nineteen male and 21 female rats were given single injections of pentobarbital 25 mg/kg one week apart. The results (figure 1) confirmed previous reports of a marked sex difference in sleeping time with this barbiturate. Male rats slept an average of 45 minutes. Female rats slept an average of 130 minutes, approximately 2.5 times as long as the males. A comparison of sleeping times the first and second week indicated that there was no residual effect of the pentobarbital in the females. The average sleeping time of male rats was about 25% longer the second week than for the original trial. Animals of this group run at a later time showed a comparable sex difference in sleeping time. A second set of controls gave similar results (figure 1).

C. *Chlorpromazine (Thorazine).*—Chlorpromazine 25 mg/kg was injected into 19 males and 11 females. A group of 10 females served as saline controls. The chlorpromazine injected animals appeared "tranquil" but did not sleep.

B. *Chlorpromazine and pentobarbital.*—One week after the injection of chlorpromazine or saline, chlorpromazine 25 mg/kg was



Comparison of pentobarbital— induced sleeping times of adult male and female rats.

FIGURE 1—Sex difference in pentobarbital sleeping time.

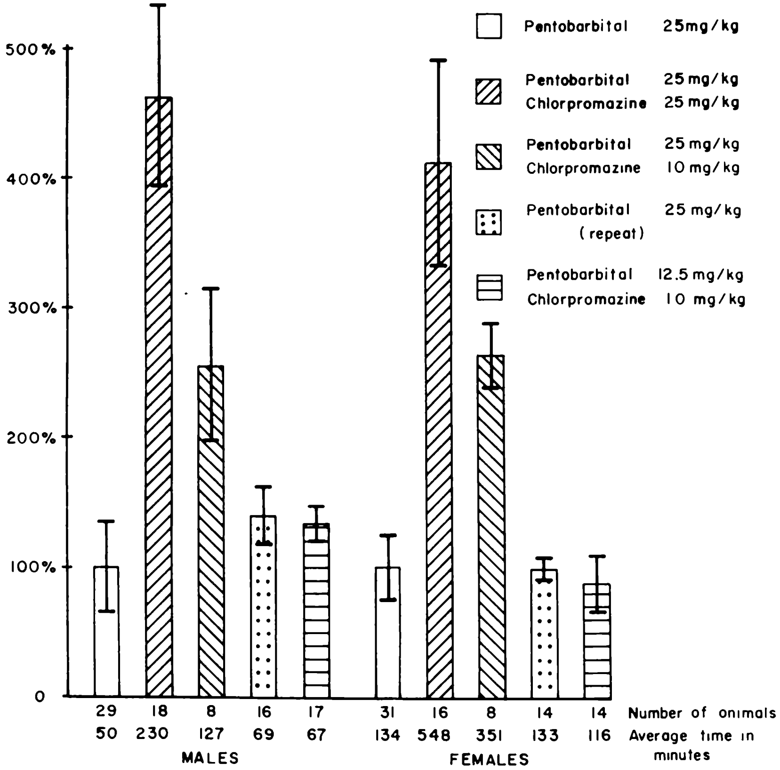
administered I.P. followed 40 minutes later by pentobarbital 25mg/kg. Sleeping times of these animals were compared with pentobarbital controls considering the control time for each sex to be 100%. Chlorpromazine prolonged pentobarbital sleeping time proportionally to 450% for males and 410% for females (figure 2). All females of the group which had served as saline controls survived, but 7 of 11 females and 2 of 19 males which had been injected with chlorpromazine 1 week previously died. A second group of male and female rats were given chlorpromazine 10 mg/kg followed by pentobarbital 25 mg/kg. This dose of chlorpromazine prolonged sleeping time to 240% for males and 260% for females (figure 2).

After two weeks, survivors of the first group were run as pentobarbital controls. Sleeping times are given as "Pentobarbital, repeat" (figure 2). A week later these rats were givn chlorpromazine 10 mg/kg and pentobarbital 12.5 mg/kg. The duration of sleep (figure 2) was approximately that of rats given pentobarbital 25 mg/kg.

II. Blood barbiturate level study—Dog.

Five dogs were given pentobarbital 25 mg/kg I. V. Blood samples were withdrawn at intervals and pentobarbital determined. Subsequently (at weekly intervals) these dogs received chlorpromazine

25 mg/kg or 10 mg/kg 40 minutes before pentobarbital 25 mg/kg and blood samples were analyzed as before. It is to be noted from the results (figure 3) that the blood barbiturate levels in dogs which had received chlorpromazine 25 mg/kg remained significantly higher than levels in dogs which received 10 mg/kg or the pentobarbital controls. Dogs which received chlorpromazine showed temperature depression. There did not appear to be a correlation between temperature depression and rate of disappearance of pentobarbital from the blood.



Comparison of pentobarbital induced sleeping times of rats with and without chlorpromazine (Thorazine). Sleeping time pentobarbital 25 mg/kg = 100 %

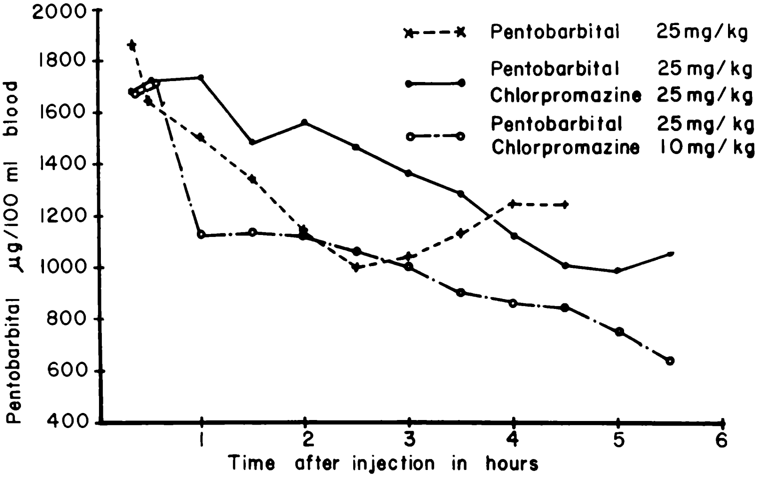
FIGURE 2—Effect of chlorpromazine on pentobarbital sleeping time.

DISCUSSION

The duration of barbiturate anesthesia is a function both of the blood level of the drug and the sensitivity of the central nervous system the barbiturate. In turn, the blood level is related to liver detoxification rates and solubility of the agent in body tissue. Also the sensitivity of the central nervous system may be modified by pathology or other drugs (3).

In rats there is a difference between the sexes in sleeping time following administration of pentobarbital or the barbiturate plus chlorpromazine. This has been attributed to the action of the sex hormones on the rate of transformation of the barbiturate into a biologically inactive form. Gaylord and Hodge (2) reported that castrated females slept a shorter time than female controls. De Boer (1) reported that castration of males prolonged their sleeping time as compared to normal males. Tureman et al. (4) reported that testosterone decreased pentobarbital sleeping time. These observations suggest, at least in the rat, that androgens and estrogens have a profound effect on certain enzyme systems in the liver.

The effect of chlorpromazine on pentobarbital sleeping time appears to have three aspects. In the first place, prolongation depends upon the amount of chlorpromazine administered, (250% of control time with chlorpromazine 10 mg/kg and 430% of control time with chlorpromazine 25 mg/kg). In the second place chlorpromazine has a residual effect lasting at least one week. When rats were given chlorpromazine 25 mg/kg and pentobarbital 25 mg/kg, of the group which had received chlorpromazine 25 mg/kg one week before, 60% of the females (7 to 10) and 10% of the males (2 to 19) died. Ten females which had served as saline controls the previous week survived. It appears further that the effect of chlorpromazine is not merely one of slowing the breakdown of pentobarbital and thus pro-



Blood Barbiturate levels in the dog following I. V. administration of Pentobarbital 25 mg/kg alone or following Chlorpromazine.

FIGURE 3—Effect of chlorpromazine on pentobarbital levels in the blood.

longing sleep. Pentobarbital 12.5 mg/kg produces transient sleep or no sleep at all in rats. However, when this dosage was preceded by chlorpromazine 10 mg/kg, the rats slept as long as with pentobarbital 25 mg/kg.

The experiments with dogs suggest that chlorpromazine decreases the rate at which pentobarbital is removed from the blood. This was observed following chlorpromazine 25 mg/kg but not with 10 mg/kg. As has been reported clinically (5), chlorpromazine lowered the body temperature of dogs. This lowering could retard or disturb enzyme systems of the liver and/or cerebral metabolism. There was, however, no direct correlation between the amount of temperature depression and the rate of disappearance of pentobarbital from the blood.

SUMMARY AND CONCLUSIONS

1. Chlorpromazine given in conjunction with pentobarbital greatly prolonged the sleeping time of rats. The duration of sleep in male and female rats remained proportionally the same.
2. A residual effect of chlorpromazine lasting at last one week was demonstrated. Of the animals given chlorpromazine one week before administration of both chlorpromazine and pentobarbital, 60% (7 of 10) of the females and 10% (2 of 19) of the males died.
3. A dose of pentobarbital insufficient to produce sleep in control rats was made effective by the concomitant administration of chlorpromazine.
4. Pentobarbital levels in the blood of dogs following single injections of pentobarbital alone or in conjunction with two levels of chlorpromazine were compared. Pentobarbital levels of dogs receiving 25 mg/kg chlorpromazine remained elevated over levels in dogs receiving 10 mg/kg chlorpromazine as well as controls receiving none.

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CHARGE TRANSFER AND STEROIDS

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In the period since the second World War interest and understanding have greatly increased in the area of intermolecular complexes. A special type of complex wherein an electron or a charge increment is transferred from one molecule to another thus forming two new molecular entities is called a charge or electron transfer complex. These entities can then be attracted to one another and form a bond. This behavior has been explained to some extent by the application of quantum mechanics particularly by Mulliken (1). Because many compounds of biological importance form these complexes and have good electron donor or acceptor properties, it seems probable that investigation of these capabilities will yield useful information about the laws that govern their biological behavior.

A group of compounds whose biological mechanisms are not fully understood is the steroids and their derivatives. Examination shows that these compounds are highly stereospecific and generally possess pi electron fragments which are available for compound formation. As an example, vitamin D_2 possesses a hexatriene system. The system is transoid from C_5 to C_8 and cisoid from C_6 to C^{10}. There is then in this area of the molecule a high pi electron density held in a relatively rigid position with little steric hindrance. Therefore, it is easy for another molecule to come into close proximity with this active site.

Earlier studies had indicated that vitamin D_2 formed a highly-colored complex with iodine. In further studies we have investigated the ability of sterols to form such complexes. The sterols had varying degrees of unsaturation. The complexing agent chosen was iodine. This was due to its convenient solubility and molecular size which allow it to approach the sterols closely without interference. In addition it has the desirable property of being able to accommodate excess charge readily. Of the sterols, or steroid derivatives, vitamin D_2, dihydrotachysterol, 7-dehydrocholesterol and ergosterol formed highly colored complexes with iodine in $CHCl_3$, CCl_4 and heptane, whereas cholesterol and dihydrocholesterol did not. The infrared spectra of those that formed color all showed a new peak at 1710/cm and a depression of the absorption peak at 3620/cm as compared to the spectra of the original sterols. From these results it seems probable that a certain amount of conjugation is necessary for complex formation. The possibility that the alcohol group is involved as a keto-enol tautomer complexing with I_3, as is the case of the cortisone-iodine system, was studied by using 7-dehydrocholesterol acetate as the sterol. This also formed a highly-colored complex with iodine in $CHCl_3$. The rate of formation and the intensity of color were similar to those obtained with 7-dehydrocholesterol although the color was different and the stability during drying was less. This indicates that the alcohol group is not a primary factor.

As might be expected the nature of the solvent has a pronounced effect on the complex formation and color (2). Using the vitamin D-iodine system as an example, it was found that the rate of formation was greater in $CHCl_3$ than in CCl_4 or heptane. The color was also darker and more intense in $CHCl_3$. In alcohol a white precipitate formed which has not been further investigated.

The thermal dependence of these complexes also showed dependence on the solvent. When a vitamin D-iodine system was frozen immediately after being mixed a yellow color was formed. On warming this changed to brown and finally to dark green. This change was not reversible. However, in heptane the solution was green at low temperatures, but turned pink on warming. This change was reversible. In this case there is probably little interaction between the solvent and solutes so that the bond formed can be broken by thermal agitation and the complex dissociates into vitamin D and free iodine which gives the purple-pink color.

The complex formed in $CHCl_3$ or CCl_4 has been isolated. It was washed with KI to remove excess iodine and with absolute methanol to remove any excess calciferol. This complex had the same absorption characteristics as the parent solution when it was redissolved in the appropriate solvent.

In these complexes the iodine does not appear to be merely adding to the double bond in vitamin D. If this were the case one would expect a colorless product. The compound upon dissolving in cottonseed oil gave a green solution. After a period of time the solution lost this color due to the highly unsaturated oil removing iodine from the vitamin D molecule. Also, if a dilute solution in $CHCl_3$ was spotted on filter paper or silica gel a colored residue was left behind. After several hours this color disappeared due to dissociation of the complex and subsequent sublimation of the iodine. Finally, the changes in the infrared spectrum are not compatible with a simple addition reaction.

Recently studies have been initiated to determine the vitamin D activity of the isolated complex. A retention of activity would tend to confirm the theory that it is more than simple addition which has taken place. We are also searching for other molecules that have the same desirable properties as iodine as basis for further studies.

Considerable information concerning the ability of certain sterols to form charge transfer complexes has been acquired. It is hoped that future investigations using sterols of different structure and sterol derivatives will point out which factors are essential for the occurrence of this phenomenon. Once these factors are known it seems logical that better explanations can be found for such actions as the hormonal and coenzymatic activity of steroids where they appear to act as reversible electron pools (3); the binding of steroids to the tyrosine residues in proteins is probably due to charge transfer, and interactions between sterols and nucleic acids may explain effects on protein synthesis. Charge transfer or electron delocalization is a useful concept for tying these actions together.

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THE HYDROLYSIS OF *o*-CHLOROACETYLSALICYLIC ACID

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INTRODUCTION

The hydrolysis of an ester is catalyzed by either hydroxyl ion or hydronium ion. Of interest is the effect of an *ortho*-carboxyl group in an aromatic ester. The hydrolysis of these esters have been postulated to proceed through a cyclic intermediate.

The rate of hydrolysis of aspirin has been thoroughly studied by Edwards (1) over the entire pH range. A graph of rate as a function of pH is shown in figure 1.

Edwards explained the observed rate by considering six simultaneous reactions. At low pH (AB) the aspirin-hydronium ion reaction is the rate determining factor while at high pH (EF) the aspirin anionhydroxyl ion reaction determines the rate. In the region (CDE) the pH dependence of the reaction can be attributed to the dissociation of a functional carboxyl group. However, it is impossible to kinetically distinguish between a carboxyl-hydroxyl ion interaction and a carboxylate ion-water interaction in the hydrolysis of aspirin.

It was decided to extend the work of Edwards by studying the

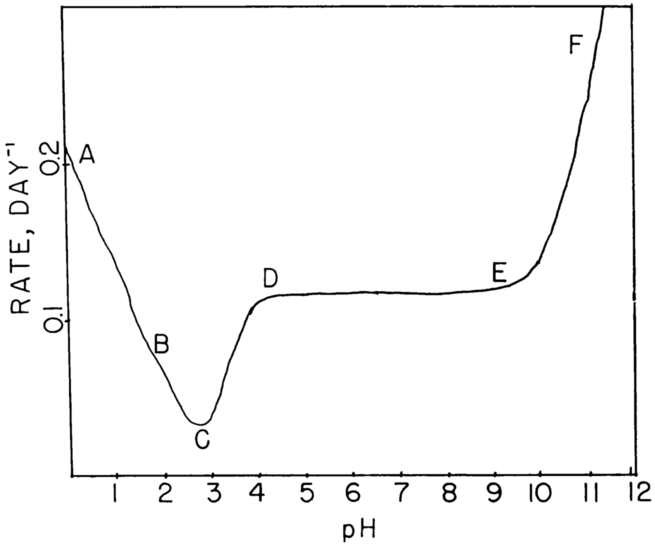


FIGURE 1—Rate of aspirin hydrolysis as a function of pH.

o-chloroacetyl derivative which exhibits a faster rate of hydrolysis than aspirin because of the powerful electron withdrawning effect of the chlorine atom. By varying the pH of the reaction solution, it was hoped that the role of the carboxyl group could be definitely proven. To elucidate the mechanism of this reaction a study of solvent and temperature effects was undertaken.

A mechanism for this reaction involving an intramolecular rearrangement has been proposed by both Garrett (2) and Bender (3). Bender studied the reaction using $H_2{}^{18}O$ and Garrett observed the effect of dielectric and spontaneous hydrolysis in ethanol-water solutions. However, ethanol proves to be an unfortunate choice of solvent. Like hydroxyl ion, ethanol is a nucleophile and an increase in the ethanol concentration effects the rate by not only changing the dielectric constant but also increasing the availability of the ethanol to take part in the reaction. Therefore, the neutral solvent dioxane was chosen to study the change in dielectric on the rate of hydrolysis of o-chloroacetylsalicylic acid.

SPECTRAL DATA

In the region of 300 mμ the absorption of aspirin and o-chloroacetylsalicylic acid is negligible while that of salicylic acid is at a maximum for all pH values. Thus, recording the growth of the salicylic acid peak at 300 mμ affords a very convenient way of studying the degree of hydrolysis at any stage.

THERMODYNAMIC DISSOCIATION CONSTANT

The thermodynamic dissociation constant of aspirin was determined spectrophotometrically by Edwards (1) to be 3.565 at 17°C. As a preliminary study this work was repeated and the dissociation constant was found to be 3.52 at the same temperature using a similar procedure. The method was modified by not taking into consideration the activities of the dissociated acid. However, there is evidence to believe that this correction is very small and the dissociation constant is therefore not effected appreciably (4). Thus, the expression for the dissociation constant reduces to:

$$pK = pH - \log \frac{e_{HA} - e}{e - e_A}$$

where e_A, e_{HA} and e are the molecular extinctions of the dissociated, undissociated and mixed forms of the acid. All data was obtained on a Beckman Model DU quartz spectrophotometer equipped with a thermostated cell compartment.

The pK of o-chloroacetylsalicylic acid at 25° ± 0.1°C, was determined at wavelengths of 270 mμ, 275 mμ and 280 mμ by the above method. Considerable difficulty was encountered in measuring the pK of o-chloroacetylsalicylic acid because of its appreciable rate of spontaneous hydrolysis. This difficulty was eliminated by studying the absorbance changes immediately following the preparation of a solution and extrapolating to zero time. The absorbance readings were then converted to extinction coefficients by using the relationship: e = A/cl, where c is the ester concentration and 1 is the cell length.

TABLE I

MOLECULAR EXTINCTIONS OF o-CHLOROACETYLSALICYLIC ACID AT DIFFERENT DEGREES OF DISSOCIATION

pH	1.20	3.10	3.45	3.70	3.90	6.40
Wavelength	e_{HA}			e		e_A
270 mμ	1078	953	915	827	792	597
275 mμ	1246	1022	965	874	773	536
280 mμ	1079	937	816	718	590	352

Substitution of the above values into equation (1) gives a mean pK for all wavelengths and pH's of 3.62. This is in good agreement with the reaction kinetic pK of 3.58 determined titrimetrically (4).

DIELECTRIC EFFECT

The dielectric effect on the kinetics of hydrolysis was investigated at pH values 3.20 and 5.10 using dioxane-water mixtures varying from 10% to 50% dioxane (v/v). These pH values were used because they are above and below the pK of the ester to prove that only one form of the carboxyl group is involved in the hydrolysis. The rate constants were determined by the Guggenheim method. The ester concentration was kept at approximately 6.5 x 10^{-4} M and the temperature maintained at 25° ± 0.1°C. The data are presented in Table II and is in good agreement with the titrimetrically determined hydrolysis rate constants in 10% dioxane at 25°C as reported by Knoblich (4).

TABLE II
CHANGE IN DIELECTRIC CONSTANT AS A FUNCTION OF RATE

	pH	3.20	5.10
% Dioxane	Dielectric constant (5)	k/min	
10	70.33	0.0131	0.0475
16.6	61.86	0.0126	0.0449
30	53.28	0.0104	0.0474
40	44.54	0.0064	0.0441
50	35.85	0.0125	0.0547

TEMPERATURE EFFECT

A study of temperature effects was made over the temperature range 10°C at pH values of 3.20 and 5.10. The Guggenheim method was again used to determine the rate constants. Arrhenius plots were made of the data and the corresponding energies of activation were calculated. These energies were used to calculate entropy changes from the relationship:

$$k = ekT/h\; e^{\Delta S^*/R}\; e^{-E/R},$$

which is valid for solution kinetics. The data we listed in Table III.

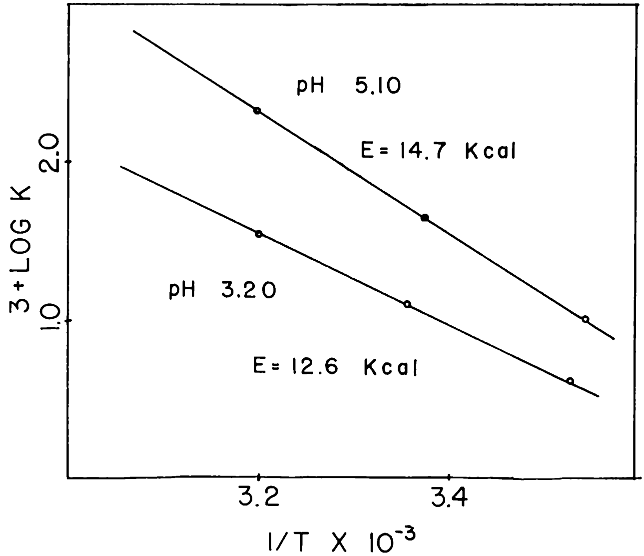


FIGURE 2—Arrhenius plot of temperature as a function of rate.

TABLE III
ENTROPIES OF ACTIVATION FOR THE HYDROLYSIS OF *o*-CHLOROACETYLSALICYLIC ACID

pH	10°	25°	40°
3.20	—24.8	—23.9	—29.0
5.10	—26.3	—21.1	—14.7

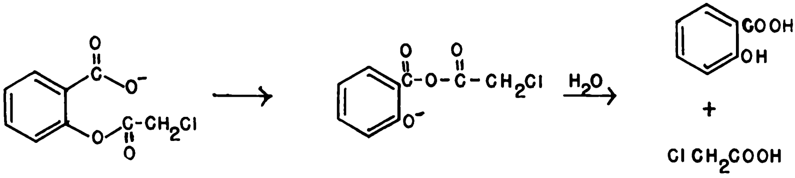
HEAT OF IONIZATION

A graph was made of temperature vs pK. The heat of ionization was calculated from the slope of the line to be —3.5 kcal.

DISCUSSION

From the experimental data obtained the following conclusions can be drawn: (a) By using dioxane the rate is independent of the dielectric constant, thus the reaction which takes place must involve an ion-neutral species interaction or an intramolecular rearrangement. (b) The negative entropy changes (see Table III) indicate that for the rate-controlled step there is probably an increase in polarity for the activated complex which becomes highly solvated thereby giving more order to the system. These entropies of activation compare very closely to those of aspirin (2). Thus the presence of the chlorine atom does not appreciably change the degree of solvation, but it appears to influence solely the carbonyl carbon making it more susceptible to nucleophilic attack. This effect lowers the activation energy by about 4 kcal, as shown by comparison of the data in figure 2 (average 14 kcal) with that for aspirin (18 kcal) as reported by Garrett (2). This lowering of the activation energy by the chlorine atom in *o*-chloroacetylsalicylic acid explains why it hydrolyzes considerably faster than aspirin. (c) Reasonably good agreement was obtained between the heats of ionization of salicylic acid (6) and *o*-chloroacetylsalicylic acid. Thus, it can be considered that the carboxylate anion is functional in the mechanism.

Bender (7) studied the effect of a neighboring hydroxyl group on the hydrolysis of *p*-nitrophenyl salicylates and came to the conclusion that the mechanism involved the interaction of water with the unionized salicylic ester. Below is outlined a mechanism involving an intramolecular rearrangement which is consistent with the present data and the previous work of Garrett and Bender.



The reaction may be pictured as a nucleophilic attack by the carboxylate anion on the electron deficient carbonyl carbon of the ester, forming an intermediate anhydride. The anhydride is subsequently decomposed by water to chloroacetic acid and salicylic acid.

ACKNOWLEDGMENT

We are deeply indebted to Jerome Knoblich for the preparation of *o*-chloroacetylsalicylic acid which enabled this work to be carried out.

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FINDING DECIMAL VALUES OF RECIPROCALS OF INTEGERS THROUGH GRAPHING

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When I started my project, I was trying to find relationships between prime numbers which would shed some light on their unique nature. I compared the prime numbers by graphing their reciprocals and comparing these graphs. This graphing was done by dividing 1 by the prime number and, after carrying out this division until it terminated or went into a pattern, translating the quotient and intermediary steps of this division into a graph. After comparing the graphs of twenty of these reciprocals, I discovered two characteristics which were found in every graph. I graphed out the reciprocals of twenty integers which were not prime to see if these characteristics were properties of just prime numbers or of all integers and they held for all integers. I studied and worked with these properties and the result was a standardized method of graphing the reciprocal of an integer which was based on the supposition that the two characteristics would hold for all integers. This method of graphing yielded the decimal value of the reciprocal in each of the forty cases I tried. I generalized my method by proving algebraically that the two characteristics would hold and that method was valid for the graph of the reciprocal of any integer. Thus, my method can be used to obtain the decimal value and the intermediary steps of the division of the reciprocal of any integer.

Since the reciprocal of an integer is 1 divided by that integer, the graph of the reciprocal would be the graph of this division. The method of translating this division into a graph is simple. As an example, the reciprocal of 7 is 1/7 which is the same as 1 divided by 7. Performing this division we obtain the results shown in figure 1.

$$\begin{array}{r l}
 & .142857 \\
7 & \overline{)1.000000} \\
 & \underline{7} \\
 & 30 \\
 & \underline{28} \\
 & 20 \\
 & \underline{14} \\
 & 60 \\
 & \underline{56} \\
 & 40 \\
 & \underline{35} \\
 & 50 \\
 & \underline{49} \\
 & 1
\end{array}$$

FIGURE 1—Division of 1 by the integer 7.

Arranging the quotient and intermediary steps of this division into a table, we obtain the results shown in Table I.

TABLE I

Quotient and Intermediary Steps of the Division of figure I in Table Form.

1. 10 =(7) (1+3/7)
2. 30 =(7) (4+2/7)
3. 20 =(7) (2+6/7)
4. 60 =(7) (8+4/7)
5. 40 =(7) (5+5/7)
6. 50 =(7) (7+1/7)
7. 10 =(7) (1+3/7)

. .

#1 #2 #3

The framework of the graph is made by using the first quadrant of a co-ordinate plane. The vertical values ascend to the value of the original integer, in this case, 7. The horizontal values ascend to 10.

If we let the numbers in column #1 of Table I be the number of the point, the numbers in column #2 be 10 times the vertical co-ordinate, and the sum of the integer and fraction of column #3 be the horizontal co-ordinate; we obtain the co-ordinates of six points. Since it is virtually impossible to compare series of points, the graph is transformed into a linear graph by connecting the points in arithmetic order, first moving vertically and then horizontally.

By comparing the graphs of forty such reciprocals ranging up to 65, I discovered two characteristics common to all of them. From these similarities I formulated two postulates from which I was able to devise a standard method of graphing. My first postulate is: The points of the graph all lie on that diagonal which con-

TABLE II

Quotient and Intermediary steps of the division of Figure 2 in table form

#1	#2		#3
1.	10	=(59)	(0+10/59)
2.	100	=(59)	(1+41/59)
3.	410	=(59)	(6+56/59)
4.	560	=(59)	(9+29/59)
5.	290	=(59)	(4+54/59)
6.	540	=(59)	(9+ 9/59)
7.	90	=(59)	(1+31/59)
8.	310	=(59)	(5+15/59)
9.	150	=(59)	(2+32/59)
10.	320	=(59)	(5+25/59)
11.	250	=(59)	(4+14/59)
12.	140	=(59)	(2+22/59)
13.	220	=(59)	(3+43/59)
14.	430	=(59)	(7+17/59)
15.	170	=(59)	(2+52/59)
16.	520	=(59)	(8+48/59)
17.	480	=(59)	(8+ 8/59)
18.	80	=(59)	(1+21/59)
19.	210	=(59)	(3+33/59)
20.	330	=(59)	(5+35/59)
21.	350	=(59)	(5+55/59)
22.	550	=(59)	(9+19/59)
23.	190	=(59)	(3+13/59)
24.	130	=(59)	(2+12/59)
25.	120	=(59)	(2+ 2/59)
26.	20	=(59)	(0+20/59)
27.	200	=(59)	(3+23/59)
28.	230	=(59)	(3+53/59)
29.	530	=(59)	(8+58/59)
30.	580	=(59)	(9+49/59)
31.	490	=(59)	(8+18/59)
32.	180	=(59)	(3+ 3/59)
33.	30	=(59)	(0+30/59)
34.	300	=(59)	(5+ 5/59)
35.	50	=(59)	(0+50/59)
36.	500	=(59)	(8+28/59)
37.	280	=(59)	(4+44/59)
38.	440	=(59)	(7+27/59)
39.	270	=(59)	(4+34/59)
40.	340	=(59)	(5+45/59)
41.	450	=(59)	(7+37/59)
42.	370	=(59)	(6+16/59)
43.	160	=(59)	(2+42/59)
44.	420	=(59)	(7+ 7/59)
45.	70	=(59)	(1+11/59)
46.	110	=(59)	(1+51/59)
47.	510	=(59)	(8+38/59)
48.	380	=(59)	(6+26/59)
49.	260	=(59)	(4+24/59)
50.	240	=(59)	(4+ 4/59)
51.	40	=(59)	(0+40/59)
52.	400	=(59)	(6+46/59)
53.	460	=(59)	(7+47/59)
54.	470	=(59)	(7+57/59)
55.	570	=(59)	(9+39/59)
56.	390	=(59)	(6+36/59)
57.	360	=(59)	(6+ 6/59)
58.	60	=(59)	(1+ 1/59)
59.	10	=(59)	(0+10/59)

nects the origin with the opposite vertex. My second postulate is: The point at which the graph changes direction from vertical to horizontal, between any two points on the graph, is on a "sub-diagonal". A "sub-diagonal" is a line segment connecting a point on the horizontal axis, having an integer horizontal co-ordinate (N), to a point having a horizontal co-ordinate one greater (N+1) and a vertical co-ordinate of the highest value in the range. Thus, in

```
    .0169491525423728813559322033898305084745762711864406779661...
59/1.0000000000000000000000000000000000000000000000000000000000
     0
    100
     59
     410
     354
      560
      531
       290
       236
        540
        531
          90
          59
         310
         295
          150
          118
           320
           295
            250
            236
             140
             118
              220
              177
               430
               413
                170
                118
                 520
                 472
                  480
                  472
                    80
                    59
                   210
                   177
                    330
                    295
                     350
                     295
                      550

550
531
 190
 177
  130
  118
   120
   118
     20
      0
    200
    177
     230
     177
      530
      472
       580
       531
        490
        472
         180
         177
           30
            0
          300
          295
            50
             0
           500
           472
            280
            236
             440
             413
              270
              236
               340
               295
                450
                413
                 370

370
354
 160
 118
  420
  413
    70
    59
   110
    59
    510
    472
     380
     354
      260
      236
       240
       236
         40
          0
        400
        354
         460
         413
          470
          413
           570
           531
            390
            354
             360
             354
               60
               59
                1
```

FIGURE 2—Division of 1 by the integer 59.

the previous graph, lines connecting the points (0,0) and (1,7), (2,0) and (3,7), and (7,0) and (8,7) are some of the 'sub-diagonals".

