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ELECTROCONDUCTIVITY OF HUMIC ACID AS A FUNCTION OF NEUTRALIZATION BY NaOH IN AQUEOUS MEDIUM[1]

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First Place Winner
A. Rodger Denison Student Research Competition

ABSTRACT

The conductivity curves obtained by titration of humic acid are presented. The results indicate some proportionality between the conductivity and the concentration of the humic acid. Significant lowering of the conductivity (up to 40%) was noted initially, followed by a much greater increase. The curves are composed of distinct linear slopes, indicating the presence of different active groups in the humic acid molecule(s). The results are discussed and interpreted.

INTRODUCTION

The humic acid (H-Ac) derived from North Dakota lignite has been a subject of industrial research in North Dakota because of possible economic importance. Youngs and Frost (1) gave a detailed discussion of the history and preparation of H-Ac, and the results of some industrial research.

It has been suggested that basic research on the physical chemical properties of H-Ac related to its structure would be of considerable importance. This paper describes an effort in involving a semi-quantitative analysis of the reactions of H-Ac with NaOH and HCl utilizing electro-conductivity techniques.

INSTRUMENTATION AND PROCEDURE

The apparatus used included a Pyrex vessel with water jacket for temperature control, dipping conductivity cell with platinized platinum electrodes, 1 KC A.C. impedence bridge with capacitive balance and oscilloscope indicator, stirring device, and an auxiliary Beckman glass electrode pH meter.

The H-Ac sample used was a non-uniform black-brown powder prepared from lignite by the Baroid Division, National Lead Company, Houston, Texas.

After careful cleaning the reaction vessel was filled with distilled water ($\Sigma = 2.5 \times 10^{-6}$ mho) and the temperature was brought

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

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to 30°C and stabilized. Stirring was started and a small sample of H-Ac in the form of a powder was added and the conductivity measured until equilibrium was reached. The resulting suspension-solution of H-Ac was then titrated in different runs with 0.1 N and 0.01 N NaOH, and 0.1 N HCl.

The titrant was added in increments and the conductivity of the suspension-solution was measured repeatedly until practical equilibrium was reached for each point. The conductivity in mho (ohm^{-1}) was plotted relative to the amount of titrant added in millimoles per gram of H-Ac (see figures 1-3). Overnight interruptions

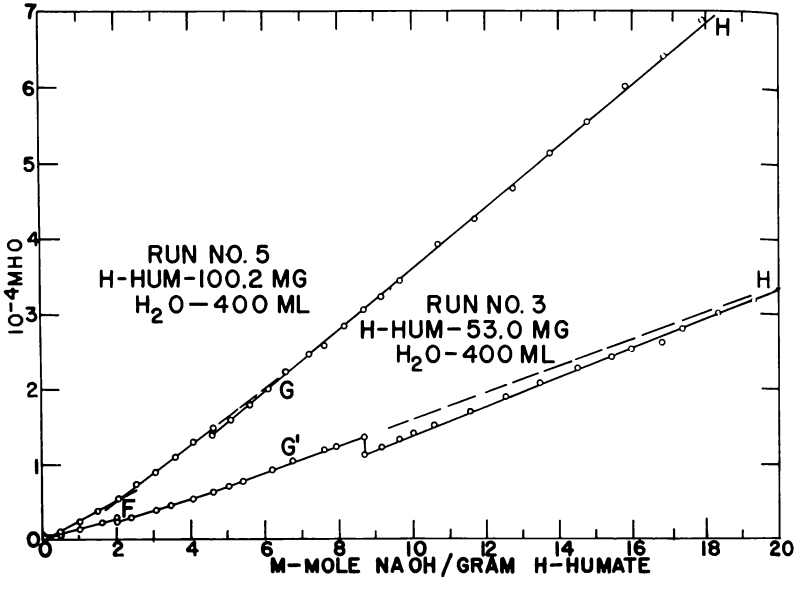

FIGURE 1—Conductivity *versus* 0.1 N NaOH added.

in the titration resulted in the discontinuities indicated by the arrows. The possible causes of such discontinuities are discussed later.

The conductivity measured in these experiments is actually the conductance of the cell in mho. As the cell constant is 1.00 ± 0.02, the conductance has approximately the same value as the specific conductivity Σ in $mho\text{-}cm^{-2}$.