These postulates are easily evident in the graph of the reciprocal of 59 which is worked out in Figure 2.

Using my two postulates, I formulated the following standardized method of graphing. Prepare the framework of the graph by using the first quadrant of a co-ordinate plane with the vertical values ascending to the value of the original integer and the horizontal values ascending to 10. This is the same framework as was used in the division method. Now draw in the diagonal and ten "subdiagonals". The first point on the graph will be that point on the diagonal which has a vertical co-ordinate of 1. This is so because the first entry of the table for any integer will always be 10. From this point you draw a line up till you come to a point of intersection with a "sub-diagonal". At this point you switch to a horizontal direction and move right until you intersect the diagonal. This point of intersection is the second point of the graph. Repeating the same procedure, you move vertically either up or down, depending on the given situation till you intersect a "sub-diagonal", change direction to horizontally and at the point of intersection with the diagonal, place the third point of the graph. This procedure is continued until the graph terminates at zero, returns to a previous point or stops at a point. The decimal value of the reciprocal is then read by taking the greatest integers in the horizontal co-ordinates of the points and arranging them in ascending arithmetic order according to the number of the respective point.

We know from arithmetic that in any division problem, the division either terminates or it doesn't. If the division doesn't terminate, it may continue in three different ways. These three types of division have definite corresponding types of graphs. The first type is when the whole sequence of the quotient is repeated to infinity. This is accomplished by the graph returning to its original point. These graphs are like the graph of 1/7 in which the whole sequence .142857 is repeated to infinity. The second type is when only a part of the sequence is repeated to inifinity. In a graph of this type you return to a point other than the initial one. This is demonstrated by the graph of 1/14 which is read .0714285. . . ; only that part of the sequence after the first zero is repeated. In the third type, the last digit of the sequence is repeated to infinity. In a graph of this type of division a point of the graph falls on a "sub-diagonal". Since you cannot move vertically and intersect a "sub-diagonal", the point is repeated to infinity. This is illustrated by the graph of 1/6 which is read .166. . . ; the 6 is repeated to infinity. If a graph or division terminates, zero is repeated to infinity. This type of division also has a corresponding type of graph. In a graph of this kind, a point of the graph has an integer for its horizontal co-ordinate. From this point you could either go up or down to intercept a "sub-diagonal".

However, if you go up, the next point of the graph would have a horizontal co-ordinate of 10. Thus, in reading the decimal value of the graph, 10 would have to be a digit in the quotient of the division. Since this is undefined and marks an error in the division process, it is also undefined in my method of graphing. Thus, from a point on the graph with an integer horizontal co-ordinate you move down vertically to the horizontal axis and then horizontally to the diagonal at the origin (zero). This is depicted in the graph of 1/8 which is read .125; it terminates at this point and zero is repeated to infinity. If a person wanted to become really technical, he could say that since types one and two both return to a previous point and repeat some or all of a sequence, they are the same type; and, since types three and four both repeat one digit (technically the fourth type repeats zero), they are the same type. However, since the first type has the added stipulation that it returns to the initial point and since the forth type stipulates that the digit be zero, I have classified the types of graphs as four different types.

Having proved experimentally that my postulates and method are valid, the next step is to prove them algebraically for the graph of the reciprocal of any integer. Since the graph provided by my method must be the same as that obtained by the division method for any given integer, any properties of a graph of the division method must also be properties of a graph of my method. You will remember that the vertical co-ordinate (Y) of a point on a division graph was found by taking 1/10 of the dividend of an intermediary step. Since the horizontal co-ordinate (X) of a point on a division graph was the dividend of an intermediary step divided by the original integer (N), we can say that $X = 10Y/N$. Solving this equation for Y we get $Y = XN/10$. This is the function which takes X into XN/10 (or $f{:}X \rightarrow XN/10$); any point of any graph must be included in this function because it was formulated from the common properties of all points of any graph. To obtain the function of the diagonal of the graph of the reciprocal of any integer (N), we use the two point form of the equation of a line, which is: $Y-Y_1 = (Y_2-Y_1/X_2-X_1)(X-X_1)$. Plugging in the co-ordinates of the origin, (0,0), and the opposite vertex, (10,N), we get $Y-0 = (N-0/10-0)(X-0)$. Simplifying this equation and expressing it in function notation we obtain $f(X) = XN/10$ which is the same function as was formulated for all points of any graph. Since these two functions are the same, any point of a graph will be found on the diagonal of the graph. Thus, my first postulate will be valid for the graph of the reciprocal of any integer.

Since my method uses two postulates to take you from one point to another, there must be a second function involved. A graph can theoretically have a point anywhere on the diagonal, therefore, this function must include all values of X for 0 to 10. Also, because of the method used in the fourth type of graph, when X is an integer,

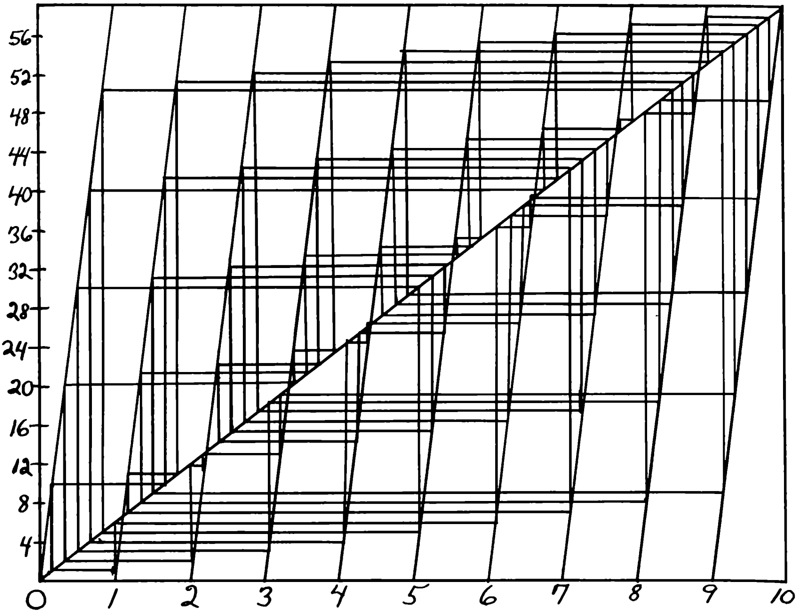

FIGURE 3—Graph of reciprocals.

f(X) = 0. Since this function must hold for the division of 1 by any integer, it must hold for any order of digits in a quotient. Thus, regardless of what any digit might be, the next digit could be any digit from 0 to 9 and the next digit after that could be any digit from 0 to 9 and so on until the graph either goes into a pattern or terminates. A digit in a quotient can be determined by either its X or its Y co-ordinate because all points of a graph must fall on the diagonal. Therefore, if this function is to include the possibility of any order of digits, it must include either all values of X or all values of Y between each integer on the horizontal (X) axis. However, by definition, between any two integers on the X axis there are only those values of X which are between the two given integers. Since there can't be all values of X between two integers on the X axis, this function must include all values in the range (Y) between each integer on the X axis. Since all points of a graph are on the diagonal, as either the X or the Y value of a point increases, its corresponding digit increases. Therefore, the X and Y values of this function must be directly proportional. To summarize, this function has four properties: (a) The function includes all X from 0 to 10. (b) When X is an integer, f(X) = 0. (c) Between every integer on the X axis, the function includes all values of Y. (d) X and Y are directly poroportional. The function stipulated by these properties is f:X→N(X—[X]). If we graph out this function for any integer (N) it is the ten "sub-diagonals" of the graph. Also as a check of this, we know that the "sub-diagonals" connect the points (0,0) and (1,N), (1,0) and (2,N), (2,0) and (3,N) . . . (9,0) and (10,N). Using the two point form of the equation of a line, we get the equations of the "sub diagonals" of the graph of the reciprocal of N to be: $Y = NX$, $Y = NX—N$, $Y = NX—9N$. Combining these equations into a function we get $f(Y) = NX—N\ [X]$ or $f{:}X \rightarrow N(X—[X])$. This is the same function as was obtained from the general properties of the points where the graph changes direction from vertically to horizontally. Thus, my second function is valid for the graph of the reciprocal of any integer.

My method of graphing uses my two postulates to determine where the graph changes direction. Thus, a proof that my method of graphing is valid for all integers would be if the two general functions which validated my postulates for any integer can be used to obtain these points for any graph by "switching" back and forth between them. This "switching" is done by taking 1 and plugging it into the first function, $f(X) = XN/10$; the image of this function is then substituted into the second function, $f(X) = N(X—[X])$. This image is then plugged back into the first function and that image back into the second function. This procedure is continued until the images either terminate or go into a pattern. You start by using 1 because the first point of any graph is always that point on the diagonal which has a vertical co-ordinate of 1. This "switching" provides the same graph as my method in every case. Using 1/7 to illustrate this proof we get: $f_1(1) = 10/7$; $f_2(10/7) = 3$; $f_1(3) = 30/7$; $f_2(30/7) = 2$; $f_1(2) = 20/7$; $f_2(20/7) = 6$; $f_1(6) = 60/7$; $f_2(60/7) = 4$; $f_1(4) = 40/7$; $f_2(40/7) = 5$; $f_1(5) = 50/7$; $f_2(50/7) = 1$; $f_1(1) = 10/7$ . . . . To translate this information onto a graph, the following method is used: The images of the f_1's are the horizontal coordinates of the points of the graph and the corresponding X value of the function is the vertical co-ordinate of that point. In the f_2's, the vertical co-ordinate is the image and the corresponding X value is the horizontal co-ordinate of the points on the "sub-diagonals" at which the line connecting two points in arithmetic order changes direction from vertically to horizontally. As before, the points, as found, occur in arithmetic order. The graph thus obtained is the same as the one determined by my method. Since the graph obtained from the general functions is always the same as that provided by my method, my method is valid for the graph of the reciprocal of any integer. It is well to remember when working in this system of graphing that because it has been validated as the division of 1 by some integer (N), the number of different points on the graph can be at most N—1.

Having proved both experimentally and algebraically that my method of obtaining decimal values of the reciprocals of integers is valid, one can see that my method is, in actuality, the graphing of functions which through their intersecting points divide 1 by a given integer. Since this is true, my method can also be used to determine the decimal value of 1 divided by a terminating decimal, a power of ten divided by an integer or even a power of ten divided by a terminating decimal. In these cases the only change in my method is to move the decimal point in the quotient the same number of places that it takes to transform the decimal to an integer and/or the power of ten to 1. Also, since the long division method remains the same regardless of what base you are working in, my method will be valid in any number base. As an example of this, 1 divided by 12 in the base of 5 yields .032412 . . . and my method also yields .032412 . . . which is equal to .142857 . . . in the base of 10. .142857 . . . is also the quotient of 1 divided by 7, and 7 in the base of 10 is equal to 12 in the base of 5. This relationship will hold true for any two graphs regardless of what two bases are used. This opens up the possibility of my method being used in a binomial system to set up circuits in an electronic brain which would divide any of the combinations of numbers for which my method is valid.

My method will eliminate the possibility of arithmetic error in calculating the decimal value of 1 or any power of ten divided by any terminating decimal but it also introduces the possibility of error in graphing (when graphing by ordinary means), . . . especially when the original integer was large. For this reason my method probably has no practical value as a general means of obtaining the decimal values of reciprocals. However, I here fall back on the privilege of all mathematicians to point out that what may not have practical value today may be accepted working theory tomorrow. Besides, if my method serves to enlighten the mind of even one individual, is that not of practical value?

LYNN BANKS McMULLEN

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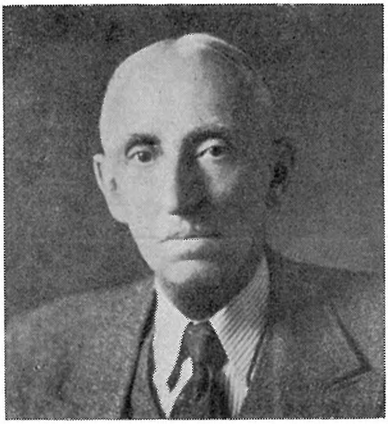
Invited Paper for the 56th Annual Meeting of the North Dakota Academy of Science, at Fargo, North Dakota, May 1-2, 1964.

The death of Dr. Lynn Banks McMullen, on May 17, 1963, at Polson, Montana, marked the passing of a noted scientist, educator, administrator, and one of the active founders of the North Dakota Academy of Science. It leaves this writer as the sole survivor of that little group of six former members of the illustrious Indiana Academy of Science who, in 1908, founded the North Dakota Academy of Science (1).

Dr. McMullen was born January 28, 1875, at Arcadia, Indiana. He attended DePauw University, at Greencastle, Indiana, majoring in physics under genial Professor Naylor who taught him to distinguish sharply between his "Knows" and his "Don't Knows." It was there, as fraternity brothers, that our life-long friendship began. In 1897 he received the degree of Bachelor of Science from DePauw; and on June 15, 1899, he married Ethel Lucretia Brown, daughter of Eli Brown, professor and author at Purdue University. For more than sixty years Ethel has been his inspiration and loyal intellectual companion; for she, too, as well as their two daughters have been teachers.



LYNN BANKS McMULLEN
(1875-1963)

Later we both taught in Indianapolis—he in the Shortridge High School and I in the Manual Training High School. For many years we managed rival athletic teams. The competition was very keen but always friendly. Our lives continued to run parallel when we both came to North Dakota—he in the fall of 1908 to become Professor of Physics at the Valley City State Normal School and I, on New Years Day, 1909, to join the faculty and Experiment Station of the State Agricultural College at Fargo. As long as he remained in North Dakota we continued to maintain close contacts and friendly relations.

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[1] Historian, North Dakota Academy of Science.

McMullen, known as "Mac" by his host of admiring friends, was very active in the organization of the North Dakota Academy of Science, serving as its secretary-treasurer during the first two years, then as vice-president, in 1911, and president in 1912. Before leaving North Dakota, Dr. McMullen had been promoted to the vice-presidency of the Valley City State Normal School. He went as a student to Columbia, and in 1919 received his Master's degree from Columbia. Then he went to Greely, Colorado, as Director of Elementary Training, and in 1920 he became President of the Northern Arizona State Normal School, at Flagstaff, a position which he held for four years. Returning to Columbia as teacher and graduate student, in 1925 he received his Doctor's degree in Education and went to the University of Kentucky as Professor of Education. Two years later he was called to be the first president of the newly established Eastern Montana State Teachers College at Billings, a position which he continued to fill with distinction until his retirement in 1945.

Dr. McMullen gained wide spread recognition as the author of the "McMullen Plan" through which professional students of Education alternate as "Observers" in public school classes with their regular college study. This plan was adopted over a wide western area, including California. After his retirement, Dr. McMullen moved to the home he had built on the east shore of beautiful Flathead Lake, Montana, near Polson. He continued to maintain his active interest in education until the time of his death, giving numerous addresses, and contributing articles and guest editorials to the press. He never lost his enthusiastic interest in sports. His favorite game was tennis, in which he was expert, and which he continued to play until a few years ago when he relinquished it for the less strenuous game of golf. He continued to play golf on his local course and on the Mesa, Arizona, course. He also enjoyed gardening, and later his fragrant cherry orchards, at his home on Flathead Lake. Always mentally keen and alert, he was ever aware of the current developments in science and in national and international affairs. His civic interests and activities found happy expression through his membership in Rotary International. He served as president of the Billings Rotary Club, and was once elected district governor of Rotary, but was compelled to decline the honor through the pressure of his academic commitments. Even after retirement he drove to Polson regularly to attend its Rotary Club meetings where his addresses and birthday talks were always awaited with keen interest, not merely for their thought provoking quality, but for their subtle humor—a characteristic gift dating back to his college days.

Surviving Dr. McMullen are Mrs. McMullen and their two daughters: Lucretia (Mrs. A. C. Hoefort) of Polson, Montana, and Katherine (Mrs. R. A. Williams) now in Curacao, Netherlands Antilles. Three grandchildren are living: Murray Williams, now in the Near East as engineer on a construction project for the Chicago Bridge and Iron Works, Lynn Hoefort, studying for her doctor's degree at Davis Teachers College, California, and Lois Hoefort, a graduate student at Montana State College.

Dr. McMullen was listed in "American Men of Science", and he lived to see the Education Building on the campus of Eastern Montana State Teachers College, named in his honor McMULLEN HALL. Dr. McMullen was a gifted educator, not a mere educationist, for his educational qualifications rested upon a solid foundation of intellectual integrity and sound scholarship. He was able to a remarkable degree to combine the realistic approach to science with the breadth of vision of a liberal culture in others. This task was not easy in a world clamoring for technologists rather than scholars. But he steadfastly refused to compromise his high ideals of character and his high standards of scholarship; although well he knew that if this scholarship is to survive, it must make a real and vital contribution to a new and changing world.

As a scientist, Dr. McMullen accepted the gospel of hard work, patient observation, and persistent inquiry. To him the universe made sense, and he did not hestitate to ascribe to it a purposeful trend toward ulimate good. He believed in the essential unity and integrity of all truth. He had the conviction that his truth will some day triumph over error, that it will help struggling humanity to get out of the jungle and finally to realize that which is called good. He believed that science was made for man, and that it must find its ultimate justification in terms of human betterment. To that end he devoted his long and useful life. Although continually pulled from the classroom to the administrator's desk, his greatest joy was gained from teaching. He was an inspiring teacher, as testified by the generations of his students who have gone forth to successful careers, who now rise in gratitude to call him blessed, and who bear the lasting imprint of his noble character and challenging life. Well may our North Dakota Dakota Academy of Science pay its grateful tribute and proudly cherish the memory of such a pioneer founder as Lynn Banks McMullen.

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INFLUENCE OF GRAZING ON FACTORS AFFECTING WATER INTAKE RATES OF RANGE SOILS

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ABSTRACT

The influence of grazing on forage production, amount of mulch present, soil organic matter, soil bulk density, and rate of water infiltration was determined on seven major range soil types in western North Dakota during the 2-year period, 1962-63. Quantitative determinations of these factors on two ungrazed or lightly grazed areas and on two moderately to heavily grazed areas on each of the seven soil types were made, a total of 28 sample areas being studied in detail.

Total forage production decreased on all sites under grazing, the the grazed sites producing only 50 to 80 percent as much as the ungrazed sites. The forage yield on ungrazed sites averaged 787 pounds dry-weight per acre, and on grazed sites, 474 pounds per acre. Total mulch, including both fresh and humic mulch, was also much reduced by grazing, averaging 3,940 pounds per acre for all ungrazed sites and 2,157 pounds per acre for the grazed sites.

Grazing influences on soil organic matter and on soil bulk density were seemingly much less significant than on forage production and amounts of mulch present. Soil organic matter in the upper 6 inches of the soil averaged 3.36 percent on the ungrazed sites and 3.32 percent on the grazed sites. The differences were not statistically significant, and it was concluded that grazing had had no appreciable effect on soil organic matter content on the sites. Bulk density values for the upper 3 inches of soil averaged 1.16 g/cc on the ungrazed areas and 1.23 g/cc on the grazed sites. The differences were consistent, though not statistically significant. There has apparently been some slight compaction of the upper soil layer on the grazed sites.

Rate of water infiltration on all range sites was dramatically reduced by grazing. Infiltation rates for the second inch of applied water averaged 6.0 inches per hour on ungrazed areas and 3.1 inches per hour on the grazed areas. Reduction in the amount of mulch present seemed to be a major influence in the reduction in rate of water infiltration on the grazed sites. An empirical formula for the calculation of rate of water infiltration as influenced by amount of mulch present was developed for the different soils, and calculated infiltration values showed high correlation with actual values determined in the field.

ANOTHER MORPHOLOGY AS RELATED TO CYTOPLASMIS MALE-STERILITY IN SUDANGRASS

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ABSTRACT

One phase of the sudangrass improvement program at North Dakota State University is the development of cytoplasmic male-sterile lines as potential female parents for crossing with normal pollen-fertile male lines in the production of hybrid sudangrass varieties. During the course of these studies, certain breeding lines, when crossed with the source line having cytoplasmic male-sterility, restored fertility to the F_1 generation whereas others did not restore fertility to the F_1 generation.

For this study, young inflorescences were collected and fixed in a solution of 1 part glacial acetic acid to 3 parts absolute ethyl alcohol. After 48 hours the materials were transferred to 70% ethyl alcohol and stored in sealed bottles in a refrigerator until used. Inflorescence samples were obtained from an A-Line which was the original source of cytoplasmic male-sterility, a male-fertile breeding line, and a cytoplasmic male-sterile and pollen-fertile F_1 line derived from crosses of breeding lines with the A-Line. Temporary slides using the smear technique and acetocarmine staining were used to study microsporogenesis in young anthers of the male-sterile A-Line and male-fertile breeding line. Microtome sections of anthers of all four lines during the period of pollen development were prepared and stained with iron-alum hematoxylin and sanfranin-fast green as permanent mounts. These were used to study the relationship between the anther tissue and the production of viable or aborted pollen grains.

Meiosis in the male-fertile breeding line and the cytoplasmic male-sterile A-Line was normal and non-defective tetrads of microspores were formed. During maturation of pollen grains in the anther, however, pollen grains aborted in both cytoplasmic male-sterile lines as compared to normal maturation of pollen in the two male-fertile lines. In male-fertile lines the tapetal cells, comprising the inner layer of the anther, degenerated as the microspores matured in contrast with non-degeneration of the tapetal cells during a similar maturation stage in the cytoplasmic male-sterile lines. The morphological and cytological details in the anthers of the male-fertile and male-sterile lines were quite similar with exception of the behavior of tapetal cells and microspores. The behavior of the tapetum in

the post meiotic stages appeared to be associated with the viable and non-viable nature of the pollen grains. This suggested that the non-degeneration of the tapetal cells in the male-sterile lines resulted in abnormal microspore nutrition and subsequent abortion of pollen grains. The results of this study are similar to those reported by Singh and Hadley (1).

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A VEGETATIONAL ANALYSIS OF THE SALT-DESERT SHRUB TYPE IN WESTERN NORTH DAKOTA

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Several woody vegetation types occur in the Badlands of southwestern North Dakota, even though the vegetation is considered to be mixed-grass prairie. Four of the principal woody types have been described earlier by Nelson (1961) and a fifth type by Potter and Green (1964).

Very recently another separate woody type has been recognized. It has been conditionally named the salt-desert shrub type, for it is somewhat similar to this type in Utah and Nevada. Tentatively, the western North Dakota type is considered to be an extension of the salt-desert shrub of northeastern Wyoming.

The type occurs along the entire course of the Little Missouri river in North Dakota but seldom closer to the River than one mile and generally at the higher elevations. It is characteristic of the steep slopes (30-45°) which are undergoing rather severe erosion. It occurs on north-, south-, east-, and west-facing slopes, but it is perhaps more frequent on, and typical of, the south- and east-facing slopes.

A sparse sprinkling of shrubs ranging from 1-3 feet in height and averaging less than one plant per square yard is characteristic of the type in western North Dakota. A scanty suffrutescent layer is present, and a scattered herbaceous layer composed of a few species of widely spaced grasses and forbs completes the type.

Eighteen stands of the type, located from Marmarth, North Dakota in the south, to the North Unit of the Theodore Roosevelt National Memorial Park in the northern Badlands, a distance of approximately 130 miles, were studied in detail during the summers of 1962 and 1963. The shrub and suffrutescent strata were sampled in each stand using the point-centered-quarter method (Cottam and Curtis, 1956; Dix, 1961). Data obtained by the use of this method were frequency, density, and percentage composition of the vegetation on the basis of numbers of individuals of each species. In addition, percentage cover and cover in square feet per 100 square yards were calculated from average crown diameter measurements and the absolute density. Average shrub height and average crown diameter were determined by measuring those plants tallied while employing the point-centered-quarter method. Forty 1 x 2-foot quadrate samples were employed in each stand to obtain percent composition and percent cover in the herbaceous layer.

Big sagebrush *(Artemisia tridentata)* was the major species on all exposures having a mean absolute density of 73.6 individuals per 100 square yards and a mean cover value of 183.7 square feet per 100 square yards. Three other shrub species, shadscale *(Atriplex confertifolia)*, long-leaved sage *(Artemisia longifolia)*, and rabbitbrush *(Chrysothamnus graveolens)*, are also important in this type, having absolute densities of 10.6, 11.0, and 10.4, respectively; and cover values of 25.4, 16.0, and 19.1, respectively. Greasewood *(Sarcobatus vermiculatus)* is as important as these latter three shrubs, for even though its density is low (5.1), it is a large shrub and ranks third in cover with a mean area of 22.3 square feet per 100 square yards. Thirteen additional shrub species were associated with the type.

Only four species were of importance in the suffrutescent stratum. They include, in order of rank, broom snakewood *(Gutierrezia sarothrae,)* Nuttall's saltbush *(Atriplex nuttallii)*, umbrella plant *(Eriogonum multiceps)*, and alkali seepweed *(Suaeda fruticosa)*. They have, respectively, density values of 41.3, 29.9, 20.8 and 10.5 and cover values of 19.3, 30.5, 7.6 and 5.9 square feet per 100 square yards.

In addition, fringed sage *(Artemisia frigida)*, was present in appreciable amounts. This species had a density of only 4.9 plants and an average cover of 1.4 square feet per 100 square yards. Only two other true suffrutescents, iron plant *(Aplopappus armeriodies)* and yellow eriogonum *(Eriogonum flavum)* were encountered, but both were insignificant in the type.

A large number of herbaceous species occurred in the type, but only a few were of significance. The type as a whole has a mean herbaceous percentage cover of only 4.35 percent with the major species, thickspike wheatgrass *(Agropyron dasystachyum)* composing 47 percent of the total herbaceous cover. Poverty weed *(Iva axillaris)*, red mallow *(Sphaeralcea coccinea)*, saltgrass *(Distichlis stricta)*, needle-and-thread *(Stipa comata)*, Hood's phlox *(Phlox hoodii,* and butte primrose *(Oenothera caespitosa)*, in order of importance, composed 20.7 percent with all other herbaceous species composing 21.5 percent of the total herbaceous cover. Squirreltail *(Sitanion hystrix)*, an important and charactistic grass in the salt-desert shrub type of Utah, Nevada, and Wyoming, was encountered rarely in the type in western North Dakota.

In general, the type as it occurs in western North Dakota is very sparse with species composition varying somewhat in regard to exposure. The relation of this shrubby type, as it occurs in the Badlands of North Dakota, to the salt-desert shrub type as it occurs to the southwest of this region is shown by comparison with representative stands from several localities.

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EFFECTS OF PARACHLOROPHENOXISOBUTYRIC ACID ON ABSCISSION OF DEBLADED PETIOLES OF *PHASEOLUS VULGARIS*

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INTRODUCTION

The antiauxin effects of *p*-chlorophenoxyisobutyric acid (PCIB) were first described by Burstrom (2) in a study of the growth and matabolism of wheat roots. Since then several hypotheses have been proposed to explain the demonstrated antiauxin action of PCIB. In his initial study, Burstrom (2) found that PCIB caused an increase in the rate of elongation of epidermal cells of wheat roots and concluded that its action was opposite to that exerted by indoleacetic acid (IAA), *i.e.*, PCIB slows down the first phase of cell elongation (cell stretching), while accelerating the second (cell wall synthesis). Since the second phase dominates, the overall effect of PCIB is to stimulate root growth. McRae and Bonner (8) have shown that the antiauxin action of PCIB can be described in terms of the methods developed by Michaelis and Menten (9) to analyze enzyme kinetics. They found, by employing the methods used by Lineweaver and Burk (6) to study competitive inhibition in enzyme systems *in vitro*, that PCIB competitively inhibited the growth-promoting action of IAA on oat-coleoptile-section growth. This was explained as a competition for auxin-reactive sites. Fransson (4) has reported that wheat roots contain, in addition to the easily extracted free IAA, a bound form from which free IAA is released. Treatment of wheat roots with PCIB resulted in an increase in the concentration of free IAA, thus providing additional evidence that PCIB competes with IAA. Since it is well known that PCIB stimulates root cell elongation (3), Fransson concluded that the competition between the antiauxin and the natural auxin takes place where auxin exerts its elongation activity and that the free, non-coupled IAA is not directly active in cell elongation.

In the present study the effect of PCIB and IAA, alone and in combination, on the abscission of debladed petioles of *Phaseolus vulgaris* L. was investigated. Evidence has been obtained that IAA under certain conditions may accelerate abscission. Application of IAA to the proximal side of an abscission zone accelerated abscission in explants of bean and cotton (1, 7) and in debladed leaflet pulvini of bean plants in the greenhouse (13). Rossetter and Jacobs (11) demonstrated that intact leaves speed up abscission of nearby debladed petioles by acting as sources of proximal auxin.

In view of the above evidence, an attempt was made to answer the following questions:

1. Does IAA delay abscission when applied to the distal portion of a debladed petiole?
2. Does IAA, when applied to the distal portion of a debladed petiole, act as a proximal source of IAA to nearby debladed petioles and thereby speed up the abscission of these petioles?
3. Does PCIB, an antiauxin, nullify the effects of IAA on abscission?

METHODS AND MATERIALS

The abscission of debladed petioles of *Phaseolus vulgaris* under the influence of exogenously applied IAA, PCIB, and combinations of these substances was investigated. The plants were grown in separate plastic pots in the greenhouse, 10 of them being chosen at random for each treatment. No special control of physical conditions was attempted, but all comparable treatments were made simultaneously and so were subjected to the same fluctuations of the environment.

Forty days after germination the first pair of true leaves of each plant was debladed and the petioles shortened to a uniform length of 2 cm. A drop of lanolin paste containing the test compound or compounds was applied to the cut surface of one of the pair of severed petioles of each plant. The abscission rate of both petioles was observed over a 20-day test period. Following the initial treatment, daily checks on the abscission of petioles were made. Just before recording abscission the pots were gently tapped in order to detach any petiole ready to abscise.

RESULTS

All debladed petioles treated with IAA at their distal tips, alone or in combination with PCIB, remained on the stem for the 20-day treatment period. In contrast, many of the untreated petioles directly opposite those treated abscised (Figure 1). Untreated debladed petioles directly opposite petioles treated with 1% IAA alone abscised within a short time after treatment. All of these petioles had abscised by the fifth day. PCIB applied simutlaneously with IAA greatly reduced abscission of the opposite untreated petioles. This influence of PCIB increased as the concentration of PCIB was increased from a low of 0.1% to a high of 2%.

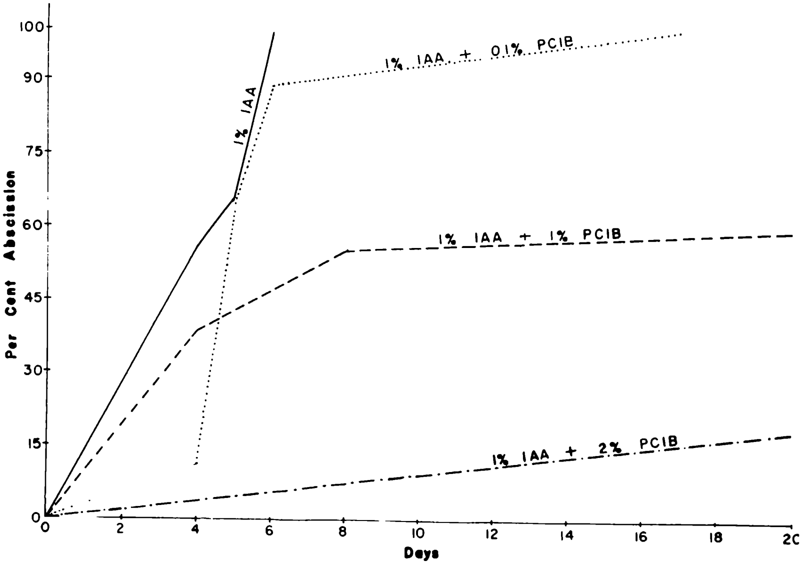

FIGURE 1—Effect of 1% IAA, alone and in combination with different concentrations of PCIB, on the abscission of debladed petioles of *Phaseolus vulgaris* L. as a function of time after treatment. The curves represent the effect of the treated petioles on the abscission of their *opposite untreated* petioles. Each curve represents ten plants (10 pairs of petioles).

A profuse callus was noted on the petioles treated with only IAA. Also a rapid shriveling of the untreated petioles was observed when 1% IAA and 1% IAA with 1% PCIB were used in treating the distal portion of the opposite petiole. It is of particular interest that where 2% PCIB was used in combination with IAA no callus tissue developed and there was no shriveling of the opposite debladed petioles.

DISCUSSION

It has been shown that applied auxin can prevent leaf abscission (5, 10). The conclusion of these studies was that the source of auxin controlling abscission was produced in the leaf blade. However, Shoji, Addicott, and Swets (12), after making measurements and comparisons of auxin concentrations distal and proximal to the abscission zone, suggested that the *auxin gradient* across the abscission zone was more important in the regulation of abscission than the auxin concentration. They found that although the auxin concentration in the blade started falling just before abscission, the concentration in the petiole remained unchanged. It was concluded that a critical auxin concentration gradient across the abscission zone, rather than the auxin concentration itself, may be necessary to prevent abscission. In support of the gradient theory, Addicott and Lynch (1) showed that distal application of auxin to debladed petioles prevented abscission while proximal application induced it.

According to this theory abscission does not occur when the gradient is steep, *i.e.*, when the endogenous auxin concentration is high on the distal side and low on the proximal side of the abscission zone. Ascission occurs when the gradient becomes slight or neutral and is accelerated when the gradient is reversed. The relationships mentioned above are shown diagrammatically in Figure 2.

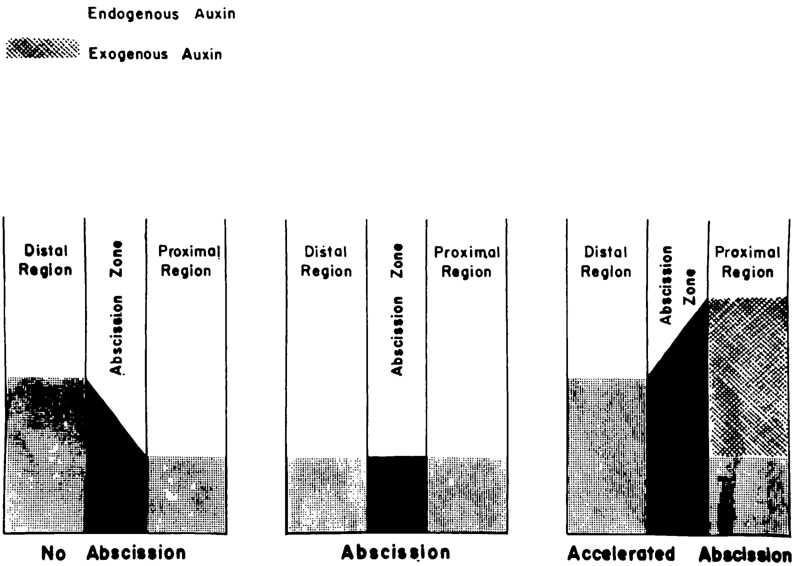

FIGURE 2—Relations between the auxin gradient across the abscission zone and abscission. Taken from Addicott and Lynch (1).

A decrease in the steepness of the auxin gradient across the abscission zone prior to abscission and the acceleration of abscission by proximal application of auxin give support to the gradient theory. Rossetter and Jacobs (11) have found that the intact leaves of Coleus speeded abscission of nearby petioles suggesting that intact leaves of Coleus act as sources of auxin.

In this study, as one might anticipate, auxin supplied to the distal portion of one of a pair of opposite debladed petioles speeded abscission of the other. One can assume that this is due to the transport of IAA from the distal region of the treated petiole to the proximal region of the untreated petiole. It is clear that auxin supplied to the distal portion of a debladed petiole acts in the same manner as an intact leaf, *e.i.*, it speeds abscission of nearby untreated petioles. However, acceleration of abscission of untreated petioles by application of IAA to stumps of nearby debladed petioles was counteracted by PCIB

It is conceivable that the counteraction of the effect of IAA by PCIB in this growth system may be due to a rapid transport of PCIB to the proximal region of the untreated petiole. A lowering of the supply of active IAA through competition with PCIB in the proximal region would retard abscission

There is also the possibility that PCIB may interfere with the transport of IAA and in this way restrict the supply of auxin to the proximal region of the untreated petiole. This would account for the fact that abscission of the treated petioles themselves was not speeded up by the interaction of PCIB and IAA. In other words, the distal portion of the petiole treated with IAA and PCIB would always be sure of having a greater concentration of IAA than the proximal region.

Here we see evidence of PCIB acting at a site (transport of IAA) other than the site of growth action. Since PCIB counteracted the callus-forming action of IAA, a growth process, one could also say that there was interaction of PCIB and IAA at the site of growth action.

SUMMARY

A study was made of the effect of IAA alone, and of the interaction of IAA and PCIB, on the abscission of debladed petioles of *Phaseolus vulgaris* L. All debladed petioles treated with IAA at their distal tip, alone or in combination with PCIB, remained on the stem for the 20-day treatment period. The untreated debladed petioles, directly opposite those treated with 1% IAA alone, abscised within a short time after treatment. PCIB, applied simultaneously with IAA, greatly reduced abscission of the opposite untreated petioles. This influence increased as the concentration of PCIB was increased from 0.1% to 2%. Callus formed on the tip of petioles treated with 1% IAA and a rapid shriveling of the untreated petioles was observed. Application of 2% PCIB simultaneously with 1% IAA counteracted the callus-forming action of IAA and there was no shriveling of the opposite debladed petioles.

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PRELIMINARY STUDIES ON MEGASPOROGENESIS, FERTILIZATION AND EARLY EMBRYOGENY IN MUGO PINE (*PINUS MUGO* TORRAS)

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ABSTRACT

Although the details of the reproductive process is generally well known for the genus *Pinus*, little attention has been given to *Pinus mugo*. This species, which is native to central and southern Europe, has been widely introduced in temperate North America largely as an ornamental, but also has some potential as a shelterbelt species because of its basal branching habit and general hardiness in a variety of habitats. As little is known of the detailed phenology of pollen formation, ovule development, fertilization, embryogeny and seed formation for this species in North America, and more

specifically for this area, the present study was designed to provide such data as obtained from well-grown specimens in the Fargo area.

Microstrobili are produced in sub-apical clusters with microspore formation in mid-May and pollen release during the first week in June with some variation due to local weather conditions. Megastrobili are initiated from sub-apical lateral buds in mid-May and undergo rapid development until early June. At this time the cone scales are slightly separated and the cones are receptive to pollen. The two ovules on the upper surface of each ovuliferous scale undergo slow development during the first summer. Within the nucellus a single megasporocyte undergoes reductional division to form a tetrad of megaspores, and the one furthest removed from the micropyle persists to develop into a multicellular gametophyte before winter dormancy. Growth of the gametophyte is resumed in late May of the following season. Several archegonia (2-6) develop at the micropylar end, although, in exceptional cases, they were also observed deep in the gametophyte and even at the opposite end. Concurrent development of the pollen tubes effect fertilization of most of the egg nuclei in the archegonia about mid-June. Several embryos are commonly developed simultaneously, but dominance is assumed by one with resulting disintegration and absorption of the others; along with the numerous sets of suspensor cells. Associated with the development of one embryo, the gametophyte tissue matures as a food reserve, and the integument differentiates into a firm dry testa. Seeds approach maturity in late July and early August. While each cone has a potential production of 40-50 seeds, a high percentage of these were observed to be abortive for reasons as yet not determined.

XYLEM DIFFERENTIATION IN ROOTS OF *MAGNOLIA GRANDIFLORA* L.

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Early vacuolation and enlargement of the cells located in the center of the stele near the root tip are common ocurrences in most dicot roots. This vacuolation and enlargement normally indicate the initial stages of differentiation of metaxylem elements in the root. In the primary roots of 10 cm long seedling of *Magnolia grandiflora*, the characteristic enlargement does not occur, although these cells show vacuolation. Thus, the parenchyma cells in the center of the stele remain undifferentiated for a considerable distance from the root apex. At a certain level away from the root apex, the entire stele becomes highly vacuolated even in the pericycle and phloem

regions. It seems apparent that meristematic activity in most of the stelar cells has ceased. Partial obliteration of parenchyma cells is easily recognizable in the stele. The obliterated cells are flattened and their walls show a characteristic wavy appearance in cross section. The remaining cells in the stele are living, for nuclei are present, and a few protoxylem elements are also discernible. Although this condition persists up to the level where the hypocotyl begins, gradually towards the base of the root these cells again assume a "normal" shape and manifest a deeply stained quality. In the root of well-developed seedling (24 cm long), near the base of the root or in the lowermost part of the hypocotyl, these cells do eventually differentiate into metazylem elements. The role of the apical meristem in inducing differentiation of the newly formed cells derived from the apical initials was discussed.

A QUANTITATIVE ANALYSIS OF THE PERENNIAL VEGETATION OF THE CENTRAL ARIZONA DESERT

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ABSTRACT

A field study of the distribution patterns of desert perennials in central Arizona was initiated in June and continued through August, 1963. Thirty desert stands were selected throughout Maricopa County, Arizona. In all cases, the stands were relatively undisturbed, except for light grazing in several floodplain areas, and represented valley, bajada, and floodplain physiography. Wash areas, river banks, and slope exposures were sampled within these major topographic regions. Elevations of the thirty stands selected ranged from 950 through 3000 feet above sea level. Where possible, 100 foot elevational belts were represented within this range.

The line strip method was used to determine relative frequency, density, and cover of the perennial species using twenty 10 x 50 foot quadrats. Thus, approximately one quarter acre was completely tallied in each stand. A total of six hundred quadrats were tabulated in the thirty stands.

In addition to elevation recordings, general descriptive notes were made on location, drainage direction, slope exposure, geological features, physical characteristics of the soil, degree of disturbance, and overall vegetational composition in each stand. Where it was possible to recognize annual vegetation during the desert summer, these species were noted. Native wildlife species were also recorded as they were observed in each stand.

Several trends are noticeable. Among these, the influence of elevation on certain species is evident. With other species, broad ecological amplitudes permit rather uniform distribution throughout the region. Vegetation along river banks and wash areas is distinctive. Soil complex, drainage, and slope exposure contribute to phytosociological patterns, and, in some cases, as along river banks and washes in the desert valley, these factors were apart from elevational influences. In other situations, as on slope and mesa areas, these effects become a part of general elevation patterns. Weather Bureau records and county soil maps furnished aid in the interpretation of the data.

Because of extensive land development in central Arizona, the distribution of some native species could someday become quite restricted. This study serves as a permanent record of the vegetation and establishes a foundation for land use practices and watershed studies.

GRAZED AND UNGRAZED WOODLOTS OF THE LOWER SHEYENNE RIVER VALLEY, NORTH DAKOTA

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ABSTRACT

A series of 20 wooded stands was selected during the summer of 1962 along a 90 mile section of the lower Sheyenne River valley, 10 of which were essentially undisturbed and 10 grazed. Each stand was a minimum of 15 acres in size, free from disturbances other than grazing, adequately drained, and bordering the river.

An arbitrary division of each stand into 4 synusiae—mature trees, shrubs and saplings, herbs, and soil—was made for purposes of systematically sampling compositional, structural, and environmental characteristics. The point-centered quarter method, in conjunction with compass lines perpendicular to the river, was employed in sampling the tree and shrub-sapling strata. The importance value, density per acre, and basal area were calculated for each tree species based on 240 individual recordings for each stand. Shrub and sapling quantitative data for each species per stand included importance value, density per acre, average height, average diameter, and percentage cover.

The herbaceous stratum within a stand was sampled with 20 quadrats, 0.25 square meters, placed at 20 pace intervals along a compass line oriented perpendicular to the river. In each quadrats

the plants were counted and recorded by species. Frequency of occurrence within each stand and density per square meter were calculated for each species encountered.

The soil profiles were sampled down to the parent material. Descriptions of each horizon in individual profiles were made in the field and samples collected for laboratory analysis. The upper horizon was analyzed for water retaining capacity (WRC), available soil nutrients, total phosphorus, available ammonium and nitrate, pH, and particle size distribution.

A three dimensional ordination of stands was constructed utilizing combined shrub-sapling and herbaceous data. Significant vegetational and environmental data were plotted on the ordination. A definite separation of the grazed and ungrazed components of the community was shown to exist. Correlation coefficients were calculated for the various combinations of axes in an attempt to derive a method of ordering stands in a linear pattern. A positive correlation was found to exist between the first and third axes of the ordination. A linear regression of the sets of values representing these two axes was calculated thus giving a new base on which the order of stands reflected continuous change from ungrazed to grazed conditions. All data plotted relative to this order of stands show quite convincingly that compositional, structural, and environmental differences exist between grazed and ungrazed stands and the changes are continuous as reflected in the gradient of values.

NINETY YEARS OF VEGETATIONAL CHANGE IN A TOWNSHIP IN SOUTHEASTERN NORTH DAKOTA

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INTRODUCTION

Original survey records of the General Land Office have been used by many workers to reconstruct regional patterns of original vegetation (Cottam, 1949; Fassett, 1944; Finley, 1951; Kenoyer, 1930, 1934; Lutz, 1930; Sears, 1925; Stearns, 1949). Such qualitative and quantitative information as is available in the surveyors' notebooks has been of great value in interpreting changes in vegetational composition and pattern, and in changes in land use under the impact of white settlement. As far as is known, the original vegetation of North Dakota is largely a matter of conjecture, based upon the often vague notes of early explorers, soldiers, Indian traders, and hunters. Hence, a long range study is now planned to utilize the survey to provide a basis for evaluation of both the pre-settlement vegetation and the changes that have occurred in the last ninety years. Botanical nomenclature used in this paper follows Stevens (1963).

THE PRE-SETTLEMENT VEGETATION

Microfilm copies of the notebooks kept by the surveyors who contracted work in the Dakota Territory are housed in the offices of the Water Conservation Commission in the State Capitol at Bismarck. The notebooks themselves are in the General Land Office in Washington, D. C. Surveys were made on a contract basis under directions issued periodically by the office of the Surveyor General of the United States. Surveyors were required to make notes on vegetation, soil, and topography as they ran the township, range and section lines. Bourdo (1956) and Stewart (1935) give comprehensive reviews of survey regulations and the utility of the notebook information for ecological purposes.

Excerpts of ecological interest were made from the survey notebooks covering Helendale Township, T.136 N., R. 52 W., in the extreme northwestern corner of Richland County, North Dakota. The county is bounded on the east by the Red River (the Minnesota border) and on the south by the joint border with South Dakota. It is the most southeastern area of North Dakota and lies in a region covered at various times during the Pleistocene by Glacial Lake Agassiz. The Sheyenne River angles generally northeast through the county and at one time built a large delta where it discharged into Lake Agassiz. Dissections of the delta now form a considerable portion of present day Richland County. Regionally, Richland County lies in a zone of transition between the eastern deciduous forest and the western plains, and prior to settlement, evidently supported a vegetational mosaic of forest, savanna, marsh, and prairie.

The exterior lines of Helendale Township were surveyed by two crews in the fall of 1870. A team led by Rollin J. Reeves completed north and west lines on September 26, 1870, and a second, headed by Miles B. Wooley, completed the south and east boundaries on October 23rd. Interior lines bounding the sections were run by John A. Burback and six men between August 9 and August 16, 1871. As the Sheyenne River runs eastward along the southern edge of the township, the associated forest lands were best noted by the records of the exterior lines, and a large proportion of the forest references are here. The interior of the township had little forested area by comparison, and a combining of the two sets of notes for ecological reconstruction could be misleading. Therefore, some of the extractions from the notebook have been separated according to whether they came from exterior or interior lines.

The pre-settlement vegetation of Helendale Township was predominately prairie, with lesser areas of savanna, forest, marsh, and shrubland (Fig. 1, Table I). Abundant references are made to "prairie" (Table II), but no species of prairie grasses are mentioned in the notes. A tall grass prairie is inferred from the regional location and contemporary studies of virgin remnants. In any event, eighty per cent of the township was prairie in 1871, broken only by scattered areas of trees, a few streams, and the Fort Totten Military Road.

TABLE I

VEGETATIONAL COMPOSITION OF HELENDALE TOWNSHIP, NORTH DAKOTA IN 1871, BASED ON THE ORIGINAL SURVEY NOTES

Vegetation Type	Acres	Per Cent of Township
Tall Grass Prairie	18,662.40	81.00
Savanna	2,103.55	9.13
Forest	1,937.66	8.41
Marsh	251.14	1.09
Shrubland	85.25	.37
Totals	23,040.00	100.00

TABLE II

SUMMARY OF REFERENCES OF ECOLOGICAL INTEREST IN THE ORIGINAL SURVEY NOTEBOOKS COVERING HELENDALE TOWNSHIP, NORTH DAKOTA, DATED 1870 AND 1871.

Numbers in the body of the table indicate the number of times an item was mentioned, separated according to exterior line surveys (1870) and interior line surveys (1871).

VEGETATION	Exterior Lines	Interior Lines	Total
Good Timber or Heavy Timber	8	5	13
Scattering Timber	8	6	14
Prairie	7	13	20
Brush and Undergrowth	1	6	7
Haymarsh	0	4	4
TOPOGRAPHY			
Sandhills and Ridges	4	9	13
Bottomland	2	5	7
Slough or Wet Ground	3	1	4
Land Level	8	29	37
Land Rolling	8	7	15
Land Broken	1	5	6
SOILS			
Soil First Rate (Deep and Rich)	12	23	35
Soil Second Rate	6	6	12
Soil Third Rate (Unfit for Cultivation)	0	3	3
Soil Sandy	1	5	6

FLORA			
Basswood (*Tilia americana*)	0	1	1
Bur Oak (*Quercus macrocarpa*)	1	6	7
Ash (*Fraxinus pensylvanica*)	0	1	1
Cottonwood (*Populus deltoides*)	1	3	4
Hackberry (*Celtis occidentalis*)	0	1	1
Elm (*Ulmus americana*)	0	2	2
Willow (*Salix spp.*)	0	4	4
Hazel (*Corylus americana*)	0	4	4
Prickly Ash (*Zanthoxylum americanum*)	0	1	1
Highbush Cranberry (*Viburnum trilobum*)	0	2	2
Raspberry (*Rubus spp.*)	0	2	2
Plumbrush (*Prunus americana?*)	0	1	1
Strawberry (*Fragaria virginiana*)	0	1	1

Savanna ("scattering timber" as per survey notes) comprised over 2,000 acres of the township in 1871, apparently dominated by Bur Oak (*Quercus macrocarpa* Michx.) with an understory of prairie grasses and forbs. Reference is also made to scattered Cottonwood (*Populus deltoides* Marsh.), normally a bottomland species, but perhaps associated with Bur Oak in the sandhill savannas.

Riverine forests comprised 8.41 per cent of the township, almost 2,000 acres (Table I). Witness trees were to be established at all corners in forest regions, and recorded as to species, size, and distance

TABLE III

SUMMARY OF SPECIES AND SIZES OF TREES NOTED IN THE GENERAL LAND OFFICE SURVEY OF HELENDALE TOWNSHIP, NORTH DAKOTA

Species	Number Measured	Number Referred to	Diameters (inches)	Average Diameter (inches)
Willow (*Salix spp.*)	1	5	6	6
White Oak (*Quercus alba?*)	2	2	10, 12	11
Cottonwood (*Populus deltoides*)	1	5	8	8
Basswood (*Tilia americana*)	1	2	18	18
Green Ash (*Fraxinus pensylvanica*)	2	3	6, 6	6
Elm (*Ulmus americana*)	4	6	10,24,8,10	13
Bur Oak (*Quercus macrocarpa*)	5	12	12,12,15,12,18	14
Hackberry (*Celtis occidentalis*)	0	1	—	—
Totals	16	36		

and direction from the corner. A total of 16 trees was so recorded, two at each of five corners and single trees at six additional corners. Summary information is given in Table III. Distance and direction notes have been used to derive an estimate of original absolute density in forests (Bourdo 1956; Cottam 1949), but due to the extremely small quantitative sample, and the six corners witnessed by only one tree, such a measure was not attempted in this study. Relative density has been computed, however, and gives a rough indication of the proportions of the various forest tree species (Table IV). Bur Oak was undoubtedly a leading dominant, with American Elm (*Ulmus americana* L.) and Green Ash (*Fraxinus pensylvanica* var. *lanceolata* (Borkh.) Sarg.) as prominent associates. Two references are made to measured White Oak (*Quercus alba* L.) although the species is not reported as part of the present flora of the state. It does occur thirty miles east in Minnesota, however. A possibility exists that the species may have been eliminated from the flora of the state by selective cutting of a desirable species by the early settlers. An alternative explanation is that the surveyor made an error in identification. This is unlikely in this instance, however, as the reference to White Oak was made by the same individual that had been making notes on Bur Oak earlier in the survey, and the assumption must be made that he could distinguish the two species.

TABLE IV

COMPARISON OF FOREST COMPOSITION IN HELENDALE TOWNSHIP, NORTH DAKOTA OVER A PERIOD OF 90 YEARS, BASED ON RELATIVE DENSITY OF THE COMPONENT SPECIES.

Data in column (a) (1871) from calculations based on 16 measured trees; those in column (b) based on a total of 36 references in the survey notebooks for the township. Data for 1961 are averages of three stands on a field sample of 160 trees in each stand.

Species	Relative Density (%)		
	1871		1961
	a	b	
Quercus macrocarpa	31.25	33.33	4.9
Quercus alba?	12.50	5.55	——
Ulmus americana	25.00	16.67	24.6
Fraxinus pensylvanica	12.50	8.33	13.4
Populus deltoides	6.25	13.90	——
Tilia americana	6.25	5.55	42.6
Salix spp.	6.25	13.90	——
Celtis occidentalis	——	2.77	1.4
Ostrya virginiana	——	——	3.7
Acer negundo	——	——	9.4
Totals	100.00	100.00	100.0

In addition to tree species measured, Hackberry (*Celtis occidentalis* L.) is mentioned in the summary description of the township as follows.

> "The quality of the land in this township is second rate excepting the southern tier of sections bordering on the Sheyenne River, which is rich bottomland, well watered by spring creeks rising in the Sandhills. Water pure.
>
> Timber: Oak, Elm, Basswood, and Cottonwood and Hackberry, chiefly in the bends of the Sheyenne River.
>
> Undergrowth: Highbush Cranberries, Hazel and Plumbrush and abundances of rasberries (*sic*). Stawberries are found in large quantities throughout the township."
>
> (Signed) John A. Burback

Understory species (Table II) include Willow (*Salix* spp.), Hazel (*Corylus americana* Walt.), Highbush Cranberry (*Viburnum trilobum* Marsh.) and Raspberry (*Rubus* spp.). These species also dominated a small area of shrub- or brushland (about 85 acres) adjacent to the galleria forest in the southeastern portion of the township.

Three marshland depressions, comprising slightly more than one per cent (251 acres) of the land area, were also present in the township 90 years ago.

The only effect of modern man on the landscape of Helendale Township in 1871 was the presence of the military road to Fort Totten referred to earlier. This was a wagon trail used to convey supplies from Minnesota to Fort Totten south of Devil's Lake. It was probably composed of two ruts in the prairie and hence its modification of either the vegetation or the topography was insignificant.

Topography and soils notations (Table II) are of interest for comparative purposes. Burback's summary description (above) classes the soil as second rate, yet the notes at section corners are predominately referring to first rate soils, and most of these corners lie in prairie, not in the bottomlands. Topographically, most of the township was level or rolling, the references to "broken land" concerned mainly with some sandhill areas and the deeply-incised valley of the meandering Sheyenne River.

THE MODERN LAND COVER

Field studies, aerial photographs and U. S. Geological Survey topographic maps with vegetational overprint (both dated 1961) were used to construct a second map of Helendale Township (Fig. 2). It is evident that the vegetation has been drastically altered. The marshes, some scattered groves of Bur Oak and Cottonwood, and the vast majority of the prairie have been completely obliterated since the advent of rural civilization. The prairie areas of 1871 have been replaced by cultivated fields, pastures, farmsteads, shelterbelts and roads. Some 92 per cent of the township has been directly altered by man and his domesticated grazing animals. About 77.5 miles of road are now present in the area, ranging in class from State High-

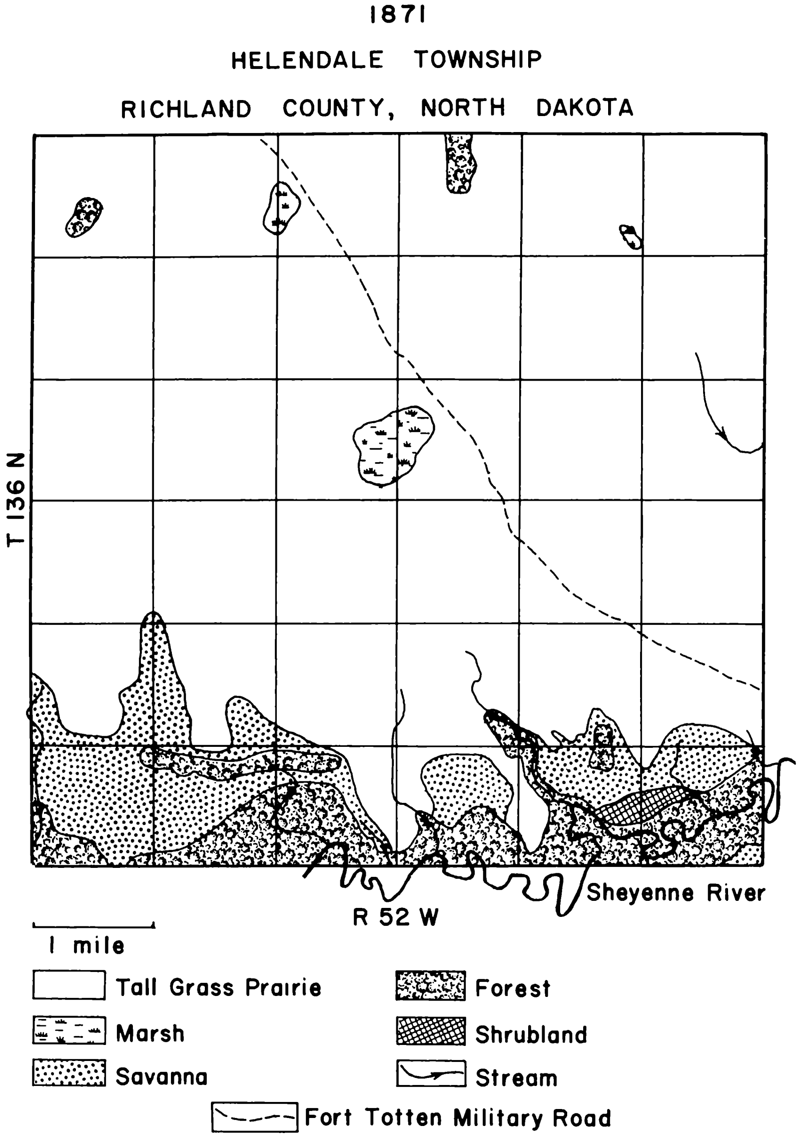

FIGURE 1—Original vegetation of Helendale Township, based on the notebooks of the original land survey.

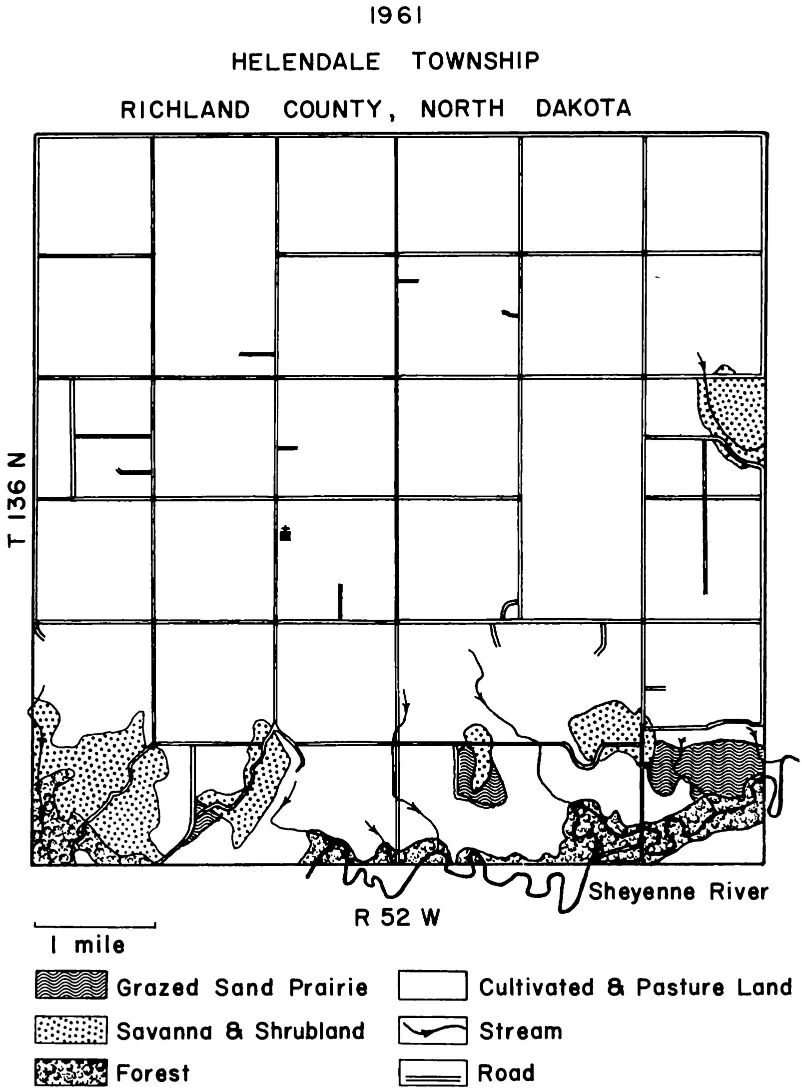

FIGURE 2—Present land cover of Helendale Township, based on field studies, aerial photographs, and topographic maps.

way 46 which forms the north boundary of the township to narrow gravel or dirt roads ending in forest, savanna, field, or pasture. Using twelve feet as a rough average width for all classes, approximately 112.7 acres are now in roads, a fantastic change from the single military road situation of 90 years ago (Table V).

TABLE V

VEGETATIONAL COMPOSITION OF HELENDALE TOWNSHIP, NORTH DAKOTA IN 1961, BASED ON FIELD STUDIES, AERIAL PHOTOGRAPHS, AND US GEOLOGICAL SURVEY TOPOGRAPHIC MAPS.

Vegetation Type	Acres	Per Cent of Township
Cultivated Fields, Pastures, and Farmsteads	20,617.01	89.50
Shelterbelts	503.95	2.18
Roads (all classes)	112.70	.48
Forest	806.40	3.50
Savanna and Shrubland	638.21	2.77
Grazed Sand Prairie	361.73	1.57
Totals	23,040.00	100.00

About 504 acres of the township are in planted shelterbelts and groves surrounding farm buildings. Many of these were planted over thirty years ago and are reaching maturity as patches of forest. A comprehensive survey of the vegetation of these shelterbelts has yet to be made, but many of the tree species were introduced from other regions, even other continents, and many of the understory plants are ruderal species that have yet to be eliminated from the shelterbelt groves through natural ecological processes, if indeed they ever will be.