THEORY

The basic theory of conductometric titrations is known (2). Basically the conductivity is proportional to the number of reactant molecules present, N, and their ionization constants and mobilities

(represented by the combined constant K) and given by the equation 1.

$$\Sigma = \frac{N}{V} K + \theta$$

where θ is the background constant due to non-reactant ions present. The slope of a plot of the conductivity, Σ, versus amount of titrant added, N, will be as expressed in equation 2.

$$\frac{d\Sigma}{dN} = \frac{K}{V}$$

where K is a constant, characteristic of the reaction, and V is the

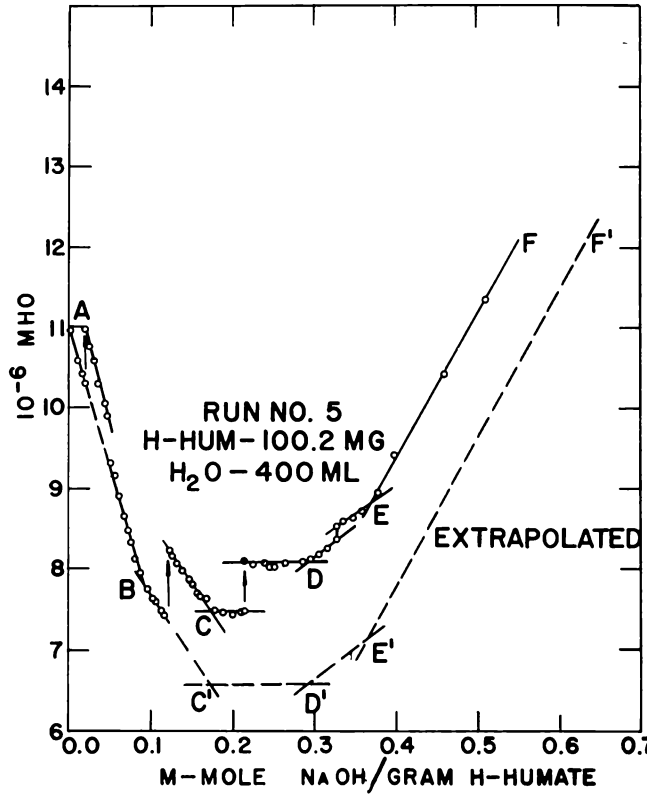

FIGURE 2—Conductivity *versus* 0.01 N NaOH added.

volume of the system. It is thus apparent that, if the volume change of the system is negligible, the plot will be linear, with the slope dependent on the reaction.

Now when several reactions are possible, for example a multibasic acid, each possible reaction will have its own particular, usually distinct, K. As the reactions will proceed serially according to the

value of each K with only a small overlapping as titrant is added, the graph of Σ versus N will be composed of several linear segments of different slopes with short transitions between each. Each segment corresponds to an acid-base reaction of a particular functional group. By measuring dN in moles of titrant per gram of unknown, determinations of the quantities of bases present may be made.

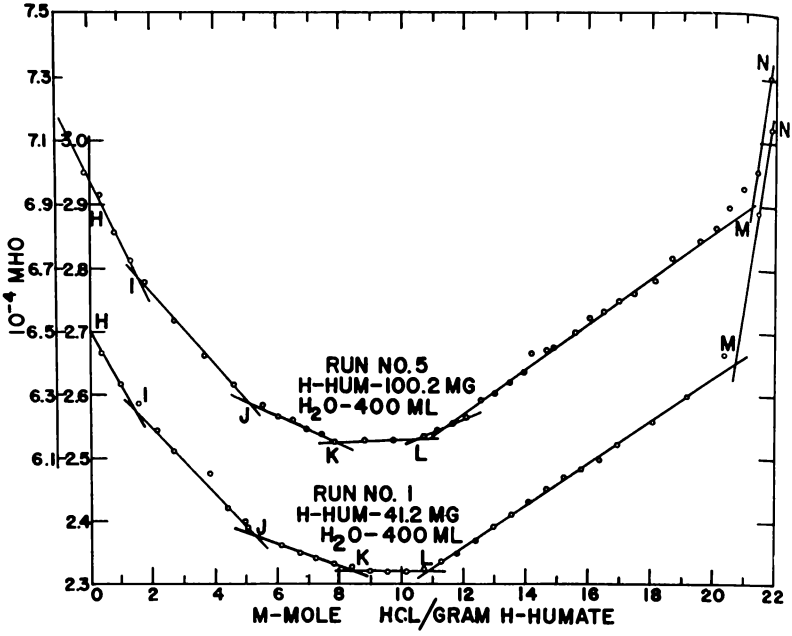

FIGURE 3—Conductivity *versus* 0.1 N HCl added.

The theory of conduction in a suspension-solution such as H-Ac is rather more complex due to the possible presence of colloidal particles. However K remains essentially constant and results may be interpreted on that basis.

RESULTS

The conductivity Σ versus millimoles titrant per gram of H-Ac is presented for a typical titration, number 5, in figures 1, 2, and 3. The results of other titrations are included where necessary for clarification.

Figure 1 shows the titration of suspended H-Ac by NaOH. Because the H-Ac is in the form of small solid particles, the active group reactions of the H-Ac are determined by exposure on the surface of the undissolved H-Ac rather than their activities. Consequently the only clear change of slope is at point "F," with a possible inflection near "G." There is no distinct endpoint, and the

forward titration was generally stopped on the basis of pH measurements.