The forest area has been cut in half since 1871, a considerable percentage reduction, but insignificant when compared to the devastation of the prairie. Present composition is similar to that in 1871, but there is evidence of successional change. The increase in the relative density of Basswood can be explained as a consequence of normal autogenic succession (Table IV). A long-lived, shade tolerant, mesic species, it forms a large component of the climax deciduous forest in western Wisconsin and Minnesota. A second example of possible succession is the presence of Ironwood (*Ostrya virginiana* (Mill.) Koch.). This species is a common understory tree in the terminal (climax) forests of the upper midwest (Curtis 1959) but was not mentioned in the original survey. It seldom reaches a size where it might have been utilized as a witness tree, however, and this may account for its lack of record in 1870 and 1871.

Box Elder (*Acer negundo* L.) is also present in the current forests but unmentioned in the notebook. Although capable of reaching considerable size, it more commonly is a small tree similar to Ironwood and may have been missed for similar reasons. An alternative hypothesis states that Box Elder, being a pioneer or "weed" tree, may have entered the township after initial settlement and since spread to its present status of importance in the Sheyenne forests. Other than these changes, forest composition is remarkably similar over the 90 year span of this study. This is not surprising, however, as these forests must have represented an oasis in the wilderness to the early settlers, who carefully husbanded their primary source of fuel and building materials.

Savanna now occupies only 638 acres in Helendale Township. It is impossible to separate modern savanna areas from shrub-dominated communities on a small scale map such as Figure 2, hence they have been combined. Savanna changes have occurred in two vegetational directions. Heavy grazing has broken the vegetative cover in sandhill areas resulting in a deterioration of the native community to shrub areas and bluegrass (*Poa spp.*) pastures. Shrub communities of today are characterized by varying combinations of Rose (*Rosa spp.*), willow (*Salix spp.*), Wolfberry (*Symphoricarpos occidentalis* Hook.), and Hazel.

Other savannas have matured to forest status with closed canopies and a replacement of the prairie understory by a deciduous forest shrub and herb component. Such forest replacement of savanna is known to occur in the prairie-forest transition region of the United States because of a cessation of natural fires which favored the growth of native grasses and were detrimental to the seedlings and saplings of tree species (Cottam 1949; Curtis 1959). The present savanna on the eastern boundary of the township may represent a "grub prairie" (Curtis 1959) where oaks existed for many years as essentially underground plants and matured as trees only with stoppage of the prairie fires.

About 362 acres of former savanna has been converted to sand prairie. It is possible that a combination of grazing and drought eliminated the trees from these areas, and that during the great drought of the 1930's much of this region was blowing sand with little (if any) vegetative cover. Recolonization since 1940 has been by species indigenous to sandy areas rather than by exotic forms, accounting for the maintenance of these areas by a depauperate native prairie rather than a less well-adapted introduced community.

CONCLUSIONS

It is evident that great changes have taken place in the vegetation cover of Helendale Township during the last 90 years. Almost total destruction of the former grassland has been accompanied by diminution of savanna and forest vegetation. Most of the changes in the landscape are attributable to the influence of the American farmer, including his agricultural practices and animal husbandry, his realistic needs for fuel and building materials from the forests and savannas, his protectional needs from shelterbelts, and his sincere though often questionable practice of building a road wherever no road now leads.

It should also be evident from the foregoing discussion that the notebooks of the original surveyors represent a vast and virtually untapped depository of ecological information concerning North Dakota prior to its carousal in wheat. It is hoped that a knowledge of pre-settlement conditions will soon be available for the entire state; knowledge that will represent a firm scientific foundation on which valid evaluations of agricultural endeavors, soil and watershed preservation plans, wildlife management schemes, urban development, and land reclamation may be based. In the long run, it is only by use of such information that we may someday be able to stand back, survey our works, and proclaim them good.

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GOOSE AND DUCK USE OF GARRISON AND SNAKE CREEK RESERVOIRS, 1955-1963

by George W. Enyeart[1]

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INTRODUCTION

Game and Fish Department personnel began regular waterfowl surveys of Garrison and Snake Creek Reservoirs July 26, 1955. Waterfowl surveys of Missouri River between Garrison Dam and Washburn were started in 1957. The Washburn to Bismarck river segment was censused once in 1958. Surveys on remaining river segments, Bismarck to the South Dakota state line and Williston to the Montana state line, were started in 1959. In 1957, the Department began managing certain suitable Garrison Reservoir lands to improve conditions for resident and migratory game species. This paper summarizes the waterfowl phase of our Garrison Reservoir project to date.

METHODS

Thirteen census segments have been established on Garrison Reservoir, Snake Creek Reservoir, and Missouri River. Spring and fall surveys are made on all segments. Summer waterfowl use counts are conducted on Van Hook Arm, Snake Creek Arm, Snake Creek Game Management Area, and Snake Creek Refuge. All counts taken have been aerial surveys. Three makes of aircraft have been used: Piper 150 Supercub, Cessna 170, and Cessna 172. These surveys are taken from an altitude of 150 to 250 feet. The spring count is made about May 15 and summer counts are conducted the last week of July and the second week of August. Fall surveys begin about September 15 and are flown at approximate ten day intervals until after the fall migration peak has been reached(1).

Selected Garrison Reservoir lands are managed for resident and migratory species through management licenses with the Army Corps of Engineers, a cooperative agreement with the U. S. Fish and Wildlife Service, and through service contracts with local farmers and ranchers. These contracts are set up on a three-quarter crop share for the operator and a one-quarter share for the Game and Fish Department. The Department crop share is used to improve these areas for wildlife and public recreation.

FALL SURVEY RESULTS

Peak fall duck numbers have varied from a low of 35,438 recorded in 1955 to a high of 465,074 birds recorded in 1957 (1). Severe weather conditions may have caused us to miss the 1958 peak count. The recorded peak for 1958 was 101,128 ducks. Low recorded peak counts during 1959-1961 are believed to be a reflection of low flyway populations during these years. In 1962 and 1963, fall counts again increased with 291,488 and 458,125 ducks recorded as peak counts for these respective years. With two exceptions (1955 & 1959) fall duck numbers have reached their peak during the last eight days of October. As the fall migration progresses, the percentage of mallards increases until they account for 90 to 95 per cent of the ducks found on Garrison Reservoir when the fall migration peak is reached.

TABLE I

PEAK FALL DUCK NUMBERS 1955-1963.

Year	Recorded peak	Date peak recorded
1955	35,438	Nov. 3 & 4
1956	59,227	Oct. 29 & 30
1957	465,074	Oct. 28
1958	101,128	Oct. 24
1959	65,777	Oct. 15
1960	121,237	Oct. 25 & 28
1961	72,199	Oct. 25 & 26
1962	291,488	Oct. 25, 26 & 29
1963	458,125	Oct. 28 & 29

Fall duck use of shoreline areas has not been uniform. Areas which have gentle sloping shorelines and an abundance of adjacent cropland are used earliest and heaviest. The Van Hook Arm and Snake Creek segments are examples of this type area. Areas with steeper shorelines see little use until the migration peak is approached. On Garrison Reservoir, a good share of the adjacent cropland in these areas is planted to corn which is grazed off by livestock. Garrison Reservoir areas which have very steep shorelines and relatively little adjacent cropland receive very little waterfowl use. Little duck use is made of Missouri River segments below Garrison Dam until after the fall migration peak is reached and passed. Depending on weather conditions, a majority of the ducks on these segments may winter in North Dakota. The number of ducks wintering on these segments varies from several hundred in severe winters to about 60,000 observed during the 1963-64 winter (2). Generally, duck use of upper Missouri River segments has increased as the reservoir headwaters come into these areas.

Until 1963, the largest fall concentrations of ducks were found on the Van Hook Arm segment of Garrison Reservoir. However, in 1963 some of the more attractive waterfowl areas on the Van Hook Arm were lost to flooding. On the Snake Creek segments, conditions for migrating waterfowl were considerably improved by flooding and land management practices used on lands managed by the North Dakota Game and Fish Department and the Bureau ouf Sport Fisheries and Wildlife. Improved land management practices will increase waterfowl use of the Van Hook area, but Snake Creek segments will continue to support the larger waterfowl concentrations.

To date geese have not made extensive use of Garrison Reservoir. Peak migration numbers have varied from a low of 169 in the fall of 1955 to a high of 1,353 in the fall of 1958. White-fronted geese and Canada-type geese account for most of the geese observed on our census segments. White-fronted geese usually reach their peak number by October 15 and depart shortly thereafter. Chronology of the fall migration peak for Canada geese is comparable to that observed for ducks. However, goose migrations on Garrison Reservoir have not been as predictable as duck migrations. Most of the geese have been observed on the Van Hook Arm, and Snake Creek segments. Geese also make late season use of the Mercer County segment and Missouri River segments below Garrison Dam.

TABLE II

Year	Recorded peak	Date recorded peak
1955	169	Nov. 3, 7, 4
1956	917	Oct. 29 & 30
1957	432	Oct. 28
1958	1353	Oct. 24
1959	289	Oct. 5
1960	648	Sept. 28 & 30
1961	1304	Oct. 9 & 10
1962	1225	Oct. 4, 5, & 8
1963	569	Oct. 16 & 17

SPRING AND SUMMER SURVEY RESULTS

Spring surveys were started in 1957 and are conducted on all segments. Spring geese numbers have varied from one observed in 1960 to 39 observed in 1957. One goose brood was observed on the Missouri River west of Williston in 1963. Duck numbers have varied from a low of 3956 recorded in 1960 to a high of 15,699 recorded in 1962. Most of the ducks have been observed on the Van Hook Arm, Snake Creek, and the headwaters segments. Table III presents results of spring surveys.

TABLE III
SPRING SURVEY RESULTS, 1956-1963

Year	Geese	Ducks
1957	39	6490
1958	21	7416
1959	2	9803
1960	1	3956
1961	6	6385
1962	10	15,699
1963	6 (3 young)	15,645

Early morning summer counts have been made on Van Hook Arm and Snake Creek segments only. Additional segments were surveyed but have been discontinued because of the light waterfowl

TABLE IV
SUMMER GOOSE AND DUCK COUNTS, 1955-1963

Year	Geese	Ducks	Duck Broods	Date
1955	0	5585	26	July 26
	0	13,377	73	August 16 & 17
1956		NO COUNTS		
1957	0	17,634	42	August 1 & 2
	0	43,235	117	August 14 & 15
1958	0	7541	57	July 31 & Aug. 1
	0	13,331	33	August 17 & 18
1959	0	18,186	93	August 3 & 4
	0	19,052	93	August 17 & 18
1960	0	11,943	177	July 27 & 28
	0	25,066	170	August 10 & 11
1961	0	41,744	81	July 19 & 20
	0	11,693	56	August 16 & 18
1962	0	18,011	123	July 27 & 28
	2	47,934	111	August 13 & 14
1963	5	48,269	265	July 22 & 23
	0	73,939	422	August 7 & 8

use found on these segments. Originally, four summer counts were made on each segment. However, peak summer use occurs during the period of late July to mid-August. Therefore, summer use surveys have been reduced to a late July and a mid-August count on each segment until 1963, the Van Hook Arm segment supported the largest summer duck concentrations. During 1963, flooding improved duck habitat conditions on the Snake Creek segments while the Van Hook segment lost duck habitat to flooding. Summer duck use of censused segments has increased markedly during the past two years. Summer use of these segments is presented in Table IV.

LAND MANAGEMENT PRACTICES

In 1957, the North Dakota Game and Fish Department began managing suitable Garrison Reservoir shoreline areas to improve habitat conditions for both resident and migratory species, to allieviate duck depredation problems on adjacent private lands, and to improve Garrison Reservoir as an outdoor recreation area. We started with three game management areas and now manage twelve such areas at various locations along Garrison Reservoir shorelines.

With the large number of ducks using Garrison Reservoir, a serious duck depredation problem can develop during late summer and early fall. This was particularly true in 1963. We have used techniques to hold these ducks on our management areas during the small grain harvest season. Planting small grains so that they are flooded during the harvest season has been our most successful technique. Swathing and stacking barley in small shock sized piles near water or leaving barley in the swath near water also create good duck holding areas. Later in the season, ducks make extensive use of flooded, picked, and grazed corn fields. Our most spectacular duck use has occurred on stacked barley (small and picked corn fields. From a recreational viewpoint, grazed corn fields provide more hours of recreation. Use of the preceeding practices held feeding ducks on the Snake Creek segments until September 22 in 1963 (3). Ducks used these segments after this date but did not go out onto private lands in any number before this date. The duck use season on our game management areas can also be extended by restricting cars to parking lots where vehicular traffic becomes a problem during the waterfowl hunting season.

GOOSE NESTING PROJECT

Early work in the state of Washington (4) and more recent work in Montana (5) and Missouri (6) has indicated nesting goose populations may be benefited through the use of artificial nesting platforms. Field work on the Yellowstone and Missouri Rivers has revealed a small nesting population of Canada geese on these rivers. In an effort to increase this wild population of geese, the North Dakota Game and Fish Department began constructing artificial nesting platforms on these rivers in the spring of 1963 (7). Fifty-eight wash tubs were fastened to flooded trees in the headwaters area of Garrison Reservoir. An aerial survey of these tubs in May 1963, indicated three of the tubs may have been used. A check of the tubs during the winter of 1963-64 revealed egg shell fragments in one of the tubs located in an area where a family group of geese had been reported during the previous summer. During the fall and winter of 1963-64, twenty-five additional tub platforms were constructed on the Missouri River banks west of Williston, and 25 hay bale platforms supported by four steel fence posts were erected on sandbars of Yellowstone River. We have also constructed 38 hay bale platforms on our Wolf Creek and Snake Creek game management areas. Ten nesting mounds consisting of four bales of native hay set on the ground have also been put in place on the latter two areas. It will be several years before success of our program can be determined, but we are hopeful of increasing the number of wild nesting geese in North Dakota through these techniques.

SUMMARY

1. Duck and goose use of Garrison and Snake Creek Reservoirs have been recorded through a series of aerial surveys beginning in 1955.
2. Peak fall duck numbers have varied from a low of 35,438 recorded in 1955 to a high of 465,074 recorded in 1957.
3. Fall duck numbers usually reach their peak during the last eight days of October.
4. Fall use of shoreline areas has not been uniform. Gentle sloping

shorelines with an abundance of adjacent croplands are used earliest and heaviest.

5. Largest fall concentrations of waterfowl are found on the Van Hook Arm and Snake Creek segments of Garrison Reservoir.
6. Geese have not made extensive use of Garrison Reservoir.
7. Spring duck numbers on Garrison Reservoir have varied from a low of 3956 in 1960 to a high of 15,699 in 1962.
8. Summer duck use of Van Hook Arm and Snake Creek segments has markedly increased during the past two years.
9. The North Dakota Game and Fish Department manages 12 game management areas on Garrison Reservoir in cooperation with the U.S.A. Corps of Engineers.
10. These areas are managed to benefit both resident and migratory species, alleviate duck depredation problems, and to improve outdoor recreation opportunities on Garrison Reservoir.
11. Management practices used on these areas have been successful in holding feeding waterfowl on these units during the small grain harvest.
12. In all, 156 artificial nesting platforms have been constructed for Canada geese. Success of this endeavor will not be determined for several years.

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APOMIXIS IN A *BOTHRIOCHLOA* CROSS[1]

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ABSTRACT

The grass genus *Bothriochloa* has chromosome numbers ranging from $2n = 20$ to $2n = 180$ and is found in Australia, Asia, Europe, Africa, South and North America. Diploids, some hexaploids, and other higher ploid species reproduce sexually. Most of the tetraploids, pentaploids, and hexaploids reproduce apomictically. A sexual form of *B. grahamii* ($2n = 40$) and an apomictic *B. kuntzeana* ($2n = 40$) was crossed to study the mode of inheritance of apomixis and relationship of the species. Cytological studies were conducted from developing microsporocytes and the F_1, F_2 plants studied were found to possess $2n = 40$ chromosomes. Morphological studies were conducted in the field and in the herbarium. All the F_2 plants were maternal in their morphological and cytological behavior, and this is believed to be due to the apomictic behavior of the F_1. It is concluded that the apomictic mode of reproduction is dominant over sexual mode of reproduction at the same ploidy level. The two species appear to be closely related and are found in India.

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SODIUM TRANSPORT BY THE SKIN OF AMPHIBIAN SPECIES FROM DIFFERENT HABITATS

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ABSTRACT

Amphibians have been shown to exhibit interspecific differences in rates of hydration and these differences have been correlated with the ecologies of the species studied: terrestrial species absorb water faster than aquatic species. Also, when held under conditions of prolonged hydration stress, terrestrial species do not live as long as do aquatic species. If salts are regulated at some steady state concentration, it might be expected *a priori* that amphibians would also exhibit differences in rate of sodium uptake.

The net rate of sodium transport was measured in isolated skin preparations for five species of anuran amphibians from different habitats. The five species included in the present study were: *Rana septentrionalis* Baird, *R. pipiens* Schreber, *Hyla versicolor* LeConte, *Bufo hemiophrys* Cope, and *B. americanus* Holbrook. The results have indicated a lack of significant differences in active sodium flux for these species. The mean rate of sodium flux (all species) was 1.05 micro equivalents of solium per cm^2 surface per hour at 25° C.

DISTRIBUTION OF AQUATIC VEGETATION AS MEASURED BY LINE-INTERCEPT WITH SCUBA[1]

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ABSTRACT

The aquatic vegetation of Long Lake (T 144 N, R 36, 37 W) in northwestern Minnesota was surveyed by means of line transects run underwater with SCUBA apparatus. Eight stations were established about the lake and twelve transects were run from each station. The transects were run along depth contours at one-half meter depth and at one-meter intervals from one through eleven meters depth. No vegetation was observed deeper than eleven meters depth. Each transect was twenty meters in length and the presence of plant species was recorded at intervals of one-third meter. The data were recorded underwater by writing on a sheet of aluminum foil held in a metal clipboard.

This survey method has provided information of (1) the general distribution of aquatic vegetation, (2) relative abundance of various species and (3) inter-species and environmental correlations. The results indicated that the vegetation is not randomly distributed and that there is a definite difference between stations of the north and south slopes of the lake bottom. Further applications of this survey technique to the analysis of aquatic distributions were discussed.

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THE REACTION OF TITANIUM (IV) CHLORIDE WITH SOME ALDEHYDES

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Titanium (IV) chloride reacts exothermally with benzaldehyde, *m*-nitrobenzaldehyde, and cinnamaldehyde to yield solid addition complexes in the ratio of two moles of aldehyde to one mole of titanium (IV) chloride. The 2:1 ratio indicates that the titanium atom in these reactions has attained its most prevalent coordination number of six.

Previously reported reactions of aldehydes with titanium (IV) halides have pertained to isolated cases in extensive organo-titanium investigations. In 1926 Scagliarini and Tartarini reported the formation of a 2:1 addition complex of heliotropin and titanium (IV) chloride in chloroform solution (1). The complex was isolated as bright red crystals, but they were unstable in air. A heat evolution of 35.0 kcal/mole was reported in the formation of a 2:1 addition complex of benzaldehyde and titanium (IV) chloride (2). The reaction of acetaldehyde with titanium (IV) fluoride yielded a 1:1 addition complex (3) and the reaction of furfural with titanium (IV) chloride also yielded a 1:1 addition complex (4).

During the course of our work studying the reactions of titanium (IV) chloride with benzaldehyde, *m*-nitrobenzaldehyde, and cinnamaldehyde, no hydrogen chloride was evolved, apparently indicating that no condensation occurred. Upon hydrolysis of the complexes, TiO_2 precipitated and the original aldehyde was quantitatively obtained. The reaction apparently proceeds as follows:

$$TiCl_4 + 2RCHO \longrightarrow (2{:}1 \text{ addition complex}) \longrightarrow TiO_2 + 4\,Cl^- + 2\,RCHO$$

Hydrogen chloride was evolved during the reaction of titanium (IV) chloride with vanillin, indicating reaction with the hydroxyl group. Attempts to prepare the 2, 4-dinitrophenylhydrazone derivative of the vanillin — titanium (IV) chloride red-orange solid reaction product yielded the hydrazone of vanillin. Apparently a titanium-oxygen bond was formed during the reaction, but the bond appears to hydrolyze readily under the conditions used in trying to form the hydrazone.

Infrared spectra were taken of the complexes and the amount of shift of the carbonyl absorption band to shorter frequencies were noted. If strong coordination bonds were formed between the carbonyl oxygens and the titanium, the resultant shift of the carbonyl absorption band would be in the order of 100 cm^{-1} (5), instead of the range 8-20 cm^{-1} observed experimentally. In previous work, a shift of from 10-60 cm^{-1} was observed for the carbonyl bands in the formation of 1:1 ketone-titanium (IV) chloride complexes (6).

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CATECHOLS FROM THE CARBONIZATION OF NORTH DAKOTA LIGNITE

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INTRODUCTION

Catechol and its methyl homologs occur in a great variety of materials. These include tea, wine, grapes and other fruit, wood products, and coal tar. Of these materials only the last two have been considered as commercial sources for catechol. For example, extensive investigation has been carried out on the destructive distillation of lignin, wood, and bark as possible sources of catechols (18).

Low-temperature carbonization of coal, especially low-rank coals, is a particularly attractive source of catechols if the process can be justified by the improved fuel characteristics of the resulting char, the tar then being treated as a by-product. Also, since catechols are water-soluble, they are found not only in the tar but in the aqueous liquor as well, from which they are easily recovered.

Recovery of catechol from tar and process water has been considered feasible even when relatively low concentrations of the chemical are found. For example, Shono (16) proposes recovery of catechol from aqueous liquor, 50 liters of which contain only 220 grams catechol or 0.5%. Williams (19) obtained only 13 grams refined catechol from almost 2 kilograms raw tar (0.6%), although he obtained an equal amount of catechol from the associated liquor.

It is the purpose of this paper to show that the low-temperature carbonization products of North Dakota lignite are a rich source of catechol and its homologs.

ANALYTICAL METHODS

Catechols were found in North Dakota lignite tar early in our assay program. In the acid portion of this tar, catechol and the 3- and 4-methyl homologs are exceeded in concentration only by phenol. The relative abundance of these chemicals resulted in their crystallization from distilled fractions of the tar acids and prompted more intensive investigation. Several methods for quantitative estimation of the catechols have been used, depending on the type of mixture involved and the accuracy required.

The most accurate and universally applicable method is column partition chromatography as described by Barker and Hollingworth (1). This consists of a stationary phase of 0.1 N HCl on a support of Celite 535. The mobile phase is cyclohexane to which n-butanol is added in 1% increments up to 8%. The effluent is examined by UV spectrophotometry in a very sensitive range so that analysis is possible with quite small samples. This method was developed for analysis of ammoniacal liquors, but with slight variations can be used to determine catechols in whole tar.

A more rapid though less accurate method is gas-liquid chromatography of tar fractions or of extracts from the process water. Quantitative results are difficult with complex mixtures, but narrow-boiling fractions of tar acids and extracts of aqueous solutions can be estimated satisfactorily.

Analysis of Low-Temperature Tar.—As previously mentioned, Williams (19) extracted catechol in the amount of 0.6% of the raw tar. Gluud and Breuer (5) report the same percentage of catechol in low-temperature tar obtained from a Saar coal. For comparison, low-temperature tar from North Dakota lignite contains 1.0% catechol and, in addition, 2.8% 4-methyl catechol and 1.5% 3-methyl catechol.

Subsequent analysis of tar distillates indicated that the catechols were subject to degradation under conditions of atmospheric distillation. Distilling under vacuum greatly reduced this effect and resulted in considerable enrichment of catechols in the distillates. Tar, vacuum-distilled at 3mm Hg to the equivalent of 240°C at atmospheric pressure, gave an overhead representing 11% of the tar: this distillate contained 8% catechol, 9% 4-methyl catechol, and 0.6% 3-methyl catechol. Based on the figures given previously for gross tar, it would appear that there is too much 4-methyl catechol in this distillate and not enough 3-methyl catechol; however, it should be kept in mind that 3-substituted catechols are notoriously unstable and rearrangement or degradation of this material is the rule rather than the exception.

Analysis of Carbonizer Process Water.—Catechol and 4-methyl catechol are very soluble in water; 3-methyl catechol, although less soluble than these, can still be classed as soluble. For this reason, virtually every aqueous solution that comes in contact with the tar will extract catechols. This is a recognized fact, as evident from the number of investigations concerning recovery of chemicals from aqueous liquor. Subbarao, et al., (17) extracted carbonized process water with butyl acetate and found, in addition to catechols, a numer of other phenolics. Morgan and Pettet (11) extracted up to 1.7 grams catechol per liter of low-temperature carbonization liquid. Similar results have been obtained by others (6, 4, 13).

Butyl acetate extraction was carried out on aqueous liquor from a carbonizer operated at Dickinson, North Dakota. This is a Lurgi-type carbonizer used for production of char briquets from North Dakota lignite (3,7). Exhaustive extraction of this liquid yielded 37 grams of extract per liter of process water. This extract, analyzed by gas-liquid chromatography, consisted principally of catechol, 3- and 4-methyl catechols with some phenol, cresols, and xylenols also present. Quantitative estimation from the chromatogram indicated 41% catechol, 19% 4-methyl catechol, and 9% 3-methyl catechol in the mixture. Thus a liter of process water contains about 15 grams catechol, 7 grams 4-methyl catechol, and 3 grams 3-methyl catechol. This aqueous liquor is somewhat richer in catechols than those mentioned in the literature, the extraction of which is regarded as commercially feasible.

YIELDS OF CATECHOLS

Carbonization of a ton of lignite at the Dickinson plant yields 5 gallons (42 pounds) of tar (70% of assay) (3), and 20 gallons of process water 8). Thus from the previous figures we can expect one ton of lignite to yield 0.42 pounds catechol from the tar and 2.5 pounds catechol from the process water for a total of 2.9 pounds catechol per ton of lignite carbonized. Similarly, a ton of lignite should yield 2.2 pounds of 4-methyl catechol and 1.1 pounds of 3-methyl catechol. The amount of catechols actually recovered depends, of course, on the efficiency of the extraction process. While these figures represent the amount of catechols available, it may not be economically feasible to extract the entire amount.

RECOVERY METHODS

The phenosolvan process (17, 12, 15, 10) is the most popular method currently used to extract waste waters. It consists of countercurrent extraction with an ester mixture composed primarily of butyl acetate. Entrained ester is recovered by steam distillation, making the process highly efficient.

This method of recovery can also be used on solutions of buffers used to extract catechols from the tar (19, 20).

Others have extracted aqueous liquors with ether (16,5) "ketone oil" (6), and cresilic acid (11).

Another interesting method of recovery involves adsorption of phenolics from liquor by activated charcoal (14) or coke dust (4). The phenolics were then desorbed by steam. Catechols were also recovered by precipitation of their lead salts before adsorption of the monohydroxy phenols on charcoal (14).

Separation of Recovered Catechols—Although catechol and 3-methyl catechol boil at about the same temperature (240° and 241° C respectively) at atmospheric pressure, they are separable by distillation under reduced pressure. Thus at 20 mm Hg, 3-methyl catechol has a boiling point of 129° C, whereas that of catechol is 134° and that of 4-methyl catechol is 147° (9).

Separation of catechol and 3-methyl catchol can also be achieved under certain conditions by precipitation of lead salts (2). Similarly 3-methyl and 4-methyl catechols can be separated by precipitation of the 4-methyl catechol with ammoniacal calcium chloride (9).

CONCLUSION

It has been shown that the low-temperature carbonization products of North Dakota lignite are a rich source of catechol, 3-methyl catechol, and 4-methyl catechol. Proven methods are available for recovery and separation of these valuable chemicals. Their potential abundance could result in development of many new uses and in expansion of present applications such as medicinals, antioxidants, plastics, glues, ore flotation, photo developers, and herbicides.

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SOME OBSERVATIONS ON THE DISSOCIATION OF N_2O_4

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ABSTRACT

Pressure measurements (1) of gaseous nitrogen tetroxide have led to the generally accepted conclusion that:

(1) $N_20_4 \leftrightarrows 2\ N0_2$ This reaction is complete to the right at 150°C.

(2) $N0_2 \leftrightarrows N0 + \frac{1}{2}\ 0_2$ This reaction begins at 150° and goes to completion at above 600°. The average molecular weight of the gases in the equilibrium mixture at 150° is in fact 46.

The observations noted below lead us to these modifications:

(3) $N_20_4 \leftrightarrows 2\ N0_2$ Complete only at 250° to 300°.

(4) $N0_2 \leftrightarrows N0 + \frac{1}{2}\ 0_2$ This reaction begins at 50° to 60°.

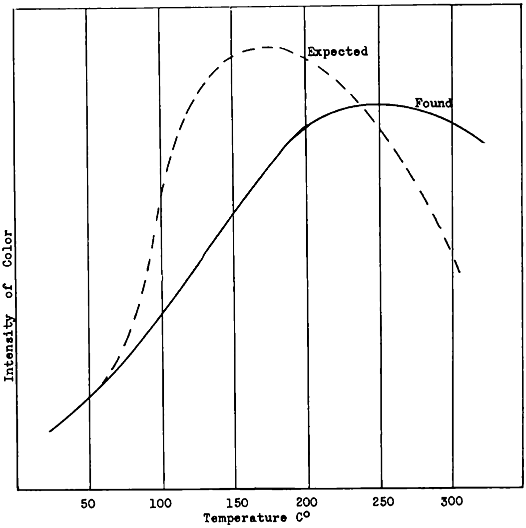

FIGURE 1

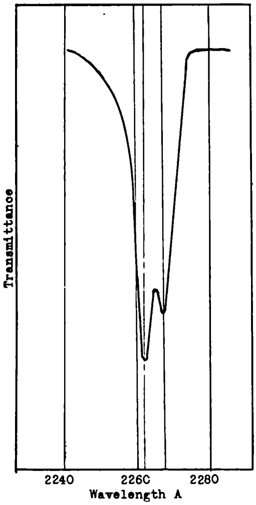

FIGURE 2

DISCUSSION OF FIGURES

Figure 1. Because all the gases of equations 1 and 2 are colorless except $N0_2$, it was to be expected that as the temperature is increased

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[1] Graduate Assistants: Mac Rhoades, William Strieb, Joseph Siefker and Cortes Perry.

upon a sample of these gases, the intensity of the brown color of the $N0_2$ should reach a maximum at 150° as shown in the dotted line. Measurements with a photo-electric cell found a maximum only at 250° to 300°. The concentration (color) of $N0_2$ continues to increase up to 250°, and since its formation is from the dissociation of N_20_4, all the molecules of N_20_4 can not be dissociated at 150° as indicated by equation 1. The average molecular weight at 150° is 46, but the gas still contains molecules of weight 92; therefore there must be light molecules present, these being $N0$ and 0_2. The "expected" and "found" curves of the figure begin to deviate at about 60°.

Figure 2. This figure shows the spectrum (2) of pure $N0$ at wavelengths between 2240 A and 2280 A, showing two sharp minima at 2261.5 A and 2267.8 A.

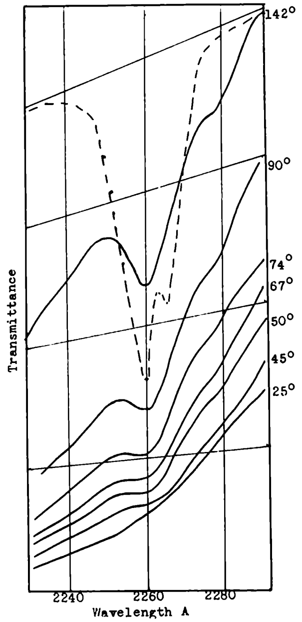

FIGURE 3

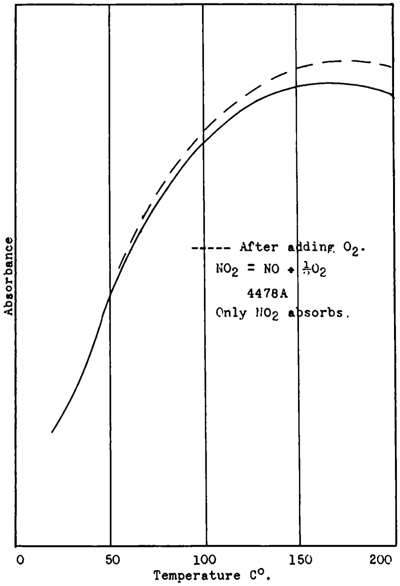

FIGURE 4

Figure 3. This figure is of a series of spectra of N_20_4-$N0_2$ gases as the temperature is raised from 25° to 142°. The dotted line repeats the spectrum of $N0$ from figure 2. At 25° the spectrum over 2261 A is almost a straight line. At 142° the spectrum shows a very deep decrease in the transmittance at 2261 A, indicating a considerable concentration of $N0$. The first indication of such a dip appears at about 50° to 60°.

Figure 4. When the temperature is high enough to form $N0$ from the decomposition of $N0_2$ as represented in equation 4, the addition

of O_2 would increase the concentration of NO_2. The O_2 will have no effect until NO is present. Typical results are shown in figure 4. Absorbance in this figure is due to NO_2 because at the wavelength used, 4478 A, it is the only absorbing substance present (3). An increase of NO_2 after the addition of O_2 is shown by the dotted line. Note that the first showing of an effect occurs at about 60°.

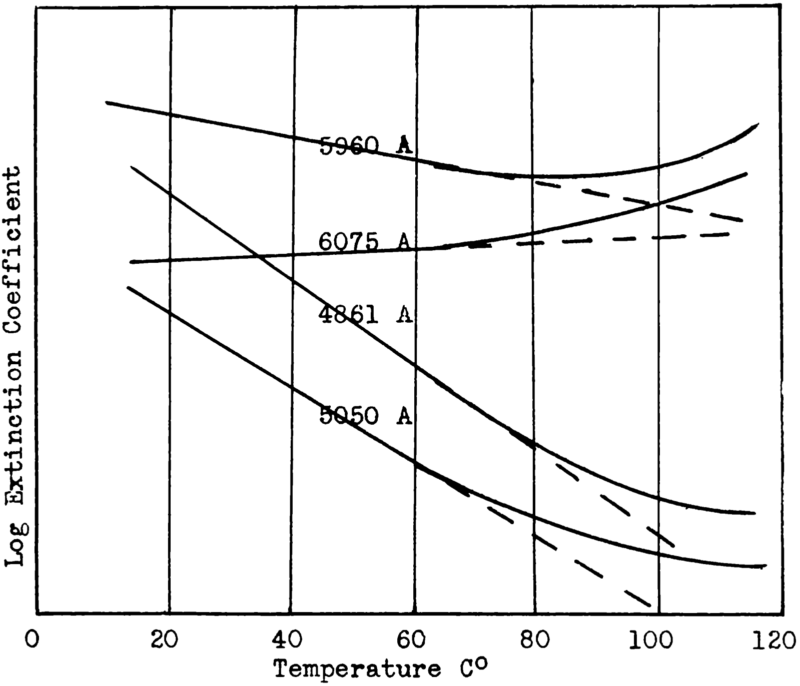

FIGURE 5

Figure 5. The values of the full line curves were all obtained by the classical assumption of equation (1) that the pressure of the gas was due to N_2O_4 and NO_2 molecules only. It is to be noted that the curves are all straight lines up to the temperature of about 60°. It would seem to be unexpected that the absorbance of these two gases for all wavelengths from the ultraviolet of 2250 A to 6075 A should change at the same temperature whether the gas was N_2O_4 or NO_2. Absorbance at 2250 A is due almost entirely to N_2O_4 while above 4000 A it is due to NO_2. (3) The same type of curve occurs in the ultraviolet as at the longer wavelengths shown in the figure.

CONCLUSIONS

Nitrogen tetroxide is not completely dissociated to nitrogen dioxide at 150° though the average molecular weight at that temperature is in fact 46. The decomposition of nitrogen dioxide begins at temperatures lower than 150°. These observations all indicate this lower temperature to be about 50° to 60°.

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SYNTHESIS OF 2-AMINOTHIOPHENE DERIVATIVES

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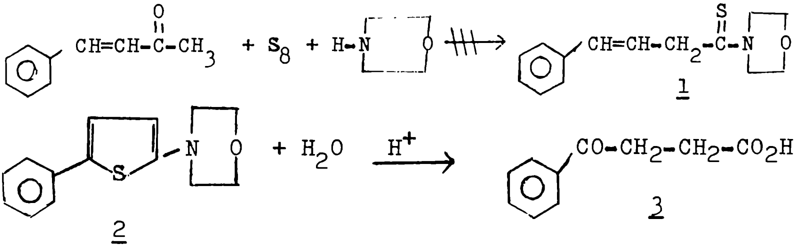
ABSTRACT

An attempt (1) to prepare 4-(4-phenyl-3-thiobutenoyl)-morpholine, 1, according to equation 1 led to a product of unknown structure. The compound has now been shown to be 2-(4-morpholino)-5-phenylthiophene, 2. The infrared and ultra-violet spectra of compound 2 are ambiguous because of the lack of appropriate models. The confirmation of structure rests upon the molecular weight, the NMR spectrum, and synthesis *via* an alternative route.



The presence of the activating nitrogen atom attached to the thiophene nucleus makes the compound susceptible to acid catalyzed hydrolysis, leading to 3-benzoylpropionic acid, 3 (equation 2).

This appears to be the first example of controlled hydrolysis of a thiophene derivative.

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IRON (III) EXTRACTION BY NITROBENZENE-BENZENE MIXTURES[1]

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ABSTRACT

It is well known that iron is extracted from aqueous hydrochloric acid by basic organic solvents as the complex acid, $HFeCl_4$(1). However little is known about the solvation of this species in the organic phase. Some work with dibutyl ether as been reported (2) and, recently, a series of papers (3,4) concerning the solvation of acids by tributyl phosphate has appeared. It is the conclusion of these workers that for strong acids only the extracted hydronium ion is strongly solvated, usually with two or three solvent molecules.

In the present work, the solvation of $HFeCl_4$ by nitrobenzene in the inert diluent benzene has been studied by observing the variation of the distribution ratio with nitrobenzene concentration while all other variables are held constant. The extracted metal species existing in the organic phase at nitrobenzene concentrations greater than 3 M may be represented as $HFeCl_4 - 14\phi NO_2 - xH_2O$ where x is being determined. At nitrobenzene concentrations below approximately 3.0 M the data are inconclusive. The large number of nitrobenzene molecules per metal atom may indicate that the anion is also solvated in this system. Further work, in progress, is necessary to clarify the situation.

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THE STEREOCHEMICAL COURSE OF THE REACTION OF TERTIARY PHOSPHINES AND STILBENE EPOXIDES

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ABSTRACT

Nucleophilic displacement reactions by phosphines on epoxides and episulfides are of interest since each appears to follow a different stereochemical course. Reaction of *cis*-2-butene episulfide and triethoxyphosphine gives *cis*-2-butene (1), while displacement on *cis*-2,3-epoxybutane by tributylphosphine yields mainly *trans*-2-butene (2). This most likely explanation for this difference is that attack occurs on sulfur in the case of the episulfide, while attack occurs on carbon in the case of the epoxide. Results reported recently by Speziale and Bissing (3) have resolved part of the contradictions in the literature. These authors have shown that *cis*-ethyl cinnamate can be isomerized readily in the presence of tertiary phosphine. This isomerization explains results which are difficult to interpret in terms of direct displacement.

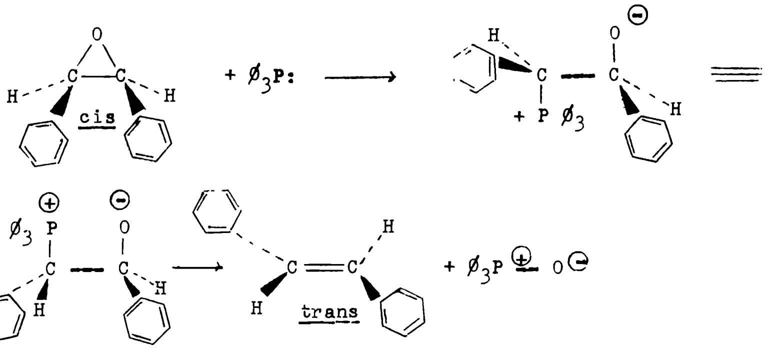

FIGURE 1—Stereochemical course of reaction.

Experimental work in this laboratory has been concerned with the reaction between tertiary phosphines and *cis*- and *trans*-stilbene epoxides. It was hoped that the reaction would proceed by predominant inversion of configuration. If this occurs, then the alkenes formed would have configurations opposite to the epoxides (Figure 1). Three phosphines were studied: tribenzylphosphine, triphenylphosphine and triphenoxyphosphine. The most efficient deoxygenating reagent is tribenzylphosphine. Triphenylphosphine and triphenoxyphosphine are about equally useful under the conditions employed. Although the temperatures employed were high (237°C), it was shown that significant thermal isomerization does not occur under these conditions.

The products of the reaction were partly separated by distillation and identified by their ultraviolet spectra. *Trans* stilbene has an absorption maximum at 295 mμ, while the *cis*-alkene absorbs at about 276 mμ. The intensity of absorption of *cis*-stilbene is also much less. By comparing the distillation product spectra to those of synthetic mixtures, it was possible to tentatively identify the distillation mixtures and to roughly determine the percent of *cis*- and *trans*-stilbenes that they contained. The evidence obtained in this manner indicates that the *trans*-epoxide is converted into an alkene mixture which is about 75% *cis*-stilbene. The *cis* epoxide is converted to a stilbene mixture which is between 75 and 85% *trans*-stilbene.

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THE PROBLEM OF LAKE AGASSIZ

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ABSTRACT

A considerable body of literature dealing with Lake Agassiz is in existence. This paper presents a summary of the pertinent literature dealing with the origin of the lake. An attempt is made to sort fact from theory so we can assess more adequately current work now in progress. Problems still unsolved are stressed with suggestions as to their possible solutions. This paper will serve as a general summary of what is known of this very fascinating geologic province and should be of interest to the layman as well as the professional geologist.

INTRODUCTION

For almost the entire time of my residence in North Dakota (since 1940) I have been interested in Glacial Lake Agassiz. This interest is natural as we live on the plain formed on its bottom; it is the dominant landscape we see every day, and in its fertile soil much of the prosperity of eastern North Dakota is rooted. The very size of the area, to say nothing of its geologic complexity, is a challenge in itself.

ACKNOWLEDGMENTS

I wish sincerely to thank Mr. Samuel J. Tuthill, Geologist with the North Dakota Geological Survey, who read this manuscript critically and supervised the drafting of the illustrations. He is not to be held responsible for any of the conclusions herein presented.

LOCATION AND ASSOCIATION WITH OTHER LAKES

Lake Agassiz plain is located in the eastern quarter of North Dakota, the western and northern part of Minnesota and much of southern and southwestern Manitoba. In total area it covers in excess of 100,000 square miles, and at its maximum probably was the largest body of fresh water in the world.

At its maximum Glacial Lake Agassiz was connected with many other proglacial lakes, some of which survive in the lowest parts of its basin. It was connected first with the Mississippi River system and later with the Great Lakes to the East, and was linked to Glacial Lake Souris and related lakes in Manitoba to the West and North. The larger remnant lakes surviving to this day are Lakes Winnipeg, Manitoba, and Winnipegosis (see figure 1).

NATURE OF THE PROBLEM

When all the literature is reviewed, it may well be wondered why any additional work is needed or even why this review is necessary. However, much of the evidence is unclear; and some appears contradictory, especially when it is not considered in context. In any event, the data on which the various hypotheses are based are fragmentary and scanty at best. In addition, topographic, geologic and pedologic mapping is yet to be completed. Great gaps in our knowledge are particularly distressing in northwestern Minnesota and southern Manitoba where the vegetation and terrain is such that field mapping is a difficult and time consuming task indeed.

LITERATURE SURVEY

Only a brief attempt will be made to review the more pertinent literature dealing with Lake Agassiz.

Dawson (1875) noted the nature of the soil in this flat area and ascribed it to fluvatile or lacustrine origin.

Undoubtedly the writer having the greatest influence on geologic thinking on Lake Agassiz was Upham (1896) whose monumental work is still a standard reference. In summary Upham stated that Glacial Lake Agassiz was caused by a confinement within the Red River Valley of a large body of water by the waning ice of the Des Moines lobe. In the higher stages, this lake drained southward by way of Lakes Traverse and Big Stone to the Minnesota River. Lower stages drained to the east when the level of the lake fell below the Traverse outlet. Lacustrine clays, silts and beach gravels were deposited in this lake. Some of the upper beaches rise in elevation toward the north where they frequently are multiple. To Upham this meant that there was isostatic rebound caused by the melting of the ice which formerly covered this area.

On the sides of the lakes are a number of deltaic deposits most of which are attributable to present day streams which apparently flowed into Glacial Lake Agassiz. These deposits are crossed by the higher beaches indicating that the deltas preceded the beaches.

Upham also believed that the silts in the central part of the basin were fluvatile in orgin, a hypothesis with which I cannot agree. In

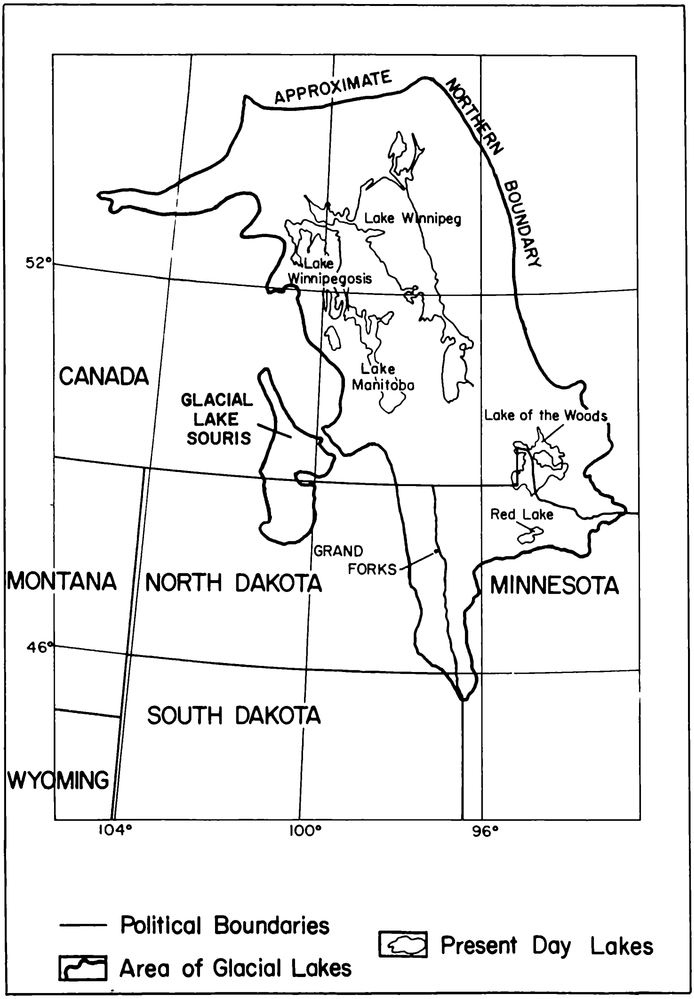

FIGURE 1—Map showing area of Glacial Lake Agassiz and Glacial Lake Souris (modified from Upham, 1896, p. 11).

essence, Upham saw basically only one stage in the lake—namely a falling stage which drained into both the Minnesota River and into Hudson Bay in the lower stages of the lake.

Leverett (1932) is probably the second most important writer on the glacial geology of this area. As to the theories concerned with Glacial Lake Agassiz he follows Upham closely with a few minor difference of opinion. For example he regards the "deltas" as modified outwash. His main contribution deals with the glacial geology of Minnesota outside the Glacial Lake Agassiz area.

Johnston (1916), on the basis of field studies primarily in the Lake of the Woods area in northern Minnesota and southern Manitoba, found evidence of two stages of Glacial Lake Agassiz and felt that the lower deposits represent a rising stage of the lake and that the upper deposits represent a lowering stage. Johnston followed the thinking of Tyrrell (1896) that the junction of the ice sheets from the north and northeast caused the ponding which resulted in Glacial Lake Agassiz (Tyrrell, 1914). Johnston (1921) also believed that these stages were separated by an erosion interval. The second stage of the lake came up to the Campbell beach and declined from there as eastward outlets opened.

Laird (1943) also found evidence of a disconformity between the laminated clays and the overlying silts as did Rominger and Rutledge (1952); although the latter writers also noted dessication surfaces in the lower laminated clay layers as well.

Elson (1958), in a study of the glacial geology of southern Manitoba, paid particular attention to the proglacial lakes associated with the ice sheet in that area. He believes that the later or upper Glacial Lake Agassiz deposits (Lake Agassiz II) were laid down in a lake formed by the advance of the Valders ice sheet.

By far the most unusual theory dealing with Glacial Lake Agassiz is that advanced by Nikiforoff (1947) who believed that the lake was formed by the coalescence of several lakes which began to form in the northern and southern part of the Glacial Lake Agassiz basin at about the same time. The earliest of these was in northeastern Minnesota. He cited the fragmentary nature of the beaches and felt that the declination of these beaches to the south is more apparent than real and could be explained by the fact that the beach fragments are disconnected and represent stages in a portion of the falling lake. Thus, he did away with the necessity of isostatic readjustment to account for the southward declination of the beaches. His theories have not met with wide acceptance.

Thus it can be seen that the theories of the formation of Glacial Lake Agassiz are conflicting particularly as to the number of lake stages represented. Again it should be noted that the conflict of hypotheses represent the fragmentary state of our knowledge.

KNOWN FACTS ABOUT GLACIAL LAKE AGASSIZ

Information available about Glacial Lake Agassiz is derived from several sources. Data on the surface is derived from geologic mapping by the North Dakota Geological Survey, the Minnesota Geological Survey, the United States Geological Survey and the Federal and Provincial Geologic Surveys in Canada. Subsurface data has been derived from the drilling done by the North Dakota State Water Commission and the United States Geological Survey in connection with underground water studies.

The entire lake bed is underlain by glacial till which was largely derived from the underlying bedrock. This till is calcareous in nature and contains large percentages of Cretaceous shale, Paleozoic carbonates, and Precambrian igneous and metamorphic rocks. The till can easily be seen at the edges of the basin where it has been planed by the waves of the lake. It is known to exist in the subsurface below the lake sediments from its presence in well borings made in the center of the lake.

End moraines crossing the lake basin are not common. The main end moraine so situated is the Edinburg-Holt moraine which crosses the axis of the valley just east of Hillsboro, North Dakota. This moraine is indistinct in the center part of the valley where it was probably laid down in water. Its relief in the axis of the Red River Valley is very low and the till has a washed appearance. To the north, near Edinburg, North Dakota, this moraine is quite a distinct topographic feature and was not wave eroded.

The lake sediments are of two main types. The lower, resting on the till is a bedded, laminated clay having a varve-like appearance. I do not think these are necessarily varves if the connotation of annual sedimentation is implied by that term. These laminated clays have suggestions of dessication zones in them according to Rominger and Rutledge (1952). If these authors are correct, at least local exposure of the lake bottom to the air must have occurred. Whether or not this is a lake-wide phenomenon is impossible to determine at present because of a lack of data. These laminated clays are found toward the center of the Glacial Lake Agassiz basin but, at least on the west side, do not extend to the outermost boundary of the lake.

Facies relationships may be present where sands, also rhymically bedded, appear at the same stratigraphic position. Such a condition is well shown in the area just east of the Grand Forks Air Base, west of Grand Forks. Such correlative relationships are hard to establish because of the lack of exposures and of adequate subsurface information.

The upper lake sediment is a brown, silty clay and silt, massively bedded and found even more concentrated in the center of the lake basin than the underlying laminated clay. In other words, the silt does not extend in an east and west direction as far as the laminated clay. The silt is in unconformable relationship with the underlying clay.

This silt was thought by Upham to be fluvatile in origin. However, aquatic forms suggesting lake environment have been found (Tuthill, Clayton and Thompson, 1962), and the distribution is so widespread that stream deposition seems unlikely.

The clays and silt are marked by peculiar lineations which have been explained several ways. Horberg (1951) attributed these to frozen ground structures formed during the retreat of late Wisconsin ice. Colton (1958) believed that the "ridges" formed when the fluctuating lake level fell, allowing ice to rest in the lakes mud thus squeezing the mud into cracks between the ice cakes.

Clayton and others (in press) think that these lineations which consist of both ridges and troughs are due to dragging of icebergs across the shallow lake bottom during the time of the spring breakup.

At best, the ridges are only 3-10 feet high and 75-100 feet wide and are often not only intersecting but curved, as well. They appear to be largely confined to the axial portion of the lake basin coincident with exposures of silt, and to a lesser degree, the exposures of the laminated clay. They are not frequently seen on the planed till surface. These lineations can rarely be seen well on the ground and their distribution has been made known and mapped primarily from aerial photographs. Their main distribution in the axial portion of the basin would suggest that they formed only where the water was shallow and where the sediment type was such that they could readily be preserved.

The beach sediments unconformably overlie the planed till surface as well as the clays and silts mentioned before. They consist largely of gravel; although sand is an important size constituent of practically all deposits. The gravel consists of material derived from the Precambrian and Paleozoic outcrops in Canada as well as the Cretaceous shale on which the glacial till rests in the western part of the lake basin.

The upper and larger beaches are found primarily on the planed till surface. These are the beaches from the Herman to Campbell Beaches, which formed when the lake drained southward through the Traverse outlet. The lower beaches from the Ojata Beach downward rest on the clay and silt in the axial portion of the lake basin. The intermediate beaches (McCauleyville, Blanchard, Hillsboro and Emerado) are mainly on wave-planed till, but conceivably they might rest on clay and silt in places. Our mapping, to date, is not complete enough to be certain of this fact.

As might be expected in an area where the prevailing wind direction during much of the year is from the northwest, the beaches on the east side are more massive than those on the west side of the lake basin. This is most marked in cases of the Herman, Tintah, Norcross, Campbell and McCauleyville Beaches.

All the beaches, especially the upper ones, are multiple in the northern part of the basin, particularly north of the latitude of Grand Forks. The various hypotheses erected to account for this have been reviewed previously. Some of the multiple beaches appear to have much the same general elevation within a given beach complex (i. e., Blanchard beaches). This leads one to believe that some of these may well have been offshore bars or similar features such as the "low and ball" features on the east shore of Lake Michigan which, perhaps, were not above water level during their formation. Therefore, all beach-like structures in Glacial Lake Agassiz may not represent separate stillstands of the lake level. The possibility should also be considered that some of these "bars" could have formed during the construction of the next higher beach which was at the edge of the lake at that time.

The beaches at elevation higher than the Ojata beach are better developed, more continuous, have steeper fronts toward the lake; and in general, suggest that the lake level stood longer at their respective levels than was the case in the beaches below the Emerado Beach. This suggests that the lower beaches represent shorter periods of stable lake level, than the upper beaches.

All the beaches are discontinuous, particularly the lower beaches, which makes correlation somewhat difficult. In places, such as at Mountain, North Dakota, the Campbell Beach is represented only by a wave cut cliff in the till with little or no gravel associated with it.

The beaches also cross the so-called deltas which will be described later. In most instances the beaches largely reworked the material of the deltas into the topographic form of a beach ridge. Thus, the deltas were obviously formed prior to the formation of the beaches. In addition, the beaches down to and including the Emerado Beach cross the Holt-Edinburg moraine indicating that it, too, was formed prior to the beaches crossing it.

On both sides of the Lake Agassiz Basin are large accumulations of sediment, predominantly sand, which have been called deltas by Upham and modified outwash by Leverett. In North Dakota there are three of these deltas, the Pembina, the Elk Valley and the Sheyenne deltas. These are better known and studied than similar accumulations on the east side of Glacial Lake Agassiz.

The northernmost delta in North Dakota, the Pembina, is clearly associated with the Pembina River system, which still exists and undoubtedly was present during the formation of Glacial Lake Agassiz. There is more coarse material in this delta than in any of the others; although probably sand-sized material predominates even here. Fragments of Cretaceous shale are very prominent in the gravels of this delta.

The relationship of these two deltas, and the Edinburg moraine which borders the Elk Valley delta on its northeastern side, would suggest that they formed concurrently when a body of ice stood in the axis of the lake basin and the area to the west was free of ice. On the east and north side of the Pembina delta, the face of the delta is abrupt and suggests an ice contact face. The same type of steep face is present, but less distinct, on the east side of the Elk Valley delta.

The Sheyenne delta in southeastern North Dakota is the largest of the three deltas in the State. It is predominantly a sand delta and is obviously related to the present Sheyenne River which was also active at the time of the formation of Glacial Lake Agassiz. The surface of this delta, and to a lesser extent the surface of the Elk Valley delta, has dune topography. These dunes presently are relatively stable, but, during dry years, they are more mobile. The eastern side of the Sheyenne delta is also more abrupt than the other sides, suggesting ice contact conditions. However, the abrupt face does not always conform to the distribution of the deltaic sediment which, according to a personal communication from Claude Baker, may extend as much as a mile or more eastward of the delta "face." Currently Dr. John Brophy is working on the Sheyenne delta and, as his work progresses, we will know even more about this feature and the relationship the Sheyenne Valley trench has to the delta.

There are other pieces of geologic information available in the Glacial Lake Agassiz Basin which it is not yet possible to relate to the whole picture. One of the unexplained problems is that dealing with occurences of gravel and sand near the axis of the lake basin, which have no readily apparent connection with lake beaches. One of these is found north of Wahpeton near the Red River where, I understand from Claude Baker of the United States Geological Survey, the deposit is not covered by anything but wind blown material and soil. Another such similar deposit is found southeast of Moorhead, Minnesota. East of Reynolds, North Dakota, a deeper gravel and sand body is found in the glacial till. This is also the case in the aquifers immediately west of Fargo and in the West Fargo area. Undoubtedly other similar deposits occur but they have not been found because of the lack of adequate subsurface information. The explanation for these gravel bodies is not immediately forthcoming. They may represent glacial distributary channels in front of an advancing ice sheet or they may be some other form of glaciofluvial deposit.

Another enigma is the presence of what apparently is an older lake deposit below the till west of Fargo. How laterally extensive in a north-south direction this is, we do not know. Is it a pre-early Lake Agassiz stage? No one knows.

RELATIONSHIP OF LAKE AGASSIZ TO THE REGIONAL GLACIAL PICTURE

Evidence gained from drilling for water in the Glacial Lake

Agassiz Basin, as well as in south-central North Dakota, would strongly suggest a preglacial northward and northeastward drainage course for many of the streams in the northern part of the great Plains north of the Black Hills. It is even possible that, at times, the Missouri drainage could have been temporarily diverted into the preglacial Red River or Lake Agassiz. Such a flood of water plus melting of the ice, would provide the water needed to cause the great amount of erosive power required to cut the trench of the Minnesota River south and east of Big Stone Lake. This erosion of the Minnesota River valley would certainly then have taken place during Pleistocene time. It seems likely that this outlet may have been used several times during the Pleistocene, as certainly pre-Wisconsinan glaciers must have come down the Red River Valley several times, and probably each time formed a proglacial lake which drained through this outlet. The glacial deposits as well as the proglacial lake sediments of these advances are not known but may be present at depth.

There seems little doubt that the subsurface evidence points to a north-flowing stream in the Red River Valley during preglacial time. In fact, with all the postulated tributaries this stream must have had, it probably was more than one single valley, more likely being a strath or erosion level developed on the Precambrian, lower Paleozoic limestones and shales and the thick shales of Cretaceous age.

Thus a lowland area was developed during the Tertiary and served as a path for the advancing ice sheet from the north and certainly controlled its course both during its advance as well as its retreat. This is quite evident from a study of the moraines, which have been mapped in Minnesota by Leverett (1932) and in North Dakota by Colton and others (1963). This lobe of the ice has been named the Des Moines lobe or the Minnesota-Iowa lobe. This lobe is only one of several such crenulations of the edge of the Wisconsin ice sheet, but it is one that extended farthest south and was no doubt channeled by the preglacial Red River Valley lowland. Another similar but smaller lobe bordered it on the west has been named the James Lobe.

The preglacial drainage system has been alluded to in some of the preceeding paragraphs. Far too little definite subsurface information is available to make more than a few educated guesses as to its nature and direction. However, it would appear from the available data that the Red River was the main stream flowing northward east of the preglacial Missouri and its immediate tributaries which flowed northeastward near the Montana-North Dakota line into the preglacial Assiniboine River. Probably all the Missouri Slope drainage in North Dakota, other than the Little Missouri River, including the Cannonball, Heart and Knife flowed eastward in preglacial time and joined the north-flowing ancestral Red River. The same is true for the Grand, Moreau and Cheyenne Rivers in northern South Dakota (Flint, 1955). In other words the present Missouri River as we know it is a latecomer in this area. It is not inconceivable that, during the several earlier advances of the ice, the north-flowing Missouri was diverted eastward through the Cannonball drainage so that at some times it, too, may have been diverting part or all of its flow into the Lake Agassiz Basin area. This was the first Missouri River Diversion project and one that was done at no cost to the taxpayers.

The dating of events in years in the Lake Agassiz basin is somewhat startling especially when radiocarbon dates, obtained from the Coteau area of North Dakota, are considered. There are certain assumptions we must make about these dates if we are to come to a theory about the late geologic history of the area. These assumptions are:

1. The Carbon-14 dates are essentially correct and reflect, at least roughly, the solar years which have elapsed since the deposition of the beds which contain the dated organic material.
2. Geologic processes, particularly the recession of the ice and the deposition of glacial materials, took place at a rate much more rapid than is commonly believed.

There are two dates in the Fargo-Moorhead area of approximately 9900 B.P. (before present). There are three dates in the Grand Forks area which are in the range of 10,050-10,960 B.P. The sediments in which they were found appear to be near the top of the bedded clay of Glacial Lake Agassiz I or at the base of the overlying silt which appear to represent Lake Agassiz II. All these dates were derived from wood samples.[1]

The samples cited are as follows:

U. S. G. S.	W-723 - 10,960±300	East of the main gate of Grand Forks Air Base, Grand Forks, North Dakota.
U. S. G. S.	W-900 - 10,080±280	
U. S. G. S.	W-388 - 9930±280	Moorhead, Minnesota
U. S. G. S.	WR-993 - 9900±400	Fargo, North Dakota

If the latest date for the Valders-Two Creeks boundary is 11,000 B. P. as suggested by Frye and Willman (1960), then these dates are all early Valders and post-Two Creeks interval in age. To the west we find that the Streeter moraine has been dated at approximately 11,650 B. P. or Twocreekan in age. This moraine is associated with much dead ice topography on which and associated with which are lake deposits and collapsed outwash deposits containing shells and other dateable material. These deposits have yielded C14 dates of 9,000 to 9,870 B. P. suggesting that the stagnant ice on which these materials were deposited stayed in the area for approximately two thousand years after emplacement of the ice.

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[1] Samples analyzed by Dr. Meyer Rubin, United States Geological Survey, Washington, D. C.

Thus, sometime between 11,650 B.P. and 10,960 B. P. the ice must have entirely ablated over approximately the eastern quarter of North Dakota and the northwestern and western part of Minnesota and parts of southern Manitoba as well. It is my considered opinion that the ice invaded a temperate climate and was relatively thin. How thick the ice was, is not known, but probably it was less than 1,000 feet thick at this time in this part of North Dakota. In this respect the ice probably resembled the conditions in the Prince William Sound area in south-central Alaska today. In addition to melting back rapidly, a great lake had to be established in this basin almost at the same time.

The dates, if true, definitely seem to put the Glacial Lake Agassiz in early Valders time and suggest, in addition, that no moraines of Valders age are known in North Dakota.

THEORY OF THE ORIGIN OF LAKE AGASSIZ

We come now to a theory of the origin of this much discussed lake. In this theory I have tried to take into consideration all the facts as we now know them as well as the theories of other workers.