Note that the slopes of the two curves show a tendency toward proportionality to the concentration of H-Ac.

Figure 2 shows the first portion of the curve of figure 1 on an expanded scale. The presence of distinct slopes indicates that there are some active groups which, through some form of dissolving or a "reopening" (disaggregation) of part of the surface molecules, are available to react with the NaOH according to the value of K for the particular functional group involved as outlined in the theory. At least five different slopes are present with the last extending to point "F" on figure 1. With the exception of the unexplained discontinuity around 0.05 mmole per gram of the slope AB, the discontinuities, indicated by arrows, appear to result partly from overnight evaporation and possible CO_2 dissolved from the air, both of which would increase the conductivity appreciably at the low values (10^{-5} mho-cm^{-1}) involved. The dotted curve represents the probable appearance of the curve had the titration been performed continuously. Note that only the value of θ (equation 1) has been affected, and that the slopes are unchanged across the discontinuities.

Figures 1 and 2 thus have a total of 6, possibly 7, different slopes.

Figure 3 contains the results of back titration with HCl. pH measurements indicate that slope HI is due to neutralization of excess NaOH, and that slope MN is due to excess HCl. Also point "M" falls within 4% of the starting point of the NaOH titration,

TABLE I

LENGTHS OF SLOPES IN MILLIMOLES TITRANT PER GRAM OF H-Ac

Forward Titration with NaOH		Back Titration with HCl	
Run No. 5 (fig. 1 & 2)		Run No. 1 (fig. 3)	Run No. 5 (fig. 3)
m moles/gm		m moles/gm	m moles/gm
Slope AB	= 0.090	IJ = 3.50	IJ = 3.80
Slope BC	= 0.083	JK = 2.85	JK = 3.10
Slope CD	= 0.120	KL = 2.65	KL = 2.40
Slope DE	= 0.077	LM = 10.80	LM = 9.90
Slope EF	= 1.63		
Slope FG	= 4.60?		
Slope GH	= no definite end point		
Total	—	19.80	19.20

indicating "M" to be the endpoint. Thus, four distinct slopes (IJ, JK, KL, LM) due to active functional group reactions are in evidence, with a possible fifth short slope bridging point "M."

DISCUSSION

It would be unwise to draw too many quantitative conclusions

from the data presented for several reasons. First, the sample may possibly contain some of the acid used in its preparation. Also the H-Ac powder tends to collect on the sides of the reaction vessel by the swirling action of the suspension, and an undetermined and variable portion is thereby partly removed from the system. Further, the effect of dissolved CO_2 which would react with NaOH, especially at high pH, and may shift the endpoints) is unkown. Nevertheless, certain useful qualitative conclusions may be drawn from Table I of approximate endpoint separations.

The most obvious feature of Table I is the lack of correspondence between the NaOH and HCl titrations. Because of the rough initially colloidal state of the H-Ac during the NaOH titration and the presence of NaCl inthe back titration, this is not unexpected.

Comparing the two back titrations with HCl it is noted that, while the total lengths agree within 3%, the individual slopes differ in length by 8 to 10%. This is much greater than the uncertainty of the endpoints, particularly on slope KL where the uncertainty on Number 5 would tend to increase the discrepency to 14-16%. This may indicate a slightly non-uniform composition of the sample.

The large (40%) decrease in the initial conductivity (figure 2), steep slop of AB and BC, and the initial acidity (pH = 5.4) of the solution-suspension indicates that these slopes may be due to hydrogen ion neutralization, with the two slopes of AB and BC resulting from different dissociation constants of the acid groups supplying hydrogen ions.

The increase in conductivity throughout the remainder of the titration (figure 1) is due partly to increased numbers of ions in solution resulting from the disaggregation of the solid H-Ac particles. The disaggregation also changes the color of the solution-suspension from nearly clear to deep brown. The proportionality between the slopes of the curves in figure 1 indicates that the solution of ions produced was not saturated at the concentrations used.

The most important conclusion that can be made about the data presented is that at least five, and possibly six or seven different active functional groups of H-Ac are involved in the reaction with NaOH. The four, possibly five, slopes evidenced on figure 3 (HCl titration) may definitely be ascribed to distinct reactions and corresponding active groups as may the first four slopes of figure 2 (NaOH titration). The remaining two or three slopes of figures 1 and 2 (EF, FG, and GH) may be due to 2-3 particular active group reactions. However FG and GH are most probably a single smooth curve resulting from simultaneous reactions with several of the less active groups as they are exposed on the surface of the solid H-Ac particles.

Further work with more finely divided and carefully purified H-Ac is required to clarify the results, particularly the NaOH

titration. Such H-Ac has been prepared in this laboratory and preliminary results are very promising.