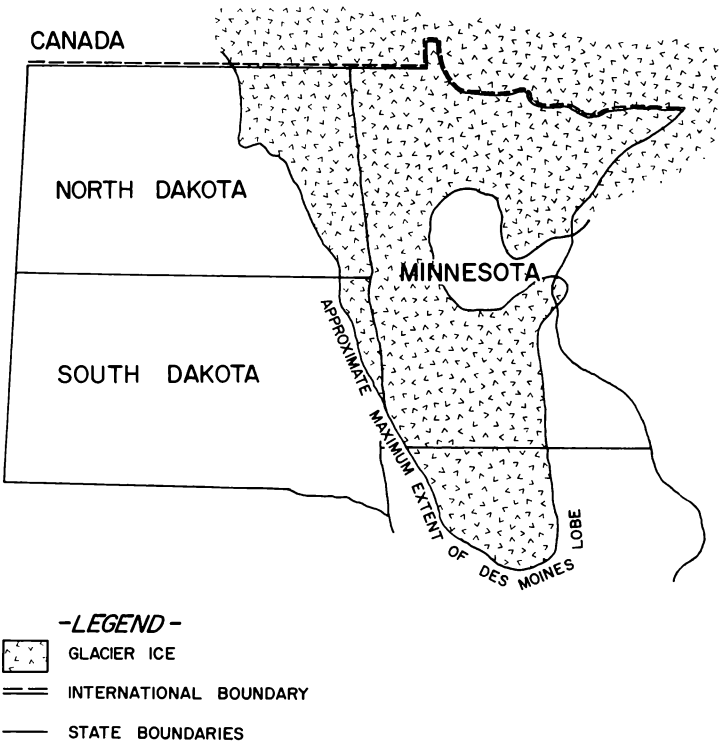

FIGURE 2—Maximum extent of Des Moines Lobe.

An early stage of Lake Agassiz prior to any stages we now know, must have existed during the rising stage of the lake when the ice first began to advance from the north. There could have been several such stages in Pre-Wisconsin time, but little evidence of them is presently known. A date of greater than 36,000 years B. P. determined from samples of peat from Kittson County in northwestern Minnesota, at a depth of 95 feet in Lake Agassiz sediments has been reported. A buried lake deposit below till, which is overlain by Lake Agassiz deposits, has been reported in the Fargo area (Dennis and others, 1949). It is not inconceivable that some of this sediment was brought into the lake from streams draining the Missouri Slope Country and possibly even contributions from the preglacial Missouri, Yellowstone and Little Missouri when their northward drainages were first blocked by the advancing ice. However, as little or no data is available on this matter, the best we can do is acknowledge a present area of ignorance and proceed from there.

The story begins with the advance of the Des Moines Lobe down the axis of the Pre-Wisconsinan Red River Valley lowland to the central part of Iowa (see figure 2). The front ceased moving forward and the entire terminus began to ablate. Our interest begins when the edge of the ice had receded back to the north end of Lake Traverse when a small proglacial lake, Lake Milnor, came into existence and had a very short life (see figure 3).

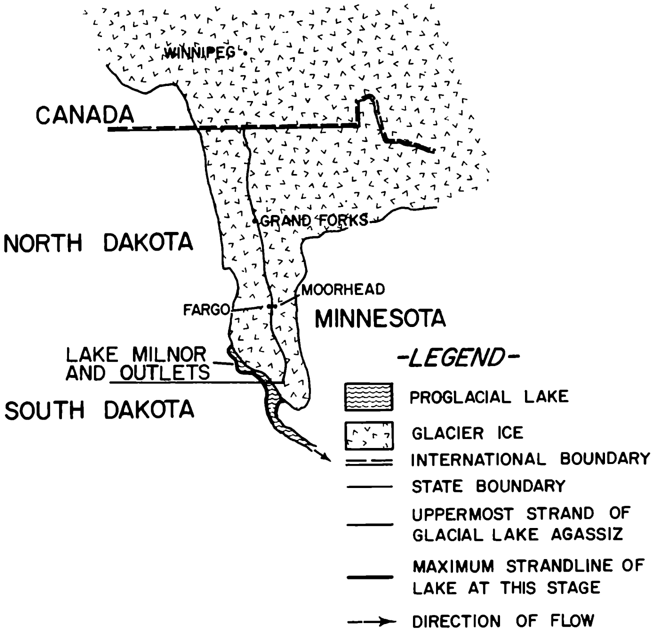

FIGURE 3—Lake Milnor proglacial stage.

Ablation of the ice mass continued and the front receded, at which time Lake Milnor, being higher, drained and a lower proglacial lake, Lake Agassiz I formed (See figure 4). At this time the ice front stood somewhere in the area between Wahpeton and Fargo, but the sides of the ice tongue had melted away from the flanks of the Red River Valley lowland so that there was open water between it and valley walls. At this time the various deltas were formed with the sediment piling up against the sides of the ice body. In this lake, the beaches from the Herman down through the Campbell formed as the lake was draining to the south via Lake Traverse.

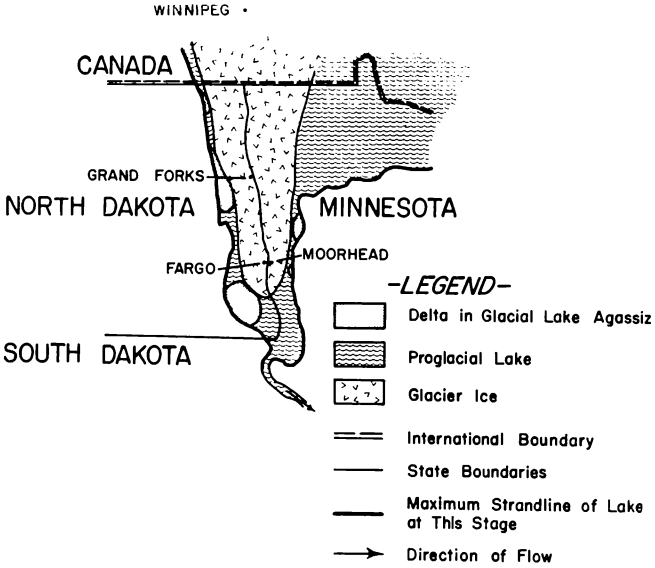

FIGURE 4—Herman to Upper Campbell proglacial lake stage.

The ice front then withdrew rapidly to the present site of the Holt-Edinburg moraine where a stillstand was long enough for the axial portion of the moraine to be deposited in the lake. Part of the moraine near Edinburg, North Dakota, on the west side of the lake basin was not under water at the time of its formation.

At or shortly before the deposition of the Holt-Edinburg moraine, clays began to accumulate in the axial portion of the lake. These are the bedded clays which appear to thicken from south to north and are the main deposits of Lake Agassiz I exclusive of the upper beaches and the deltas. The clays were derived from the reworking of till on the edges of the lake and from material carried in by streams flowing into the lake which flowed through glacial sediments (see figures 5 and 6). The clay deposition continued until the ice had drawn far enough north to open eastern outlets draining

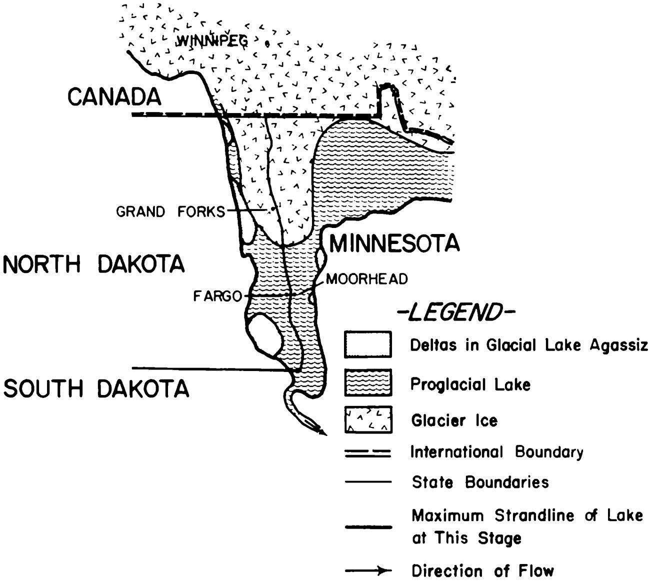

FIGURE 5—Holt-Edinburg moraine stage.

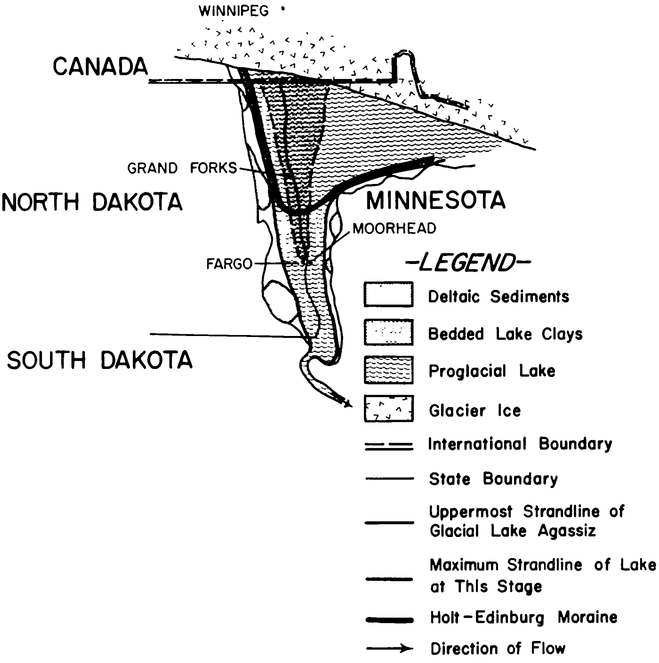

FIGURE 6—Deposits of bedded clays.

the lake in that direction (see figures 6 and 7). Then the lake bottom was dry, the streams extended their courses across it, and the north-flowing drainage of the Red River was re-established. A slight disconformity was thus produced at this time (see figure 8). Apparently, if the radiocarbon dates are valid, all the history of the lake up to this point took place prior to 10,960±300 years B. P.

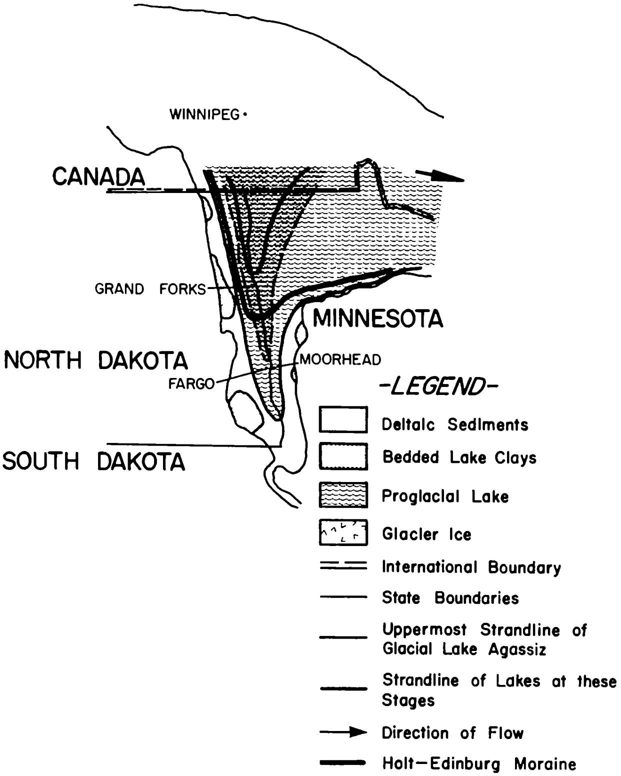

FIGURE 7—Successively lower stages after opening of eastern outlet.

Then the eastward outlet was blocked and the lake rose rapidly and south-flowing drainage by way of the Lake Traverse outlet was probably re-established (see figure 9). If the lake rose again to this outlet, it rose to at least the lower Campbell strandline. This is the most prominent beach level in the Lake Agassiz basin, showing not only prominent beach deposits but also wave cut cliffs. These facts suggest either double occupancy of this position or a longer stand of the lake at this particular level. This rise of water marks the beginning of Lake Agassiz II.

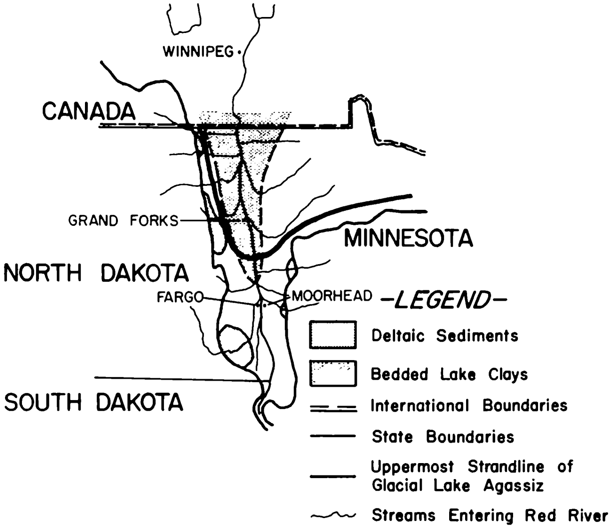

FIGURE 8—Complete drainage of lake with establishment of extended consequent streams, alluviation along streams and development of terraces.

The lake level began to decline from this point. As no beaches below the Campbell enter the Lake Traverse outlet, it can only be assumed that beaches below this were formed when the lake drained to the east.

The most prominent beach below the Campbell is the Emerado Beach. Below this beach, in the axial portions of the basin are upper silts, the deposits of Lake Agassiz II (see figure 10). These sediments are coarser and rest unconformably upon the Lake Agassiz I bedded clays. They are also tan in color, less well bedded, and quite different in appearance to the underlying sediments. From this time on, the lake drained rapidly and the beaches below the Emerado are so poorly developed that it appears the lake level did not remain at any one level very long (see figure 11).

As Upham and others have noted the beaches rise in a northward direction, the upper beaches more rapidly than the lower. Furthermore, the beaches become multiple toward the north. Upham suggests this was caused by isostatic rebound, due to the removal of the weight of the glacier by melting. It would appear this tilting is not an important aspect of the mode of origin of the lake so it will not be discussed in detail as part of this present theory.

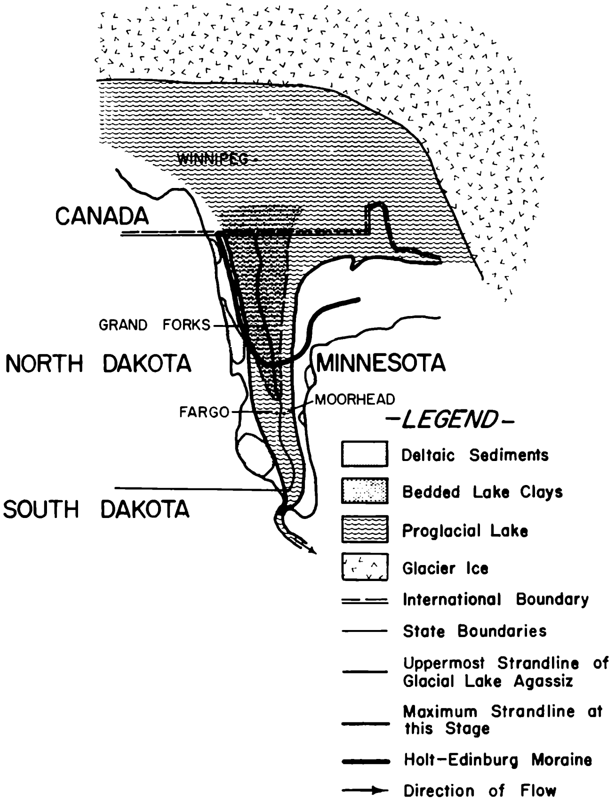

FIGURE 9—Blocking of eastern outlet and rise to Campbell Beach with southern outlet.

PROBLEMS DEALING WITH GLACIAL LAKE AGASSIZ STILL WAITING SOUTION

It has been pointed out repeatedly in the foregoing pages that significant data is lacking in many places and that all theories are extremely tentative. There are a number of problems, some of which I have mentioned, that require more data. These are:

1. The direction and location of preglacial drainages.
2. The nature and distribution of proglacial lake beds, formed in the rising stages of Lake Agassiz.

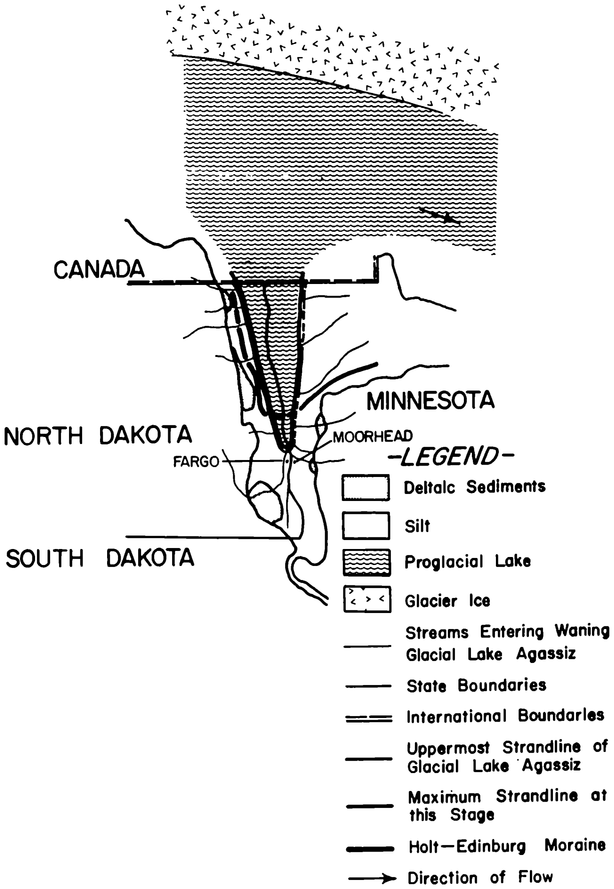

FIGURE 10—Emerado stage: eastern outlet open, lake declining, extended consequent streams depositing silt into lake.

3. The relationship of the terraces, along the present streams, to lake deposits, including some of the lower beaches.
4. The relationship of the Pembina and Elk Valley deltas.

The solution to these problems and many unmentioned await accurate geologic surface mapping and subsurface data derived from drilling. It is my fervant hope that in the near future some of this work will be done and that our knowledge of Glacial Lake Agassiz will someday be much better than it is now.

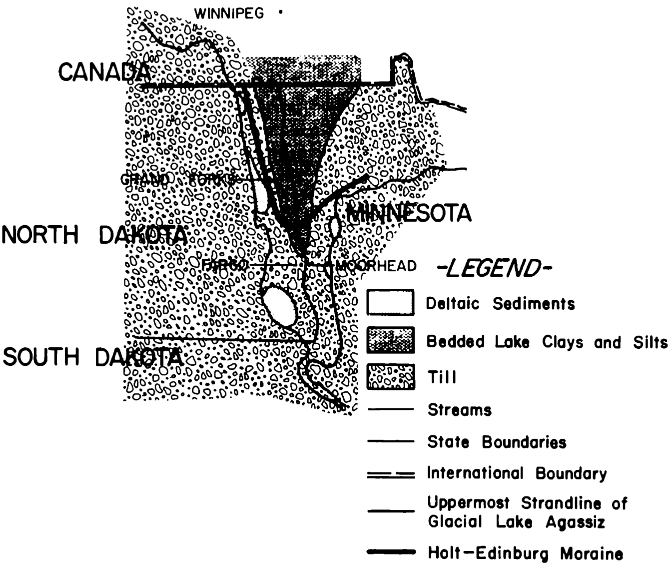

FIGURE 11—Present Lake Agassiz Basin showing approximate limits of clays and silts.

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FOSSILIFEROUS MARL BENEATH LOWER CAMPBELL (GLACIAL LAKE AGASSIZ) BEACH SEDIMENTS[1]

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INTRODUCTION

Fossil mollusks occur in a ten inch thick sandy marl beneath six feet of sand of the lower Campbell beach, a strandline of Glacial Lake Agassiz, in Turtle River State Park, north of Arvilla, Grand Forks County, North Dakota. This fossiliferous site is here informally designated the Turtle River State Park site. Forty-seven feet of till is exposed beneath the marl and all of these units are of late Wisconsin (Pleistocene) age.

It is likely that the mollusks lived in Glacial Lake Agassiz, but at present it is impossible to assert definitely that they did so, because

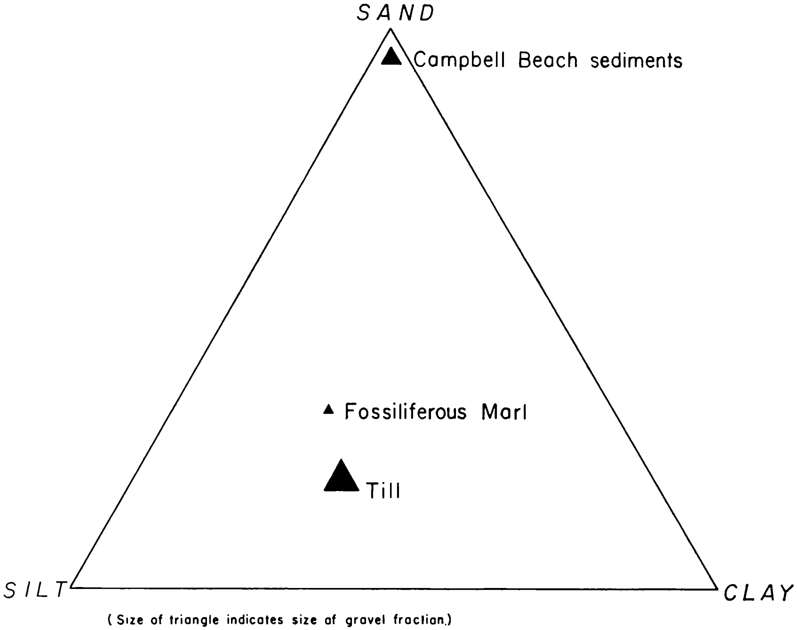

FIGURE 1—Triangular diagram showing grain-size distribution of the three lithological units at the Turtle River State Park site.

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of the incompleteness of our knowledge of the sequence of events which comprise the history of Glacial Lake Agassiz.

LOCATION AND DESCRIPTION OF THE LITHOLOGIC UNITS

The Turtle River State Park site is located in a west-facing exposure forty-seven feet above the east bank of the Turtle River in the SE 1/4, SE 1/4 Sec. 36, R. 53 W., T. 152 N., in Turtle River State Park near Arvilla, Grand Forks County, North Dakota. From the base of the exposure at the river level, the section consists of poorly

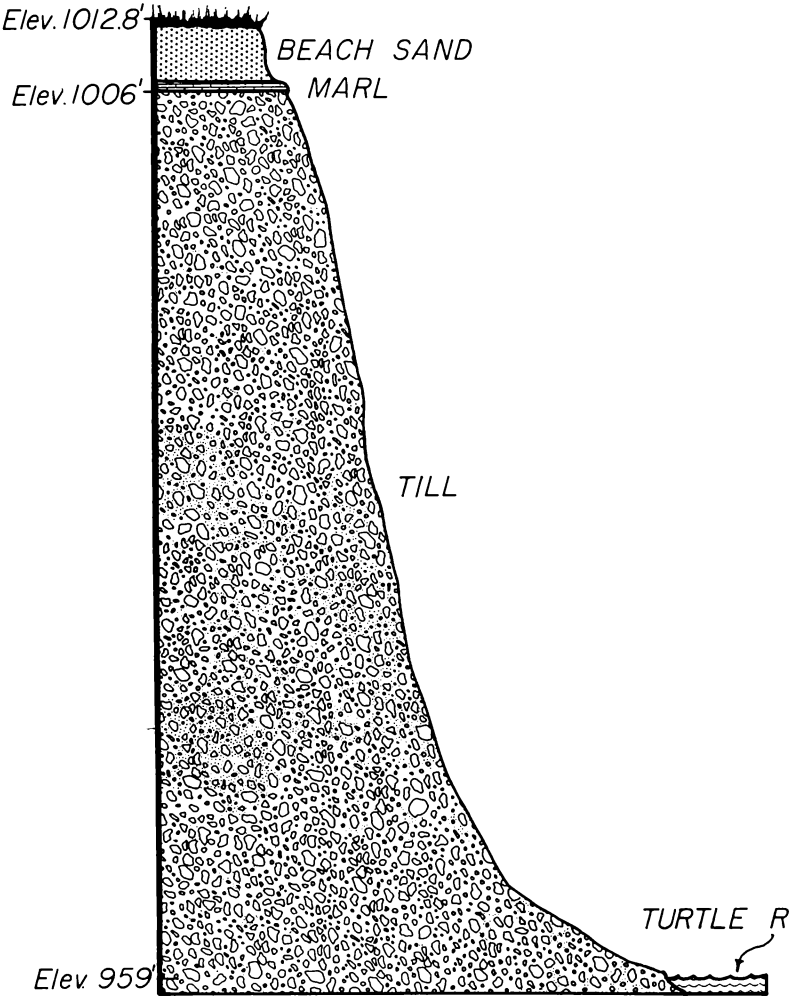

FIGURE 2—Geologic section at the Turtle River State Park site.

A. Back-beach lagoon, storm waves deposit sand over marl.

B. Marl deposit, lake level changes, sand deposit.

C. Lake drained, marl deposited. Lake refilled, beach forms.

D. Marl deposited as forebeach sediment.

FIGURE 3—Possile modes of deposition of marl.

indurated till, 47 feet; silty-marl (68.8% insoluble in dilute HCl) 10 inches; very well sorted, quartz sand, 6 feet. Figure 1 is a triangular diagram showing the results of mechanical and hydrometer analyses of the three lithologic units. Figure 2 shows a diagrammatic representation of the section and a description of the units. The marl unit contains the only fossils found at the site.

DISCUSSION OF THE ENVIRONMENT OF DEPOSITION OF THE MARL

The fossil mollusks occur in marl at an elevation of 1,005 feet above mean sea level. The environment of deposition was clearly lacustrine, but which of the following situations explains their occurence is not possible to determine at this time (see figure 3): A) a back-beach lagoon over which beach sediments were spread during storms, or B) deposition in a previous stage of Glacial Lake Agassiz, overlaid by a later beach formed at the strand of a rising lake, or C) deposition in a temporary pond developed in a depression during the period when Glacial Lake Agassiz was completely drained, or D) deposition as fore-beach sediments, overlaid by beach sediments during a lowering stage of the Lake. Table I shows the typical habitats in which living members of the species are found.

Not enough fossil shell material has yet been recovered to provide material for a radiocarbon date, but it is hoped that different methods of collection may make this possible. The discovery of this fauna should serve to encourage careful search for similar deposits in and under the beaches of Glacial Lake Agassiz as a means of correlation within the basin which will not be subject to correlation errors induced as a result of postdepositional tilting, presumably due to isostatic rebound, or minor errors in leveling. As all the species

TABLE I
TYPICAL HABITATS OF SPECIES OF GASTROPODS

GASTROPODS	TYPICAL HABITATS													GEOGRAPHIC RANGE				
	Permanent standing water	Ephemeral standing water	Small streams	Rivers	Terrestrial margins of water bodies	Low suspended detritus	High suspended detritus	Low dissolved solids	High dissolved solids	Water depth <5m	Water depth >5m	Water (summer) temp.<20°C.	Water (summer) temp.>20°C.	Center N. of Lat. 37°N.	Center S. of Lat. 37°N.	Center E. of Long. 95°W.	Center W. of Long. 95°W.	Ubiquitous in North America
BRANCHIATE GASTROPODS																		
Valvata tricarinata	X		X	X		X		X	X	X	X	X		X		X		
V. lewisi	X					X		X	X	X				X				
Amnicola limosa	X		X	X		X		X	X	X		X	X	X		X		
PULMONATE GASTROPODS																		
Lymnaea humilis	X	X	X	X	X	X	X	X	X	X		X	X	X		X		
Gyraulus parvus	X	X	X			X		X	X	X			X					X
Helisoma anceps	X		X	X		X		X		X				X				
Physa sp.	X	X	X	X		X	X	X	X	X		X	X					X
Totals	7	3	6	5	1	7	2	7	6	7	1	4	4	5		3		2

of mollusks are still living in North Dakota, correlations will have to depend upon radiocarbon dates taken from the shell material rather than on biostratigraphic methods.

MOLLUSCAN FAUNA

Nine species of freshwater mollusks are represented in the fossil fauna from the Turtle River State Park site. Three are branchiate gastropods, four are pulmonate gastropods and two are pelecypods.

Systematic List
Phylum Mollusca
  Class Pelecypoda
    Order Prionodesmacea
      Superfamily Naiadacea
          Fragments
    Order Telodesmacea
      Family Sphaeriidae
        Genus Pisidium
          Pisidium nitidum Jenyns 1832
  Class Gastropoda
    Order Mesogastropoda
      Family Valvatidae

Genus Valvata
- *Valvata tricarinata* (Say) 1817
- *Valvata lewisi* Currier 1868

Family Hydrobiidae
Genus Amnicola
- *Amnicola limsoa* (Say) 1817

Order Bassommatophora
Family Lymnaeidae
Genus Lymnaea
- *Lymnaea humilis* (Say) 1822 (*sensu* Hubendick, 1951)*

Family Planorbidae
Genus Helisoma
- *Helisoma anceps* (Menke) 1830

Genus Gyraulus
- *Gyraulus parvus* (Say) 1817

Family Physidae
Genus Physa
- *Physa* sp.

*The Lymnaea grouped above under Hubendick's concept of the species *L. humilis,* are represented in this fauna by shell forms which are referable to *Fossaria dalli* (Baker) 1906, *sensu* Taylor and Hibbard, 1960.

The molluscan fauna indicates that the environment in which they lived was a shallow, clear, temperate body of standing water. As suggested in the introduction, it is possible that these mollusks lived in Glacial Lake Agassiz or that they occupied a pond on the surface of the lake plain during the period when the lake had completly drained by way of the eastern outlet. As this complete drainage is hypothetical it does not provide an adequate basis for speculation about this particular site. If other fossiliferous sites are discovered at the same stratigraphic horizon, the fossils may provide evidence by which Johnston's (1916) hypothetical drainage of Glacial Lake Agassiz (i.e. previous to the formation of the Campbell Beach complex) can be evaluated. A single site cannot be expected to provide conclusive evidence of this type. The development of marly sand at the Turtle River State Park site substantiates the idea that these mollusks occupied a shallow, clear, temperate pondment. Table I shows the ecologic affinities of the living relatives of the species represented in the fossil molluscan fauna. This fauna is essentially similar to that found in the Norcross Beach sediments in Minnesota and reported by Tuthill (1963, p. 98) except that the Turtle River State Park site fauna lacks terrestrial forms which were present in the Norcross Beach fauna.

SUMMARY

If the nine species of freshwater mollusks reported here lived in Glacial Lake Agassiz, they are strong indicators that the lake contained clear, seasonally warm, freshwater. While this single site is too isolated to provide any conclusive evidence about the history of Glacial Lake Agassiz, it does encourage geologists concerned with the study of Glacial Lake Agassiz sediments to look carefully for fossil material which may provide paleoecologic information and radiocarbon datable material of great usefulness.

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FOSSIL MOLLUSCAN FAUNA FROM THE UPPER TERRACE OF THE CANNONBALL RIVER, GRANT COUNTY, NORTH DAKOTA

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INTRODUCTION

The age of the drifts west of the Missouri River in North Dakota has long been in question. Paleontologic evidence is lacking and all estimates of the age have been based on the amount of dissection of the tills and their geographic position in central North Dakota. The discovery of fossiliferous sands beneath colluvium at two sites in the uppermost terrace (approximately 70 feet above the present floodplain) along the Cannonball River in Grant County has produced evidence that the alluvium was deposited during Yarmouthian time. While this does not date any of the tills in the western part of the state, the age of the terrace can be used as a starting point for studies relating the terraces of the Cannonball River to those in the valley of the Missouri River and the various drift borders west of the Missouri River.