The results compare with the results of H_2O vapor sorption experiments on the dry solid done by W. S. Hnojewyi (3) in which at least three different active sorption sites, corresponding to active groups, were identified. Also the preliminary sorption experiments on solid H-Ac done in the laboratory with ammonia and ethyl alcohol indicate the presence of different active sites in H-Ac.

SUMMARY

Experiments in the physical chemistry of H-Ac indicate the presence of at least five active groups in acid-base reactions and three in H_2O sorption. These functional groups have not been identified, nor have correlations between the base-acid active and the sorption active groups been established at this time.

ACKNOWLEDGMENT

Thanks are due to Dr. D. Schwartz for partial support of G. B. during the course of this investigation.

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THE PRESENT GENERAL LACK OF "SCORIA" IN TWO BURNING LIGNITE AREAS IN NORTH DAKOTA

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INTRODUCTION

The formation of baked and melted sediments, locally called "scoria", by the action of burning lignite has long been recognized in North Dakota. As early as 1805, Lewis and Clark reported the occurrence of "pumice stone" and "lava", and related it to burning lignite beds. They not only recognized the process by which "scoria" was formed, but actually produced synthetic "scoria" by baking and melting samples of the parent sediment in a furnace (Reid, 1948).

Their early observations and experiments proved far more accurate than some later observations attributing "scoria" to volcanic action. Subsequent investigations by the author and others, indicate that the basic factors necessary for "scoria" formation are: a suitable grade and thickness of combustible lignite; an overburden of alterable sediments or rocks; dissection through the overburden and lignite; and an agency of ignition. However, in the two largest areas of burning lignite in North Dakota, which appear to possess these necessary factors, no appreciable amount of "scoria" has formed.

The purpose of this paper is to determine other factors of "scoria" formation that are lacking in two presently burning lignite areas. This study is a part of a larger investigation of "scoria" which has been supported during the past three summers by the North Dakota Geological Survey. The writer also wishes to acknowledge the constructive criticism of Dr. W. M. Laird, State Geologist, the direction of the Geology Department faculty, University of North Dakota, and the help of the National Park Service and residents of Medora, North Dakota.

AREA OF INVESTIGATION

In the field study of "scoria", a proportionally large amount of time has been devoted to investigation of the "natural laboratories"

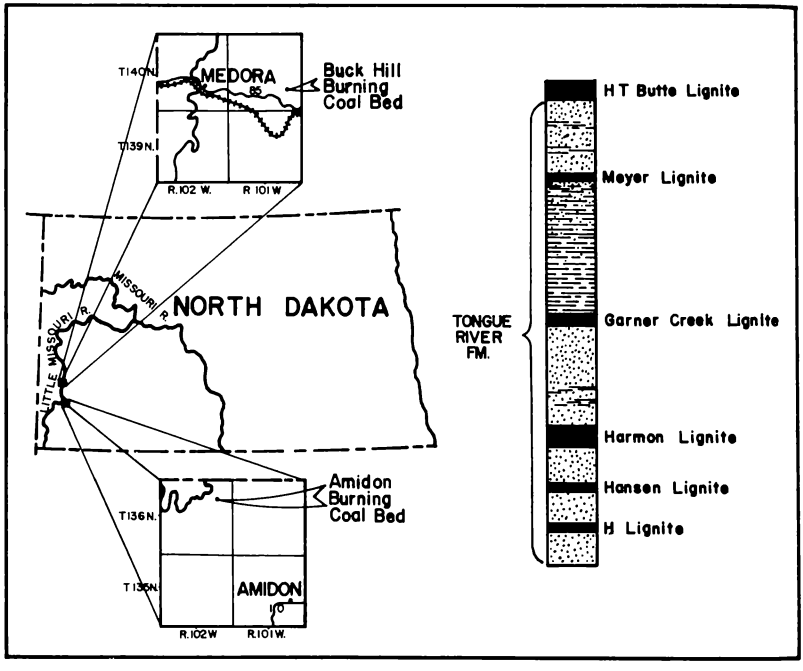

FIGURE 1—Location map and stratigraphic column.

provided by two large burning coal beds in southwestern North Dakota. One of these, the Buck Hill Burning Coal Bed, is located in the South Unit of Theodore Roosevelt National Memorial Park, SW¼, sec. 23, T. 140 N., R. 101 W., and the other, the Amidon Burning Coal Bed, is located approximately 11 miles northwest of Amidon, North Dakota, SE¼, sec. 11, T. 136 N., R. 102 W. (figure 1). The Buck Hill "burn" covers about six acres, almost twice the area of the Amidon Burning Coal Bed, and has burned at a more rapid rate. Active burning in the Buck Hill area was discovered in 1951, while the Amidon Burning Coal Bed area has apparently been burning for considerably more than sixty years.

The actual "burn" in the Buck Hill area is an almost flat surface, bordered to the north by a ridge having relief of about fifty feet. It is bordered elsewhere around its periphery by "scoria" which formed during an earlier period. While the general relief of the "burn" area is less than five feet, several small buttes do occur within it. A collapsed butte in the center of the burned area near its southeast border marks the area of initial ignition. Two other buttes having relief of less than twenty feet are located in the southwestern corner of the pit. A small amount of "scoria" was found to have formed near the more westernly of these buttes in 1963. Burning is presently taking place along the ridge to the north; along a front moving in a northwesterly direction, and to the west in a narrow tongue, in the southwest end of the pit.

In the Amidon Burning Bed area the surface is more irregular and slopes towards the east from a centrally located high area. During the past three years, areas of active burning have occurred in the north and northwest end of the pit; sporadically along the front facing towards the east, and in the southeast end. Small amounts of "scoria" have formed in the northern end of the of the pit.

"SCORIA"

Scoria is a local name applied to baked and melted sediments which show an extremely wide range of alteration, texture and appearance. As reviewed by Sigsby and Holland (1964) however, scoria is a misnomer for this material. From a petrologic standpoint the name scoria is restricted to a specific type of pyroclastic ejecta. Throughout this paper, the baked and melted sediments will be referred to as "scoria".

In the opinion of the writer, the minimal characteristics necessary to constitute "scoria" are cohesiveness through the action of fritting, sintering or melting, and a color change, if iron or organic compounds are present in sufficient quantity. In both burning areas there are small amounts of reddish to pinkish material which has the general appearance of "scoria", but when handled easily crumbles to material of ash-like consistency. The arbitrary decision not to classify this material as "scoria" is based on the observation that it weathers rapidly and would not be preserved long. Some "scoria",

however, may not undergo a noticeable color change if the compounds which change color under the influence of heat are not present. In this case, the classification as "scoria" rests on demonstrable cohesiveness caused by pyrometamorphic activity.

INVESTIGATION

During the past three summers, numerous measurements of firing temperatures were made in the two burning areas. Temperatures were measured by the use of a chromel-alumel thermocouple, connected to a direct reading potentiometer by insulated chromel-alumel leads. Corrections for this equipment, before and after the field seasons, indicated a variation of less than 10°F from known temperatures in the range of measurement.

The results of some thirty measurements, in the hottest zone of burning gases, indicates the temperature range to be from about 1,400-1,600°F in the two areas; the maximum temperature recorded was 1,622°F.

At this point, it became evident that the actual temperature range of "scoria" formation should be investigated. To accomplish this a series of Tongue River Formation sediments were selected, including samples from the two burning areas, which were representative of the range of petrographic composition and geographic location of sediments known to have formed "scoria". Individual small samples of these sediments were then heated in an electric furnace by progressive increments of 50°F to beyond the point of complete fusion. New, preheated and sized samples were used for each trial. Lignite was fired along with the samples in experiments with the same series to provide mild reducing conditions.

These tests indicated that reddening or darkening takes place between 1,500-1,600°F. Under mild reducing conditions, the same sediments attained the same coloration at slightly lower temperatures, and also tended to fuse at temperatures slightly lower than the same sediments in oxidizing conditions. Initial fusion, usually the first indication of cohesiveness, took place in a range between 1,900-2,100°F, and complete fusion occurred between 2,100-2,300°F.

Considering the probability that effects of color change and fusion are accentuated and accelerated in samples of small size, it is obvious that temperatures which would produce significant amounts of "scoria" are presently only rarely attained in the two burning lignite areas. It is also evident, from several lines of evidence, that the sediments overlying the burning areas are capable of being metamorphosed to "scoria". As indicated by the heating experiments, synthetic "scoria" can be formed from these sediments. Secondly, similar sediments have previously been metamorphosed to "scoria" in nearby areas. Thirdly, as compared to numerous other samples and thin sections, these sediments fall within the range of composition of sediments which are known to have formed "scoria".

A second possibility for the lack of "scoria" in these two burning areas might be an insufficient thickness and/or grade (relative quality) of lignite. It should be noted, however, that "scoria" has formed over these same lignites in nearby areas. The Harmon Lignite, which underlies the Amidon Burning Coal Bed area, has an average thickness of about sixteen feet. The Buck Hill area is underlain by what appears to be the HT Butte Lignite which has an average thickness in this area of about four feet. The Harmon is one of the thickest lignites in the southwestern part of the state, however, and even the bed underlying the Buck Hill area is thicker than some lignites which are known to have been involved in "scoria" formation elsewhere. The Harmon bed is a high grade lignite with a heating value of 6,062 B.T.U. (Brant, 1953). The lignite underlying the Buck Hill area has a heating value of about 7,000 B.T.U. (North Dakota School of Mines, Lab. No. 20,574). Both of these lignite heating values are in the upper heating value range of lignites which have formed "scoria". It would appear then, that neither the heating value or thickness of the lignites nor the composition of the overlying sediments will account for the lack of "scoria" formation in the two burning areas.