TABLE II

DESCRIPTION OF LITHIC OR MINERAL GRAIN TYPES EMPLOYED TO EVALUATE THE ORIGIN OF THE SEDIMENTS AT THE MARPLE RANCH AND CRULL RANCH SITES.

(Frequency and suggested source of these grain types is given in Table III).

1. Dark chert grains less than 1 mm diameter.
2. Pale reddish brown (10 YR 5/4) chert.
3. Pale orange (10 YR 7/2) chert.
4. Moderate brown (5 YR 4/4) chert.
5. Pseudo-quartzite.
6. Light colored chalcedony.
7. Pale brown and cream petrified wood.
8. White and gray petrified wood.
9. Light colored quartzite pebbles.
10. Angular quartz grains less than 1 mm diameter.
11. Slightly rounded doubly terminated quartz.
12. Angular quartz grains greater than 1 mm diameter.
13. Limonite stained, well rounded quartz grains greater than 1 mm diameter.
14. White to clear, well rounded quartz grains greater than 1 mm diameter.
15. Angular, fresh feldspar grains less than 1 mm diameter.
16. Grayish orange (10 YR 7/4) feldspar grains greater than 1 mm diameter.
17. Clear to white, well rounded feldspar grains greater than 1 mm diameter.
18. Pink granite.
19. Grayish red (10 R, 4/2) volcanic pebbles with feldspar phenocrysts.
20. Moderate bluish gray (5 B 5/1) quartzose phyllite.
21. Shiny dusky red (5 R 3/4) hematitic grains.
22. Dull moderate reddish brown (10 YR 4/6) limonitic grains.
23. Limonitic sandstone.
24. Calcareous sandstone.
25. Flaggy calcareous sandstone pebbles.
26. "Scoria".
27. Fiberous calcite.
28. Orange garnet.
29. Red garnet.
30. Well rounded pale pink garnet.

31. Muscovite.
32. Biotite.
33. Epidote.
34. Talc.
35. Calcareous fine sandstone tubes, ½ mm in diameter.
36. Caliche.
37. Soil grains.
38. Gypsum nodules.
39. Gastropod shells.
40. Plant fragments.

TABLE III
LITHOLOGIC ANALYSIS OF SEDIMENTS FROM MARPLE RANCH AND CRULL RANCH SITES COMPARED

	SAMPLES									SUGGESTED SOURCES											
	Marple Ranch Site			Crull Ranch Site			L. Missouri R. Golden Valley, Slope and Bowman Counties														
Lithic or mineral grain type (see Table II).	Unit 1	Unit 2	Unit 3	Unit 2	Unit 3 (soil)	Unit 4	Sec. 18, T. 138 N., R. 101 W.	Sec. 2, T. 139 N., R. 99 W.	Sec. 14, T. 130 N., R. 106 W.	Locally derived	Pierre Shale Formation	Fox Hills Formation	Hell Creek Formation	Ludlow Formation	Cannonball Formation	Tongue River Formation	Golden Valley Formation	White River Formation	Canadian Shield	Western Gravels	Autocthonous grains
1.	C	F			F	C	F	F		X		X	X	X	X	X					
2.				R																X	
3.	R	F	R	F			C													X	
4.	F	F																		X	
5.	F	F								X											
6.									R	X								X			
7.	R	R					R	R												X	
8.								C		X						X					
9.							C		F	X								X			
10.	C	C				A	C	C	C	X		X	X	X	X	X	X				
11.	R	R	R		R					X			X	X	X	X					
12.	F	F	C	C			F			X						X					
13.	C	C	C	C			C	C	C	X						X					
14.	C	C	C	C	C			R	R	?						?					
15.	C	C			C	A	C	C	F	X		X	X	X	X	X	X				
16.	F	C	R	R	R		R													X	
17.	C	C	C	C	C		C	C		X						X					
18.									R										?	?	
19.							R													X	
20.	R																		?	?	
21.	C	C	F	C	F		C	R	C											X	
22.	F	C	F	C	F	C	R	C		X	X	X	X	X	X	X					
23.	F	F		C			R			X		X	X	X	X	X					
24.	R		F			C	R	R	R	X		X	X	X	X	X					
25.								C	F	X								X			
26.		R							R	X				X		X					
27.		R								X	X		X	X							
28.					R					X		X	X	X	X	X					
29.	R	R								X		X	X	X	X	X					
30.	R	R								X		X	X	X	X	X					
31.						C			R	X		X	X	X	X	X	X				
32.						R	R			X		X	X	X	X	X					
33.	R	R								X		X	X	X	X	X					
34.	R	R				R				X		X	X	X	X	X					
35.						A				X											X
36.			F	C						X											X
37.					A					X											X
38.					C					X											X
39.	R			C						X											X
40.	R	R	R		C		F	F	C	X											X

R= less than 0.01%
F= 0.01 to 1.0%
C= 1.0 to 10%
A= greater than 10%

DISCUSSION OF THE MOLLUSCAN FAUNA

The distribution and typical habitat types of living relatives of the fossils may be seen on Table IV. It is readily apparent that the fauna constitutes a combination of species which would be found along a stream in the northern Great Plains in the United States. All but one of the species (*Pupilla sinistra*) are currently found in the central part of North America and while eleven species have geo-

TABLE IV

SPECIES OF MOLLUSKS AT THE CRULL AND MARPLE RANCH SITES, GRANT COUNTY, NORTH DAKOTA

	OCCURRENCE			TYPICAL HABITATS																	GEOGRAPHIC RANGES				
				Aquatic													Terrestrial								
	Occurs both in fossil & living form	Occurs only as fossil	Occurs as living form only	Permanent standing water	Ephemeral standing water	Small streams	Rivers	Terrestrial margins of water bodies	Low in suspended detritus	High in suspended detritus	Low in dissolved solids	High in dissolved solids	Water depth <5m	Water depth >5m	Water temp. < 20°C (summer)	Water temp. > 20°C (summer)	Wooded areas	Grasslands	Subhumid-semiarid climate	Humid climate	Center N. of 37° N. Lat.	Center S. of 37° N. Lat.	Center E. of 95° W. Long.	Center W. of 95° W. Long.	Ubiquitous in North America
BRANCHIATES																									
Valvata tricarinata		X		X		X	X		X		X	X	X	X	X						X		X		
Amnicola limosa			X	X		X	X		X		X	X	X		X	X					X		X		
PULMONATES																									
Lymnaea humilis	X			X	X	X	X	X	X	X	X	X	X	X	X	X					X		X		
Physa sp.	X			X	X	X	X		X	X	X	X	X		X	X									X
Gyraulus parvus	X			X	X	X		X	X		X	X	X			X									X
Helisoma anceps			X	X		X	X		X		X		X										X		
TERRESTRIALS																									
Vallonia gracilicosta	X																X	X	X		X			X	
Gastrocopta armifera	X																X	X	X	X	X		X		
G. holzingeri			X														X		X		X				
Pupilla muscorum		X															X		X		X				
P. sinistra		X															?		?		?				
P. blandi			X														X	X	X		X			X	
Vertigo modesti		X															X	X	X	X	X				
V. ovata	X																X		X	X					X
Succinea avara	X																X	X	X	X					X
Zonitoides arborea	X																X		X	X					X
Euconulus fulvus	X																X		X	X	X				
Discus cronkhitei		X															X		X	X	X				
Deroceras laeve		X															X		X	X					X
Cionella lubrica	X																X		X	X					X
Nesovitrea binneyana		X															X			X	X		X		
PELECYPODS																									
Pisidium nitidum		X		X		X							X		X						X		X		
P. walkeri		X		X		X	X														X		X		
Sphaerium sulcatum			X	X		X	X														X				
Totals	10	9	5	9	3	9	7	2	6	2	6	5	7	2	5	4	15	5	14	10	16	0	8	2	7

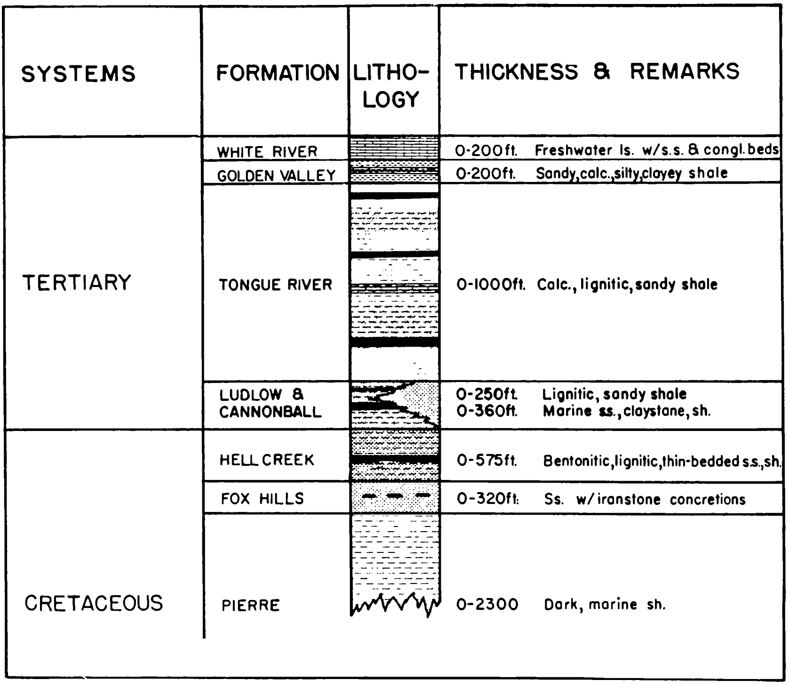

FIGURE 5—Geologic formations which crop out west of the Missouri River in North Dakota.

graphic ranges which extend south of the 35th degree of north latitude, they all have ranges north of this parallel. Eleven species are more-or-less northern elements, not now having an appreciable southern range.

Species which are noted in the literature as being usually found in less moist habitats (i. e., *Vallonia gracilicosta, Pupilla muscorum,* and *Euconulus fulvus*) would be expected to occur as fossils with those usually being found in or near streams. Currently in North Dakota, just such comingling of mollusk shells in fluviatile sediments is found. Both *E. fulvus* and *V. gracilicosta* were found in the litter on the present floodplain, along the Cannonball River (Table I). Thus the fossil assemblage can be considered as having resulted from fluviatile concentration of separate assemblages from locally, closely associated microhabitats, and not as a major thanatocoenose. The situation inferred would therefore, be very much like that which now exists along the streams in western North Dakota.

The geologic ranges given by several authors are given on Table I and those which we consider valid on Table IV. Taylor (Hibbard and Taylor, 1960) has suggested that the geologic range of

Pupilla sinistra extends to the Recent. His suggestion is based on the presence of seven specimens of this species in floodplain litter from Beaver Creek, Wibaux, Montana. In one of these specimens, all of the periostracum still remains. The specimens are from a collection of mixed age, some being obviously fossil and other Recent. As fossil *P. sinistra* have not been reported from the Montana-North Dakota area previously, it was more reasonable to assume that the shells were of Recent age at the time Taylor considered the problem of the age of *P. sinistra*. Eight of the twenty-five fossil specimens taken from the Crull Ranch site still retain all or most of their periosracum. Thus we believe that Taylor's conclusion was unfounded and that the Montana specimens were reworked fossil material.

As the sediments (Unit 2) containing the fossil mollusks is beneath colluvium and unquestionably associated with a terrace which is now approximately 70 feet above the present floodplain we do not feel that they can have lived during Recent time. The presence of *P. walkeri, D. laeve, P. sinistra, Vertigo modesta, Succinea* cf. *S. avara,* and *Euconulus fulvus,* all of which are considered to have appeared in the Mid-Continent during the Kansan-Yarmouthian time seems to us a sufficient indication that Unit 2 is no older than Yarmouthian age and therefore the age of the terrace is most likely not older than very late Kansan or more probably early Yarmouthian.

PALEOECOLOGY

Ten species occur in both the Yarmouthian and the Recent molluscan faunas of the Cannonball River. Seven of these are terrestrial snails and three are aquatic. Nine species occur only as fossils. Six of these are terristrial. And five species occur in the Recent fauna only.

Aquatic gastropods:—Of the four species of fossil aquatic gastropods the branchiate one, *Valvata tricarinata,* cannot survive ephemeral water conditions. Thus, while three-fourths of the species of aquatic snails can survive dry spells, the presence of the branchiate precludes this possibility. While all of the aquatic snails can live in limnitic habitats as well as fluviatile ones, the sediments clearly indicate a running water environment. The pulmonate *Lymnaea humilis* probably lived predominantly on the wet muddy or grassy margins of the river as is their modern habit. While two species (*Physa* and *Lymnaea humilis*) can tolerate continuous conditions of heavy silting, *Gyraulus parvus* and *Valvata tricarinata* can tolerate only moderate (approximately 50 ppm) turbidity. It is probable that during periods of flooding the water was very turbid, however. The sediments in which the fossils occur are very well sorted, thus the fines were carried in suspension at least seasonally. Protracted muddiness, would have undoubtedly exterminated most of the aquatic species represented in the fossil fauna or caused them to exist in local, relatively clear microhabitats in the river.

The evidence in the matter of whether the water of the Cannonball River during the time the fossil mollusks lived, was charged with high concentrations of dissolved mineral substances is not clear. All species can live in either high or low concentrations. The number of *Valvata tricarinata* represented in the fauna is low (1/2% of the fauna in Unit 2 (sand) at the Crull Ranch Site). Tuthill and Laird (1963-64, p. 83) have found *V. tricarinata* as a minor element of molluscan faunas in central North Dakota in waterbodies having as much as 5210 ppm total dissolved solids. Thus it seems likely that the area was one of a negative evapotranspiration-precipitation ratio, and some degree of salt concentration occurred in the Cannonball River drainage system at the time the fossil mollusks lived, but the present evidence permits nothing more than a guess about this aspect of the environment of the fossil mollusks.

Water depth was very likely quite shallow: all of the aquatic species are usually found in shallow water. The fact that three species are rarely found below 5m and one, *Valvata tricarinata,* is found frequently in both deep and shallow water, seems to be conclusive evidence that the river was essentially a shallow body.

Water temperature is not an environmental factor which can be succinctly characterized in fluviatile environments. The backwaters and edges of a stream may have much higher temperature than are found in the main current or in the spring holes. Thus, *Valvata tricarinata,* which is a cool water form, could have lived in the same river in cool spots, with *Gyraulus parvus,* which typically inhabits warmer water.

Terrestrial and Aquatic Gastropods:.—The center of the present geographic range of a species roughly catagorizes it as a boreal, transitional, or southern organism. The usefulness of this is significant only in the case of marked dislocations. None of the mollusks, aquatic or terrestrial, listed here appear to be grossly out of place. Little question exists that the fauna is comparable to the present fauna of the region. Of the 13 non-ubiquitous species the present ranges are all centered north of the 37th parallel, which approximates the July 80° isotherm. One species has the center of its geographic range west of 95° of west longitude, which approximates the evapotranspiration-precipitation zero isopleth in northern North America, and two species have the center of their range east of 95° W. longitude. This does not provide a sufficient basis for determining whether the Yarmouthian fauna clearly had western or eastern affinities. Thus it must be concluded that western North Dakota during the Yarmouthian was a zone of transition just as it appears to be today. If *Valvata tricarinata* was a more dominant element in the Yarmouthian fauna, we could assume that the climate was more humid than it is at present, but this does not appear to be the case.

Terrestrial Gastropods:—Four of the 14 species of terrestrial gastropods are found in either woods or grasslands today. The remaining ten species are usually found in wooded areas. Thus it is clear that the Yarmouthian Cannonball River was bordered by woods in which the terrestrial gastropods flourished. The situation was probably not much different than that which exists in Grant County today. The availability of local moist microhabitats under debris in a semiarid area is common in western North Dakota and readily explains the presence of the nine species in the Recent fauna. The greater size of the Yarmouthian fauna may be explained by the fact that the number of microhabitats has been reduced as a result of farming and grazing, even though the number of habitat types has not been reduced by climatic changes.

STRATIGRAPHIC CONSIDERATION

The molluscan fauna at the Marple Ranch site contains no diagnostic fossil species with which the minimum age of the sand unit can be determined but as five of the species are known to first appear in the Yarmouthian interglacial the maximum age of the sand is firmly established. The molluscan fauna at the Crull Ranch site includes *Pupilla sinistra* which Leonard (1950, p. 28-29) stated is named from, and restricted to, the Yarmouthian interglacial beds in Kansas. If this species is a valid index to the Yarmouthian throughout the Mid-Continent, the sand unit (2) is of Yarmouthian age and not younger. If *P. sinistra* ranged through more recent time in North Dakota, as is believed by Hibbard and Taylor (1960) then the sand unit could have been deposited at any time since the beginning of the Yarmouthian. As no evidence exists, at these two sites, to indicate that the terrace was cut subsequent to the deposition of the sand (Unit 2), it is reasonable to assume that the terrace is of the same age as the sand, i. e. Yarmouthian.

The establishment of the age of this terrace is of great significance and it is imperative to an understanding of the history of glaciation to the east, to trace this terrace down stream to discover its relationship with the various terraces of the Missouri River and the various drift borders.

In summary then, the Yarmouthian Cannonball River was a relatively clear (except in times of flooding), permanent, shallow stream flowing through western North Dakota and the climate of the area was essentially the same as that which exists there now. Woods bordered the stream, but the general area was probably grassland like those which existed when the white man first came to this part of the New World. The degradation of the topography represented by the depth of the Cannonball River Valley below the Yarmouthian terrace is the result of erosion during the Illinoian and Wisconsin glaciations and the Sangamonian and Recent nonglaciations.

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A PRELIMINARY THIN-SECTION ANALYSIS OF GLACIAL TILL FROM EASTERN WELLS COUNTY, NORTH DAKOTA[1]

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INTRODUCTION

The landforms of eastern Wells County, central North Dakota, are the result of Upper Wisconsinan (Pleistocene) continental glaciation. Characteristics of the surface glacial till in this area include

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similar lithology, approximately constant depth of oxidation and the presence of non-integrated (youthful) surface drainage.

Since megascopic studies have indicated no basic dissimilarities in the drift sheets, a microscopic thin-section analysis as a possible method of till differentiation was attempted. In major ice advances separated by large time lapses, buried soil profiles which have developed on the older tills as well as depths of oxidation are used in differentiating the drift sheets. However in central North Dakota, where the tills are youthful and of relatively the same age, compositional dissimilarities along with indicators of ice direction of advance and post-depositional changes which have occurred in the till are the accepted means of differentiation. The conventional method for determining the direction of glacier advance, in addition to surface lineations, is the macrofabric till analysis.

The procedure for this technique involves measuring the strike and plunge of elongate pebbles within the till. The results are plotted on rose or point diagrams and usually necessitate the presence of a major trend of pebble orientation in order to determine the direction of ice advance. The classical work was done in New York (Holmes, 1941) and in Illinois (Harrison, 1957) and these writers concluded that the measurements reflected original deposition by the glacier ice.

The orientation is commonly two-fold and at right angles to each other. The primary orientation usually coincides with the direction of glacier ice advance. In microfabric analyses of thin sections of till, Ostry and Deane (1963) have revealed a significant preferred orientation. This analysis was made on tills in the Toronto area and has proved as reliable as macrofabric analysis in determining preferred orientation.

The purpose of this preliminary study in eastern Wells County was two-fold: 1) to study the microscopic petrography of glacial till from eastern Wells County and 2) to determine the texture (microfabric and post-depositional changes) of these tills.

SAMPLING AND THIN SECTION PREPARATION

In order to assess the microfabric analysis technique, oriented till samples were taken from areas of washboard moraines where the direction of ice advance was already known. Samples were also collected at a site where two lithologically different tills were discovered. Oriented till blocks, about 6 inches on a side, were extracted from below the zone of root and frost action to avoid collecting samples with disrupted till fabric caused by these agents. The block orientation was marked and the samples were wrapped in aluminum foil to retain original moisture. Samples were then allowed to dry slowly for several weeks in the laboratory. Because of the unconsolidated nature of the sediments, smaller portions of the samples were subsequently impregnated with Lakeside #70 cement. Thin-sections were then cut in three mutually perpendicular planes: the horizontal plane, the east-west vertical plane and the north-south vertical plane.

PETROGRAPHY OF TILL

Microscopic petrographic analyses were made from three washboard moraine tills in eastern Wells County. For convenience they are designated washboard moraines "X", "Y", and "Z".

Washboard Moraine Site "X".—This washboard moraine is located 0.3 miles north of the southwest corner of Sec. 8, T. 147 N., R. 68 W. Thin section analysis of this till showed minor microfabric preferred orientation in the northeast-southwest direction. The sediment is a silty till and microscopic examination reveals that it is composed of about 10 per cent sand and 20 per cent silt. The sand is angular to sub-rounded. The sorting of the sediment is poor and the porosity is low. The matrix, which occupies about 70 per cent of the thin section area, is composed or birefringent yellow clay (probably either montmorillonite or illite) and siltsize calcite. The sand particles include about 40 to 60 per cent quartz, 20 to 30 per cent feldspar (including orthoclase with inclusions, microcline, and plagioclase) and 10 to 20 per cent lithic fragment of shale, carbonates and acidic igneous rocks in equal proportions. Small percentages of amphibole, lignite, biotite, limonite and various other opaques are also present. Jointing was not evident in the thin sections of this particular till. The only apparent post-depositional change was the migration of iron oxide stain from limonite fragments into the adjacent clay matrix.

Washboard Moraine Site "Y".—This washboard moraine is located 0.6 miles south of the northeast corner of Sec. 17, T. 148 N., R. 70 W. and is composed of a silty till. Thin-section analysis of this till did not reveal any preferred orientation. Further analysis revealed that the till contains 20 per cent silt and 8 per cent sand with angular to rounded grains. The matrix is 70 per cent birefringent clay with some silt-size calcite. Mineral composition of the sand includes 10 to 25 per cent feldspar, 50 per cent quartz and small percentages of gypsum, limonite, amphibole, biotite, lignite and various other opaques. The rock fragments comprised 6 to 10 per cent of the total particles and included carbonates, acidic igneous sand grains and minor amounts of shale. Post-depositional changes in this till involved the formation of parallel joints and the growth of gypsum crystals in these joints. Iron staining of the clay matrix around limonite grains also occurred (Figure 1).

Washboard Moraine Site "Z".—This washboard moraine is located 0.2 miles south of the northeast corner of section 18, T. 149 N., R. 70 W. Very minor preferred orientation of sand particles was revealed in this sample. This silty till contains 8 to 12 per cent sand and about 20 per cent silt. Sand-size particles include 50 to 60 per cent quartz, 20 per cent feldspar and accessory amounts of amphibole, biotite, garnet, lignite and limonite. Lithics comprise 10 per cent of the sand particles and include granite, shale and carbonate fragments.

MICROFABRIC ANALYSIS OF WASHBOARD MORAINE TILL

A cursory microscopic thin-section examination of the three washboard moraine tills indicated no preferred orientation of sand and silt particles. The orientation of elongate sand and silt particles from washboard moraines "X" and "Y" plotted on rose diagrams, however, shows two prominent directions of preferred orientation at right angles to each other. The surface trend of washboard moraine "X" (N 70°E) indicates that the ice advance was from N 30° W. The microfabric preferred orientation for this moraine is N 30° W. The

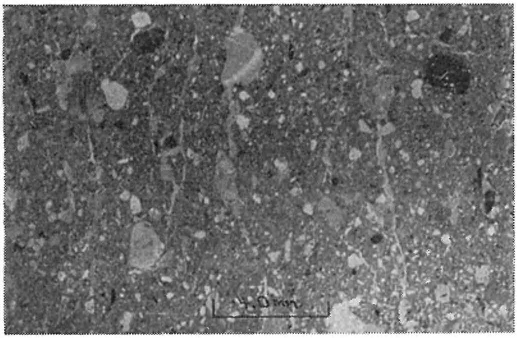

FIGURE 1—Parallel joints of washboard moraine "W" from 0.6 miles south of the northeast corner of section 17, T. 148 N., R. 70 W. Note iron-staining from limonite grain in upper right of photo. Photo taken under crossed nicols.

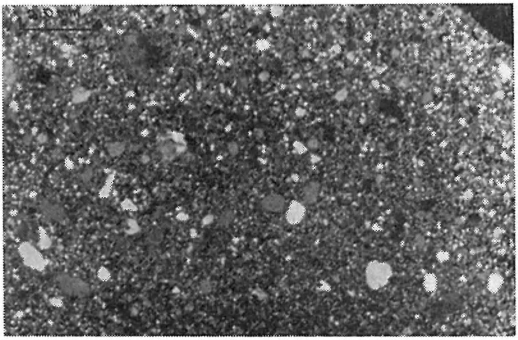

FIGURE 2—Texture of upper silty till 0.3 miles east of the northwest corner of section 6, T. 149 N., R. 69 W. Note the abundance of silt particles in this photo and the relative scarcity of silt particles in the section shown in figure 3. Photo taken under crossed nicols.

fabric pattern of washboard moraine "Y" on the other hand, shows a preferred orientation 45 degrees to the linear trend of this moraine. The macrofabric analysis of washboard moraine "X" complements the microfabric analysis of this moraine. If the till from this site was not indeed lodgement till, such an inconsistency in the fabric pattern would be expected.

THIN-SECTION ANALYSIS OF TWO TILL SITE

The area 0.3 miles east of the northwest corner of section 6, T. 149 N., R. 69 W. contains two lithologically different tills. Three feet of fissile, silty till overlies twelve feet of compacted, well-jointed clay till. The clay till rests on an undetermined thickness of washed sand. Thin-section analysis of the upper silty till revealed 15 per cent fine to medium sand, 35 per cent silt and 3 per cent lithics. No post-depositional changes were noted in this till (figure 2). Thin-section analysis of the lower till shows 10 per cent sand, 10 per cent silt and 15 per cent lithics. Post depositional changes in this clay till include the migration of limonite staining into the adjacent clay matrix and the development of two mutually perpendicular sets of joints (figure 3).

On the basis of these distinct differences it is concluded that the tills represent separate advances. Another possible conclusion would be that the lower till represents a lodgement till and the upper till is an ablation till. However the high degree of induration of the lower till, which is unlike the average till in the area, as well as the well-developed frature pattern, favor the former conclusion.

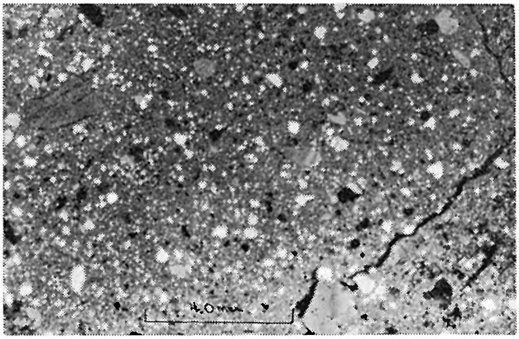

FIGURE 3—Texture of lower clay till from 0.3 miles east of the northwest corner of section 6, T. 149 N., R. 69 W. Note the joint pattern (almost ninety degrees) in the lower and upper right hand corners. Photo taken under crossed nicols.

CONCLUSIONS

Thin-section analyses of glacial till in northwestern Pennsylvania and northeastern Ohio has yielded conclusive evidence of direction of ice advance and successive reworking of older tills by a later ice advance (Sitler, 1963, and Sitler and Chapman, 1955). Similar conclusions could not be reached in eastern Wells County because of an inadequate amount of sampling. Ostry and Deane (1963) have concluded that microfabric analysis of glacial till gives results which are just as reliable as macrofabric analyses. The limited microfabric analyses made in eastern Wells County partially support their conclusions.

If microfabric analyses of tills of washboard moraines in eastern Wells County prove to be comparable with the results of macrofabric analyses of the same sediments, the micro-technique should be used since thin-section analysis of glacial till permits a detailed petrographic and fabric analysis and provides a permanent thin-section record for future reference. This technique would also conserve valuable field time which would normally be spent in making macrofabric till analyses.

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SOME TEKTITES FROM SOUTH VIET NAM

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ABSTRACT

Tektites are small obsidian-like objects scattered in several fields over the earth's surface, probably of extraterrestrial and perhaps of lunar origin. They have recently attained notice because of interest in space exploration, and in particular in connection with Project Apollo to place an astronaut on the Moon.

Tektites were first discovered and characterized as distinct from

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obsidian by Joseph Mayer in 1788 near Ceske Budejovice, and named *moldavites*. Later discoveries include the australites, javanites, indochinites, philippinites, bediasites, and Ivory Coast Tektites.

In Indochina, tektites were first described scientifically by La Croix in 1932, and later by Saurin. In October, 1962, Chao, Saurin and the present author collected nearly two thousand specimens in the vicinity of Dalat at an elevation of about 5,000 feet. In June, 1963, the present author alone collected further samples at Dalat, as well as farther north at Kon-Tum, Dak-To, Dak-Sut and Tou-Ma-Rong. Other samples were found near Plateau G and at Dak-Pek, as well as at Mang-Buk. Almost all of these tektites appear to be of the Muong Nong type found in Laos, and include shapes known as tear drops, batons, boats, pears, dumb-bells, disks, and ovaloids. One good fragment of a hollow spheroid of about 2 in. diameter was also found.

SHEET MORAINE IN BURLEIGH COUNTY, NORTH DAKOTA[1]

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INTRODUCTION

The glacial landforms of Burleigh County were mapped by the North Dakota Geological Survey during the fall of 1960 and the field seasons of 1961 and 1962. The interpretations and results of this mapping will be published in a detailed report by Kume and Hansen (5). During the course of mapping the Napoleon Drift, it became apparent that a new landform mapping unit was necessary. The purpose of this report is to introduce and discuss this proposed new landform unit, sheet moraine, as it occurs in Burleigh County.

MORPHOSTRATIGRAPHIC UNITS

The Napoleon Drift is a glacial morphostratigraphic unit of Wisconsin Age. The morphostratigraphic unit was proposed by Frye and Willman (4) and was modified and used by Clayton (1) for glacial drift. The unit was used in this study as modified by Clayton, except that it was referred to as a glacial morphostratigraphic unit. A glacial morphostratigraphic unit is defined as a body of drift identified primarily by its surface form and position, and it is composed of all the drift deposited as a result of a significant glacial advance. The basic unit of this formal nomenclature is drift rather than moraine since the drift refers to the lithology of the glacial

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advance rather than to any particular geomorphic unit. The drift of a glacial advance may include several moraines as well as the associated outwash. Commonly, the glacial morphostratigraphic unit is named for and identified by a prominent moraine which occurs in the unit.