"Scoria" crops out as a resistant capping or partial capping on buttes, ridges and stream banks, and as an irregular bed of limited extension into the subsurface, below the summit of a ridge, butte or stream bank. Typically, the extension of "scoria" into the outcrop is twenty feet or less, and while an extent of fifty feet is not uncommon, "scorias" of greater extension are rare. The writer has found no "scoria" beds of large areal extent, as might be expected from "burns" of the Buck Hill or Amidon type in western North Dakota. Several old collapsed "burns" of this type have been found, however, in which no "scoria" but some ash occurs.

The association of "chimneys", and the coke-like layers at the base of much of the persistent "scoria" afford a clue to the conditions for formation of "scoria". "Chimneys" are the more strongly metamorphosed zones of melted sediments which tend to remain as erosional remnants above surrounding "scoria". The coke-like layers are composed of de-volatilized lignite and impurities.

From the observations above, and the previous elimination of some other factors it would appear that the reason for the lack of "scoria" in the two burning areas probably lies in the character of the overburden.

Publications by Rogers (1917) and May (1954) have explained the formation of "scoria" as the result of chemical processes, as well as thermal effects. From their theories, and personal observation, I would suggest the following ideal model for the formation of extensive "scoria".

Upon ignition, either spontaneously or through external agencies, fire spreads laterally along the exposed lignite and into the subsur-

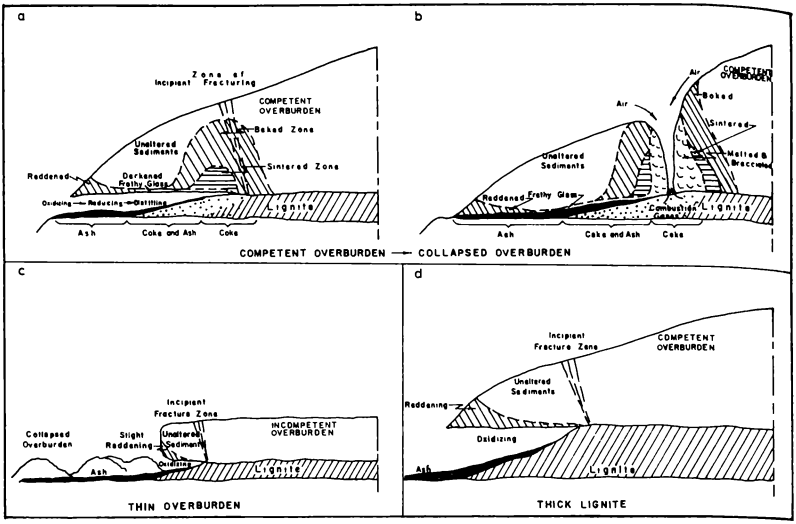

FIGURE 2—Generalized model for the formation of "scoria".

face back of the outcrop. At this time the air supply is unrestricted, providing strongly oxidizing conditions which may redden the sediments above for several inches. As burning continues, a small cavern forms in place of the burned out lignite. At this point, the overburden, if relatively thin and incompetent, will tend to collapse in thin slabs. exposing more lignite to the atmosphere (figure 2c). Burning takes place in a continuously oxidizing environment, and the effects on overlying sediments, if any, are caused by conduction of heat from the burning lignite and simple oxidation. If the overburden is thicker or more competent, the lignite will continue to burn back into the subsurface, extending the cavern and restricting the access of oxygen, causing a tendency towards reducing conditions. This reducing atmosphere may be enhanced by partial slumping of overburden, but if slumpage is complete the fire will be extinguished. Increasingly reducing conditions and the conservation of heat by the surrounding, poorly-conductive sediments provide an environment in which lignite undergoes partial distillation (figure 2a). Evidence of this process may be seen in the coke-like layers, or in the partially burned, but de-volatilized lignite which underlies much of the persistent "scoria."

Combustion of lignite with restricted oxygen supply produces carbon monoxide and water as the main products. Water is immediately reduced to hydrogen and oxygen, and the oxygen promptly unites with carbon to form more carbon monoxide. These two combustion gases, along with sulfur dioxide (contributed by gypsum and iron sulfide in the lignite), are all strong reducing agents. This potent mixture moves upward through interstices and fractures in the

sediments, transmitting heat by contact, rather than by conduction. Heat from the combustion gases may be great enough to melt the first few inches of sediment or rock to a frothy glass. Rapid heat loss results in a further gradation upward from simple melting, to fritting, and finally to baking without melting (figure 2a). The gases also tend to reduce oxidized elements to lower valence forms. Hydrous iron oxides are reduced to ferrous compounds in melted material, and to magnetite, as the gases tend to lose reducing capacity in higher zones. With continued migration, the hydrogen and carbon monoxide oxidize to water and carbon dioxide which retain enough heat to bake the sediments and oxidize iron compounds. The reduced iron compounds contribute dark coloration to the metamorphosed material, while the reddish and pastel colors are produced by oxidized iron compounds and organic bleaching.

As excavation continues beneath the overburden by burning-out of lignite, structural resistance to collapse is eventually exceeded and the overburden yields through fracturing (figure 2b). These fractures provide avenues for rapid access of air and it is probable that accumulated combustion gases and the newly supplied oxygen combine at a very high reaction rate, providing heat to fuse rock for many feet along the fracture.

Upon melting, some of this material may flow into the fracture, and in combination with collapsed rock fragments, clog the opening. Thus, the gases are diverted into other fractures to act on an increasing volume of material. These strongly fused and brecciated fracture zones are often preserved as erosional remnants ("chimneys") above the level of less resistant "scoria".

Therefore, the lack of "scoria" in the two burning areas is apparently due to an insufficient thickness or competence of overburden. In the Buck Hill area, the overburden averages about six feet in thickness, and with only slight excavation in the burned-out lignite, tends to collapse in narrow slabs (figure 2c). The thermal effects, if any, are produced by the limited conduction of heat from the burning lignite to the overlying sediments in a continuously oxidizing atmosphere. As the present combustion temperatures in the two areas are below those necessary to form "scoria", it would not be expected to form under these conditions. Beneath the larger butte in the southwest corner of the pit, however, a small amount of "scoria" has formed. Here the overburden is from fifteen to twenty feet thick, which must be very near the minimal thickness necessary for structural competence to permit distillation and allow gaseous transfer of heat to the surrounding sediments. The minimal thickness of overburden necessary to promote formation of "scoria" in any area varies with the structural competence of the overburden and the thickness of the underlying lignite. Structural competence is dependent on variables of texture, composition and cementation of the overburden.

The same general conditions exist over much of the Amidon Burning Coal Bed Area. To the west, however, the overburden thickens, and it is in this area that small amounts of "scoria" have formed. Deep collapse fractures indicate that the overburden thickness may attain twenty-five feet, but even here "scoria" is rare. In this case, the great thickness of the Harmon Lignite produces very large caverns upon burning, which provide relatively unrestricted air supply and oxidizing conditions until overburden collapse occurs (figure 2d). Once again, the slight thermal effects would be produced by conduction of heat from the burning lignite and simple oxidation. As the lignite under this thick overburden has not been burning actively for some time; and as the fire burning in a large cavern at the periphery of this area has been extinguished by overburden collapse, it does not seem likely that extensive "scoria" will form there in the future. In any case, a very thick, competent overburden will be necessary to sustain the large excavations until distillation of lignite could take place.

SUMMARY

The characteristics of the overlying sediments and the underlying lignites in the two areas both appear to be within the range of variation characteristic of known "scoria" formation. As the temperatures in the two burning areas are generally below those necessary to form "scoria", the lacking factor appears related to the thickness and structural competence of the overburden. Structural competence is dependent on composition, texture and cementation of the sediments; the minimal thickness of overburden is dependent on structural competence and thickness of the lignite. This minimal thickness of overburden which is necessary to sustain distillation of lignite, and to provide gaseous transfer of heat, is lacking in the two burning areas.

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PARASITES OF THE WHITE-TAILED JACKRABBIT IN SOUTHWESTERN NORTH DAKOTA[1]

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Third Place Winner

A. Rodger Denison Student Research Competition

A parasite survey was made on a population of white-tailed jackrabbits (*Lepus townsendi campanius* Hollister, 1915) from June, 1964, through February, 1965, in conjunction with an ecology and life history study being done by one of us (T. R. J.). The objective of this survey was to determine what parasites were present.

METHODS AND MATERIALS

Monthly collections of rabbits were made in the vicinity of Amidon, North Dakota, using .22 caliber rifles and 12 gauge shotguns. Examination of carcasses included: the external surface of the hide, subcutaneous musculature and connective tissue, viscera, heart, and 2 blood smears per rabbit. The intestinal contents were examined with a dissecting microscope; other portions of the carcass were dissected and examined macroscopically. Ectoparasites were collected by hand. Standard techniques were used in the preservation, staining, and mounting of the parasites.

RESULTS

Ten species of parasites were recovered in the survey. These and their incidence are presented in Table I.

DISCUSSION

Serial sections of greyish intestinal spots revealed the tissue stages of *Eimeria* sp. in the epithelium and connective tissue of the villi. Oocysts were commonly seen in fecal pellets, but several attempts to induce sporulation were unsuccessful. At least 5 species of *Eimeria* are known to occur in the intestines of wild and domestic rabbits of North America, all of them causing some degree of pathogenicity (Levine, 1961).