The three glacial morphostratigraphic units which extend into Burleigh County are the Napoleon Drift, Long Lake Drift, and Burnstad Drift which includes the minor "Streeter drift" (Figure 1). The Napoleon Drift was proposed by Clayton (2) who has designated the type area in western Logan County. The Napoleon Drift extends into the western and central parts of Burleigh County. It is composed of a thin, discontinuous, layer of drift draped over a stream-eroded, rugged, bedrock surface. In Burleigh County this drift was mapped as sheet moraine. Sheet moraine is a new geo-

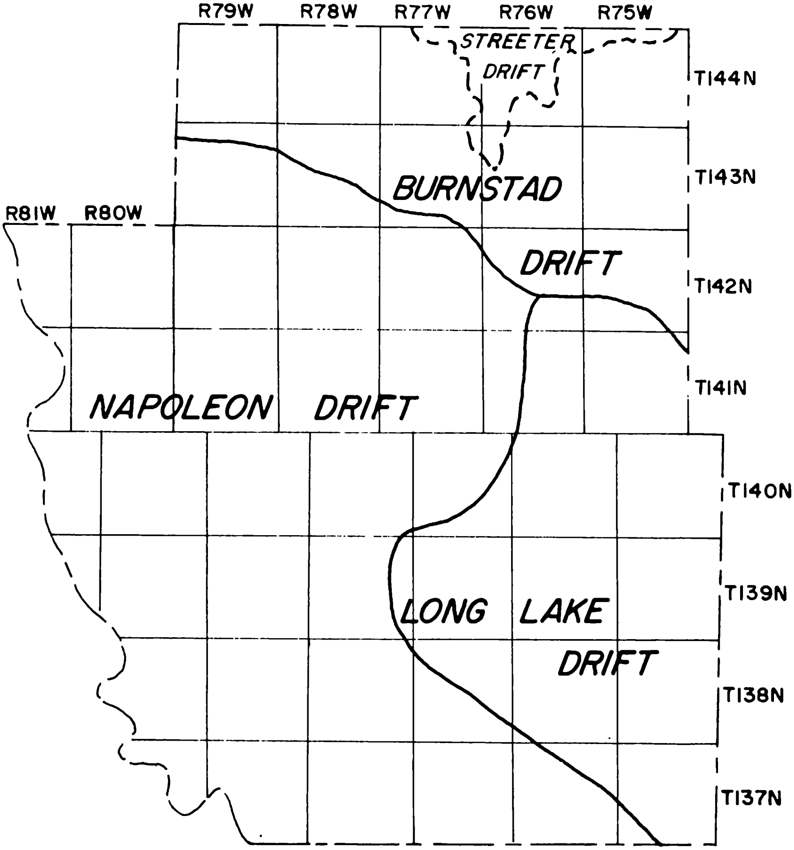

FIGURE 1—Glacial morphostratigraphic units in Burleigh County.

morphic unit and is proposed for the type of glacial landform that comprises a very thin accumulation of glacial drift.

GLACIAL LANDFORMS

Moraines are glacial landforms which were deposited directly from glacial ice, have a drift composition which is chiefly till and have constructional relief. Three major types of moraines have been recognized in North Dakota: end moraine, dead-ice moraine, and ground moraine. During the course of the Burleigh County study an additional type of moraine was recognized and subsequently named sheet moraine (Figure 2).

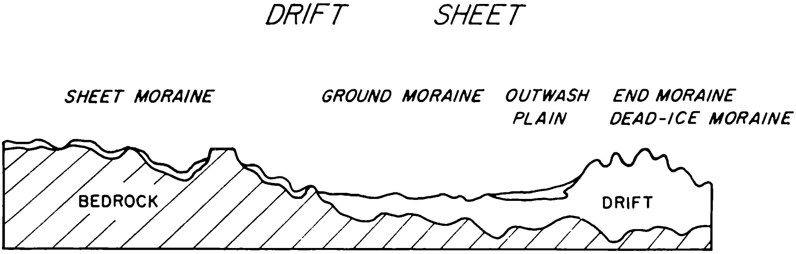

FIGURE 2—Diagrammatic profile and cross section of a drift sheet.

Sheet moraine is defined as a glacial landform composed of a mappable, blanketlike, thin accumulation of drift which is chiefly till. It is deposited directly from glacial ice, and it contains low to very low constructional relief that is generally less than 10 to 15 feet per square mile. Because the drift is thin, kettles are generally lacking. The near absence of kettles, therefore, is a diagnostic characteristic in the recognition of sheet moraine.

In the New England area of eastern United States, Flint (3) reported a similar thin drift that had been deposited upon bedrock hills. This body of till lacked its own topographic expression other than that of the immediate underlying bedrock surface. Because he has defined moraines as being independent of the underlying surface, he stated that thin drift of this nature is not properly ground moraine. It is part of a drift sheet—a stratigraphic but not a topographic unit. Mapping in Burleigh County has shown that moraines are not always independent of the underlying surface. In fact the bedrock surface has influenced the drift emplacement and accounts for part of the relief of the Long Lake and Burnstad moraines.

A drift sheet, as shown in figure 2, is composed of the accumulations of the moraines and associated outwash plain. As used in this study, it refers to the drift accumulation resulting from a significant glacial advance. A drift sheet unit is used as an informal designation. A morphostratigraphic unit also refers to the drift accumulation of a glacial advance, but it is identified on the basis of surface form. It is formally named and defined with a designated type area.

Sheet moraine differs from ground moraine by having a lesser amount of glacier constructional relief, an absence or near absence of linear elements such as ridges, and a near lack of kettles. The mode of deposition of these two types of moraines probably is quite similar, as it is assumed that deposition occurred in back of the ice margin. However, the amount of time required to deposit sheet moraine was probably much less than the time required to deposit ground moraine. This is suggested by the widespread thinness of the drift deposited. It is understood that other factors can influence the amount of drift deposited. A thin drift sheet could be caused by an abundance of meltwater with integrated distributary routes and a small glacial load of material. Other influencing factors, especially in Burleigh County, are the rugged bedrock uplands upon which the drift was deposited and the geographic position of the county which is near the limit of the Wisconsin glaciation.

Sheet moraine in Burleigh County occurs within two physiographic districts. It occurs throughout the bedrock uplands of the Coteau Slope district, and it occurs upon the dissected valley walls of the Missouri River Trench district (5).

The sheet moraine in Burleigh County generally occurs in the upland areas which have high relief of 80 to 100 feet per square mile. Frequently, the local relief exceeds 100 feet per square mile but in either case the relief is due mainly to the rugged bedrock surface upon which the sheet moraine lies. The sheet moraine is composed chiefly of till, the thickness of which ranges from 5 to 20 feet, but commonly the thickness averages about 10 feet or less. Scattered surface boulders are present, and in local areas they are quite numerous. Generally in most areas only a few, if any, kettles are present, but in a few areas, where the drift is thicker, a slight increase in the number of kettles is noted.

CONCLUSIONS

It was concluded, as a result of mapping the glacial landforms of Burleigh County, that the use of the proposed geomorphic unit was justified. It is hoped that the use of this unit will serve a mapping need especially for those workers doing detailed glacial landform mapping in the areas near the limit of the Wisconsin drift.

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GEOLOGIC STUDIES OF BURLEIGH COUNTY, NORTH DAKOTA[1]

Jack Kume

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INTRODUCTION

A comprehensive investigation of the geology and occurrence of ground water in Burleigh County was undertaken by the North Dakota Geological Survey during the fall of 1960 and the field seasons of 1961 and 1962. The major portion of this investigation was devoted to the mapping of various glacial landforms and exposed bedrock formations. In addition to this surface mapping, adequate subsurface data was collected from the drilling samples of 146 shallow test holes. The interpretations and results of this study will be published in a detailed report by Kume and Hansen (6). The purpose of this report is to present a proposed physiographic classification and to briefly summarize the glacial geology and surficial bedrock geology of Burleigh County.

PHYSIOGRAPHIC CLASSIFICATION

Burleigh County, according to Fenneman's physiographic classification (4) (5), lies in the Interior Plains major division, the Great Plains province, and the Glaciated Missouri Plateau section. Clayton (1) included the county in the Missouri Coteau and Coteau Slope districts. In this study Burleigh County is included in a proposed third district referred to as the Missouri River Trench.

The Missouri River Trench district comprises the Missouri River floodplain and the dissected walls of the river trench. In Burleigh County it occurs along the western county boundary. Adjacent and east of the river trench is the Coteau Slope district. It is the largest physiographic district in the county and comprises a glaciated slope that is subject to active erosion and has integrated drainage. East of this glaciated slope is the Missouri Coteau district which comprises a high morainic belt that has non-integrated drainage. It occurs in the northeastern part of the county.

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A physiographic classification on the subdistrict level is proposed for that part of the Coteau Slope district which occurs in Burleigh County and the adjacent counties. The physiographic subdivisions were based upon the drainage and predominant topography.

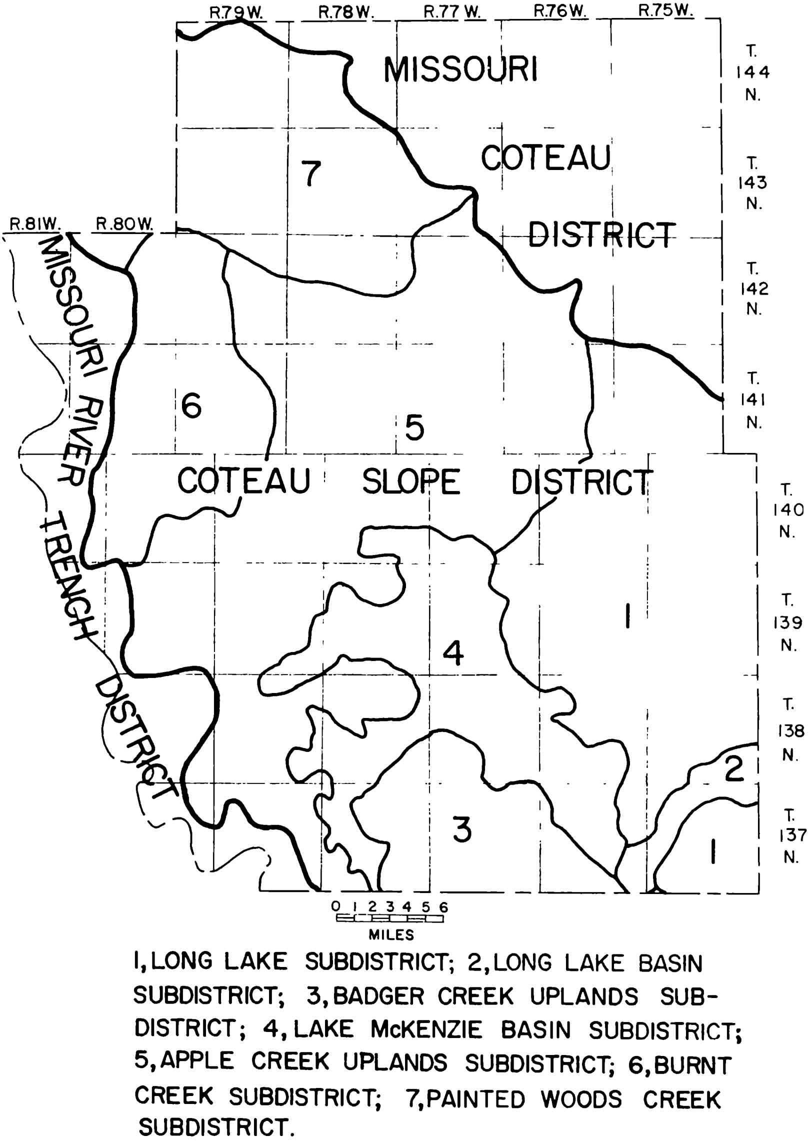


FIGURE 1—The physiographic subdivisions of Burleigh County.

Seven subdistricts of the Coteau Slope occur within or extend into Burleigh County. These subdistricts are shown on figure 1, and include: (1) Long Lake subdistrict, an end moraine, associated ground moraine, and outwash plain; (2) Long Lake Basin subdistrict, an extensive outwash and lake plain; (3) Badger Creek Uplands subdistrict, a steeply dissected drainage basin and bedrock upland ridges; (4) Lake McKenzie Basin subdistrict, an extensive lake plain with an outwash plain and minor dune sand; (5) Apple Creek Uplands subdistrict, a steeply dissected bedrock upland with an extensive sheet moraine; (6) Burnt Creek subdistrict, a steeply dissected drainage basin and bedrock uplands with minor sheet moraine; and (7) Painted Woods Creek subdistrict, an extensive dead-ice moraine, associated collapsed outwash topography, and sheet moraine.

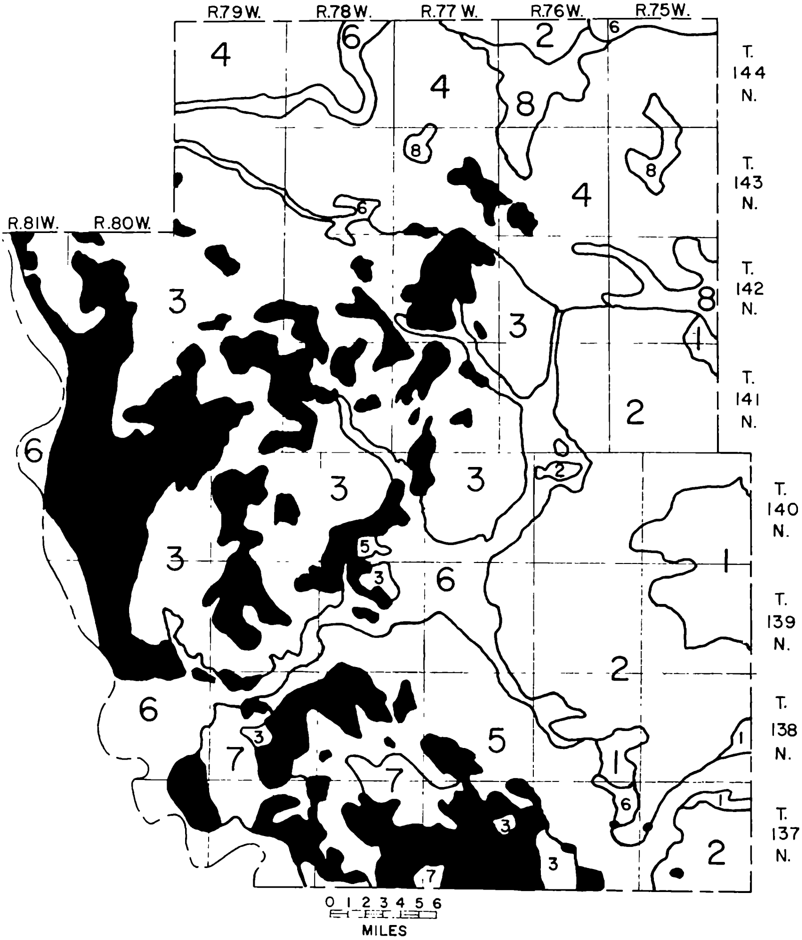
GLACIAL GEOLOGY AND HISTORY

Studying the relationships of the various glacial moraines and proglacial landforms, it was concluded that four glacial advance of the Wisconsin Age occurred in Burleigh County. Evidence for glaciations older than the Wisconsin was not found in this county. The earliest glacial activity was the Napoleon glacial advance which was later followed by the Long Lake, Burnstad, and Streeter glacial advances (figure 2).

The glacial deposits of the Napoleon advance consist mainly of a thin layer of drift draped over a rugged bedrock surface. In this study this drift was mapped as sheet moraine. The exact date of the Napoleon advance is unknown, but Clayton (2) estimated that it occurred about 38,000 to 28,700 years ago. This and other estimates of the age of the glacial deposits are based on radiocarbon dates. That the Napoleon advance probably did not persist for any extended period of time is suggested by the absence of end moraines and the uniform thinness of the drift. After the Napoleon ice melted there was a period of non-glaciation which lasted about 25,000 to 15,000 years.

The period of non-glaciation ended when the Long Lake glacier advanced into the northern and eastern part of the county. This advance occurred about 13,000 to 12,000 years ago. Considerable glacial debris was deposited along the active ice front, and three loops of a prominent end moraine were formed. A large proglacial and ice-marginal lake, Glacial Lake McKenzie, developed ahead of the Long Lake glacier; and an extensive lake and outwash plain was deposited.

The Burnstad glacier followed shortly after the recession of the Long Lake ice front. It advanced into northern Burleigh County about 12,000 years ago. The glacier truncated the north loop of the Long Lake end moraine. After considerable glacial debris had accumulated upon and within the ice, the glacier stagnated, and the buried ice slowly melted and deposited a prominent dead-ice moraine. The Burnstad advance was soon followed by the Streeter advance

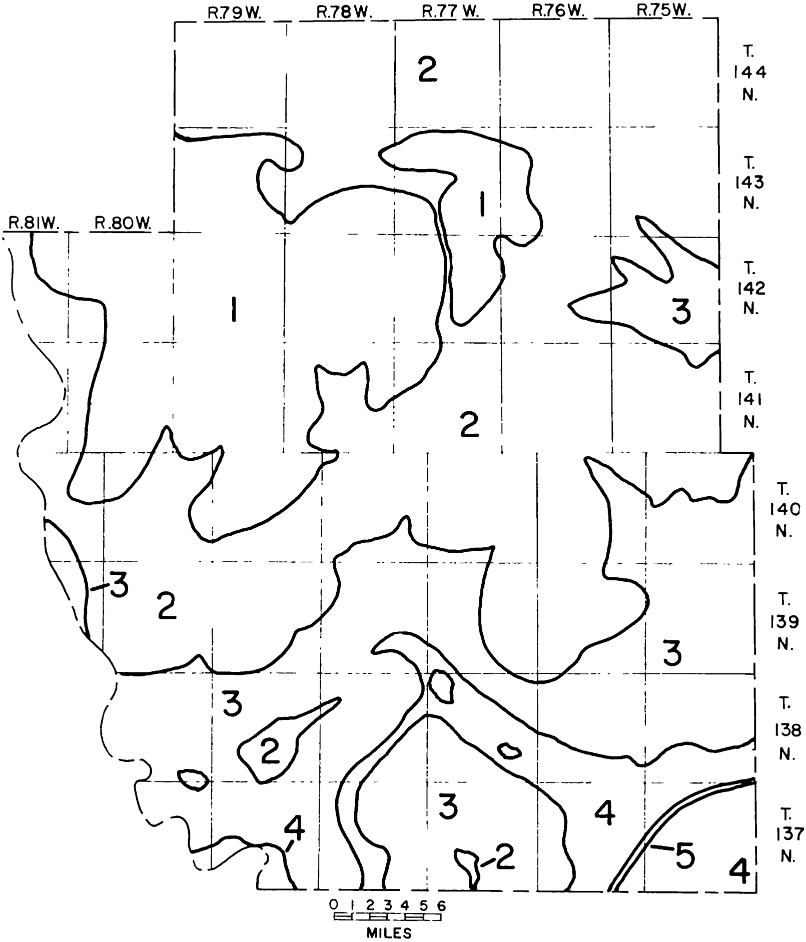


1, GROUND MORAINE; 2, END MORAINE; 3, SHEET MORAINE; 4, DEAD-ICE MORAINE; 5, LAKE PLAIN; 6, OUTWASH PLAIN; 7, DUNE SAND; 8, COLLAPSED OUTWASH TOPOGRAPHY; (BLACK) EXPOSED BEDROCK.

FIGURE 2—Glacial landform and exposed bedrock map of Burleigh County.

even while stagnant drift-covered Burnstad ice remained. The Streeter ice front was quite lobate, and a number of narrow end moraine loops were deposited along its margin. Part of one of these loops was deposited along the northern boundary of the county. Proglacial outwash was deposited upon and adjacent to the stagnant Burnstad ice. As this buried ice melted, the outwash was "let down" or "collapsed" into a hummocky topography. Clayton (3) estimated the melting of Burnstad ice may have continued for over 2,000 years.

Data collected from test drilling were used to prepare a drift isopach map. The proglacial drift is thickest in the buried channels of the southern and eastern parts of the county. The valley fill



TERTIARY: 1, TONGUE RIVER FORMATION; 2, CANNONBALL FORMATION.
UPPER CRETACEOUS: 3, HELL CREEK FORMATION; 4, FOX HILLS FORMATION; 5, PIERRE FORMATION.

FIGURE 3—Bedrock geologic map of Burleigh County.

ranges from about 100 to 200 feet thick. The drift in the Burnstad and Long Lake moraines ranges in thickness from zero where the bedrock crops out to a maximum of 165 feet and 173 feet, respectively. The average drift thickness in both moraines is about 60 feet. The drift in the Napoleon moraine ranges from about 5 to 20 feet thick and averages about 10 feet thick.

BEDROCK GEOLOGY

The mapping of the bedrock exposures showed that the Late Cretaceous Fox Hills and Hell Creek Formations, and the Tertiary Paleocene Ludlow, Cannonball, and Tongue River Formations are present at the surface in Burleigh County. Data from test drilling has shown that the Late Cretaceous Pierre Formation, the oldest bedrock directly underlying the glacial drift, occurs in a buried channel in the Long Lake area (figure 3). The Fox Hills Formation crops out along the valley walls of Long Lake and occurs beneath the buried channels in the southern part of the county. The Hell Creek Formation is exposed in the bluffs south and southeast of Bismarck, Menoken, and McKenzie, and it underlies part of the Long Lake moraine. The Cannonball Formation, including the very thin Ludlow Formation, is the most widespread surface formation in the county. It is exposed along the Missouri River Trench and in the bluffs north and south of Bismarck, Menoken, and McKenzie. It underlies the drift of the Burnstad and Napoleon moraines. The Tongue River Formation is exposed in the buttes and ridges east and west of Baldwin and Wilton.

A topographic map of the bedrock surface, prepared mainly from drilling data, clearly shows the preglacial stream patterns. The major stream patterns in the southern and eastern parts of the county include numerous tributary and main channels of the ancestral Heart and Cannonball Rivers. The main channels can be traced from Morton County, across Burleigh County toward Long Lake, and into Kidder County. Another major stream pattern occurs in the northwestern corner of the county, and it includes a tributary channel of the ancestral Knife River. During the Wisconsin glaciation these preglacial stream channels were nearly filled with proglacial accumulations.

CONCLUSIONS

It was concluded from the study of the geology of Burleigh County that a physiographic classification on the subdistrict level can be quite useful and justified. This was found to be especially true when an attempt was made to organize and explain the geology on a county-wide basis.

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THE INFLUENCE OF AMINO ACID CONCENTRATION ON INTESTINAL EFFLUX OF AMINO ACIDS[1]

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We reported some evidence (N. Dak. Acad. Sci., 17:72, 1963) for the efflux of amino acids into the lumen of the small intestine of the rat perfused with physiological saline. At that time we also reported that this efflux was affected by the presence of one or more amino acids in the perfused solution. Since that time we have perfused as many as seventeen amino acids simultaneously. These amino acids were perfused at concentrations near the level found in the free form in the plasma of the rat. A series of perfusion experiments were carried out at multiples of this concentration; namely, 1) one-half the concentration found in the plasma, 2) comparable to the concentration in the plasma, and 3) twice the concentration found in the plasma. In the current experiments the amino acid mixtures were carried in Krebs Ringer bicarbonate buffer rather than sodium chloride. Measurements were made by column chromatography, and by simultaneous radioactive monitoring of the various amino acid mixtures. Evidence indicates that there is a relationship in the efflux of certain of these amino acids dependent upon the concentration of the material perfused and perfusion time. In all cases tested, except for aspartic acid, net absorption was demonstrated by the intestine. In this latter case here was a definite net secretion or efflux of the amino acid into the lumen during the experimental period; however, by tracer studies it has been shown that aspartic acid was absorbed even though the net amount was greater in the direction of efflux. Evidence is also presented that these amino acids are transported against concentration gradients.

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THE EFFECT OF EXCESS MERCURY CHLORIDE ON SERUM ALBUMIN SULFHYDRYL STABILITY AT pH 2

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ABSTRACT

Exposure of Bovine Serum Albumin (BSA) to pH 2 conditions (no salt, adjusted with HCl) caused no significant change in sulfhydryl content whether mercury chloride was present or not. Where excess mercury chloride was added to the 1-2% BSA exposure systems a back titration with standard BSA gave the net titer for the exposed material. The titrations were amperometric—in some instances at the dropping mercury electrode, but in most cases at the more sensitive rotating platinum wire electrode, mercury coated, RPtWE(Hg). Some pH 2 titrations had to be performed in the presence of air, which had no significant effect on the titers. Simply applying a potential more positive than that of the interfering oxygen cathodic wave was all that was required. Low chloride ion concentrations necessary for titrations at pH 2 extends the mercury wave to more positive potential.

The extreme stability (>600 hrs) of the BSA —SH titer at pH 2, whether mercury chloride was added or not seems to preclude the presence of the Linderstrøm-Lang thiazoline ring in BSA. In fact, preclusion of any masked —SH of the ovalbumin or β-lactoglobulin type also seems reasonable. As well as avoiding "Huggins crossing", if any, our use of more dilute solutions should explain our inability to observe the rapid disappearance of —SH found by Simpson and Saroff under similar conditions. A manuscript covering the present and more recent work is in preparation.

CHEMICAL AND PHYSICAL STUDIES ON THE PIGMENTS PRODUCED BY THE DWARF BUNT FUNGUS, *TILLETIA CONTRAVERSA* KUHN

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Tilletia contraversa Kuhn is a smut fungus which grows parasitically on wheat and other grasses. It causes a disease on wheat know as dwarf bunt. When this organism is cultured for several days on artificial media, a reddish-orange color develops. On solid media, the pigmentation is first formed in the vicinity of the fungus, then it gradually diffuses or forms throughout the entire medium. In aerated liquid cultures, the pigmentation is first noticeable as a tan coloration throughout the medium. This color gradually becomes dark reddish-orange over a period of one or two days.

At least one other pigment is present. It is water soluble and can be extracted from the actively growing mycelium with methyl or ethyl alcohol.

The reddish-orange pigment is not homogeneous. Part of it passed through a cation exchange resin and part passed through an anion exchange resin.

Electrophoresis experiments at pH 7.5 indicate that it bears an overall negative charge. When the culture filtrate was passed through a DEAE cellulose column it was clear that more than one pigment was present because seven colored bands were detected. Four of these bands contained small amounts of a compound which appeared to be chlorogenic acid.

Lyophilized samples of the culture filtrate (representing 100 ml of solution) were dissolved in 5 ml of water and placed on columns of Sephadex (G-25 and G-50). Loci of dark particles formed in the gel as the pigmented material moved down the column, giving a speckled appearance to the white column. These dark particles tended to coagulate when placed in a magnetic field. Some were attracted to a magnet. Analysis showed an iron content of approximately 20%. Other reactions indicated that this pigmented substance exists as a coordination complex of Fe^{+++}.

LAGOON STABILIZATION OF POTATO WASTES BY PHOTOSYNTHETIC PURPLE SULFUR BACTERIA

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ABSTRACT

The lagooning of domestic sewage and its subsequent stabilization depends on the metabolism of facultative bacteria which convert organic materials into bacterial cells. The organic loading of these lagoons is usually of such a nature that the conversion, from an anaerobic winter environment to an aerobic summer environment, is a rather rapid process and one which depends on the development of photosynthetic algae.

Sewage lagoons receiving potato wastes have generally been loaded to a level which cannot be quickly converted to an aerobic environment. Since anaerobic conditions prevail, proteinacious materials are converted to, amongst other things, hydrogen sulfide. In two such lagoons in North Dakota (Grafton and Park River) the preponderance of hydrogen sulfide resulted in the selecting out of the purple-sulfur, photosynthetic-bacterium *Chromatium.* This organism is able to convert hydrogen sulfide to carbohydrate compounds and elemental sulfur.

The results of determinations on a lagoon receiving potato wastes and city sewage in which the photosynthetic *Chromatium* was selected out, suggests that these organisms are very efficient in removing organic loading of the lagoon. The biological oxygen demand (BOD) reduction in the lagoon was about the same for the photosynthetic bacteria as it was during the later period in which aerobic conditions prevailed in the presence of algae.

PURIFICATION AND IDENTIFICATION OF THE INTERNAL CORK VIRUS OF SWEET POTATOES

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ABSTRACT

The internal cork virus of sweet potato was isolated and concentrated by a differential filtration method. The cork viral preparation was separated into 3 nucleo-protein components by gradient elution chromatography on a DEAE cellulose column. All three components were radioactively labeled by the incorporation of P^{32} phosphate from a nutrient medium. The major component was found to be infective and produced internal cork symptoms in the tuberous roots of sweet potatoes. The two minor components were not completely characterized. All three components showed viral-like particles in the electron microscope.

THE MECHANISM OF LIVER MICROSOMAL INORGANIC PYROPHOSPHATASE, PYROPHOSPHATE-GLUCOSE PHOSPHOTRANSFERASE AND GLUCOSE 6-PHOSPHATASE[1]

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ABSTRACT

From studies of inorganic pyrophosphatase (1,2), glucose 6-phosphatase (3), and pyrophosphate (PP_i)—glucose (glu) phosphotransferase (2), it is concluded that the three microsomal activities are catalyzed by one enzyme. Experimental support for this conclusion is 1) the failure of conventional means of enzyme purficiation to separate the three activities; 2) the parallel effect on the three activities by molybdate concentrations as low as 10^{-6}M and by mild heating in the absence of substrate; 3) the excellent agreement among Michaelis constants determined either directly or from inhibition studies; and 4) the close agreement between the observed rates of hydrolysis of PP_i and glu-6-P, when incubated with the enzyme individually and together, and the theoretically calculated rates based on the assumption of the involvement of a single enzyme.

Kinetic studies of PP_i-glu and mannose (man)-6-P-glu phosphotransferase reactions have been conducted. Glu inhibition of PP_i, man-6-P, and glu-6-P hydrolysis also was studied. Lineweaver-Burk plots of kinetic data obtained for the transferase reactions show increases in both slopes and intercepts accompanying decreases in concentration of second substrate held constant, and all lines converge on the x-axis. Maximal reaction velocities(V_m) for the transferase reactions extrapolated to infinite substrate concentrations were equal, and further, were equal to the V_m for the hydrolysis of PP_i, man-6-P, and glu-6-P in the absence of added glu. Added glu appears to function as a non-competitive inhibitor of the three hydrolysis reactions. These results are consistent with a mechanism which involves a) the formation of a binary enzyme phosphoryl donor complex, b) a dissociation leaving a phosphorylenzyme intermediate, and c) transfer of phosphoryl group from enzyme either to water (hydrolysis) or to glu or other sugar (transferase). Excellent agreement was found between experimental data for the various

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[2] Holder of a National Defense pre-doctoral fellowship.

hydrolysis and phosphotransferase reactions studied and theoretical relationships predicted by steady-state treatment of this mechanism.

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GUINEA PIG LIVER MITROCHONDRIAL AND SOLUBLE FRACTION PHOSPHOENOLPYRUVATE CARBOXYKINASES[1]

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ABSTRACT

Phosphoenolpyruvate carboxykinase (1) has been shown to occur both in the mitochondrial and soluble fractions of guinea pig liver (2). The mitochondrial and soluble enzymes have been purified 50 and 7 fold, respectively. The two enzymes were characterized for both the forward and reverse reactions and were found to have identical K_m's for ITP, oxalacetate (OAA), phosphoenolpyruvate (PEP), and IDP. In the direction of PEP synthesis the mitochondrial enzyme had the following metal specificity expressed in terms of activity observed with Mg^{++}: Mg^{++} 100%,Mn^{++} 91%, Co^{++} 67%, Fe^{++} 65%, Ca^{++} 15%. For the soluble enzyme values were Mg^{++} 100% Mn^{++} 160%, Co^{++} 68%, Fe^{++} 92%, Ca^{++} zero. These results were observed both with purified and crude preparations, thus minimizing the possibility that the differences were due to non-specific protein binding.

In the rat the soluble fraction enzyme has been shown to respond to hydrocortisone and insulin administration and alloxan diabetes (3). The mitochondrial enzyme did not respond to these treatments. The relative distribution of the carboxykinase in guinea pig mitochondrial and soluble fractions is not altered when the fractions are isolated at room temperature rather than 0°C.

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A TOXIC EXTRACT OF GOLDENROD, A PLANT USED MEDICINALLY BY NORTH DAKOTA INDIANS[1]

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ABSTRACT

Roots or air dried leaves and flowers of eight plants used medicinally by western North Dakota Indians were obtained from the Standing Rock Reservation. The results of tests for alkaloids were negative. Hot water extracts were concentrated by evaporation and tested for toxicity by intraperitoneal injection into mice. One of these, extract of leaves and flowers of *Solidago altissima* (goldenrod), was quite toxic. The water extract of 0.05 g dry leaves was fatal within an hour after injection. Mice survived oral administration of an extract of 0.1 g leaves. The poisonous material, which is dialysable, heat stable and non-volatile, has not been obtained in crystalline form. It is soluble in hot or cold water and methanol, but insoluble or only slightly soluble in other solvents tried (including ethanol, chloroform and ether). Infrared spectrophotometric study indicated that the methanol and water soluble fractions were the same, and suggested that it is a straight chain carbon compound.

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