Three tapeworms of the genus *Cittotaenia* were found in the small intestine of 2 juvenile rabbits. Although the key given by Wardle and McLeod (1952) indicates that our specimens are *C. pectinata* and *C. p. americana*, we do have some doubt as to their specific identity. *Cittotaenia* spp. are widespread in North American lagomorphs. In areas adjacent to North Dakota they have been re-

[1]This study was supported in part by Federal Aid funds under Project W-67-R-5 of the North Dakota Game and Fish Department.

TABLE I

PARASITES OF THE WHITE-TAILED JACKRABBIT IN SOUTHWESTERN NORTH DAKOTA

	Examined No.	Infected No.	Infected Per cent
Protozoa			
Eimeria sp.	60	20	33.3
Tapeworms			
Cittotaenia sp.	60	2	3.2
Multiceps sp.	61	5	8.2
Raillietina (Raillietina) loeweni Bartel and Hansen, 1964	60	9	15.0
Taenia pisiformis Bloch, 1780	60	2	3.2
Roundworms			
Filariid sp.	72	5	6.9
Fleas			
Cediopsylla inaequalis (Baker) Jordan, 1925			
Hoplopsyllus affinis Baker, 1904			
Pulex irritans L., 1785			
Ticks			
Dermacentor andersoni Stiles, 1908	16**	9	56.3

*All rabbits (108) were infected with fleas; a few of each species were collected.

**After examining 16 animals, ticks were no longer collected, even when present.

ported from cottontails (*Sylvilagus floridanus mearnsi*) and snowshoe hares (*Lepus americanus*) in Minnesota (Erickson, 1944, 1947) and the latter host in Manitoba (Boughton, 1932).

Tapeworm coenuri of *Multiceps* sp. were found in the skeletal muscles, the thoracic cavity, and in the right ventricle of the heart. Although Christenson (1929) and Lyons, *et al.* (1960) have reported coenuri from the pericardial cavity and heart muscles of lagomorphs in Minnesota and Kansas, our discovery of a larval cyst in the ventricular chamber appears to constitute a new site of infection. Scoleces from the musculature were 2½ times larger than the ventricular forms (2.0 X 1.5 mm versus 0.8 X 0.6 mm), but measurements of the hooks showed overlapping ranges and no morphological distinctions. It is generally recognized that the coenuri of rabbits (*M. packi* and *M. serialis*) cannot be distinguished by their larval forms (Christenson, 1929; Baylis, 1932; and Erickson, 1944, 1947).

Raillietina (Raillietina) loeweni was found in the small intestine. This cestode was recently described by Bartel and Hansen (1964) as a common parasite of the black-tailed jackrabbit (*Lepus californicus melanotis*) in western Kansas. Although a very large genus,

only 3 species of *Raillietina* are known from North American lagomorphs.

Cysticerci of *Taenia pisiformis* were found in the visceral mesenteries and clustered around the rectum. This tapeworm is a cosmopolitan species commonly found wherever there is a predator-prey relationship between canines and lagomorphs. An appreciation of the parasite's wide distribution can be gained from Erickson's (1947) field and literature survey of helminths of cottontail rabbits.

Blood smears showed the presence of a sheathed microfilaria which could not be positively identified. Of the 3 filariid nematodes described from North American leporids, our specimens most closely resemble that of *Micipsella brevicauda* from the black-tailed jackrabbit in southwestern Kansas. Our stained specimens averaged 0.113 mm in length compared with 0.156 mm for *M. brevicauda*. The technical quality of our slides did not permit a critical morphological comparison with the description given by Bartel and Hansen (1962); however, our specimens definitely are too short to be *Dirofilaria scapiceps* or *D. uniformis*. Since the ranges of the white- and black-tailed jackrabbits overlap in South Dakota, Nebraska, and Kansas (Hall and Kelson, 1959), one might expect the same filarid in both hosts. Such a geographic and host distribution has occurred in the case of *Raillietina (R.) loeweni*.

All 108 rabbits checked for ectoparasites had one or more of the following species of fleas: *Cediopsylla inaequalis, Hoplopsyllus affinis,* and *Pulex irritans*. Since freshly killed rabbits were not kept separate, data are not available on the number of species of fleas per host.

Dermacentor andersoni is known to parasitize rabbits in the larval, nymphal, and adult stages (Matheson, 1950), but our collections contain only adults. The ticks were most frequently seen on the head and neck region, but as the summer progressed their relative abundance declined. Western North Dakota possesses overlaping ranges for *D. andersoni* and *D. variabilis*, however none of the latter species were seen.

Although only a facet of a larger ecological problem, the present study is the first attempt at surveying the parasitic fauna of any North Dakota lagomorph. Obviously, much work remains to be done on this and other species of rabbits.

SUMMARY

Portions of 108 white-tailed jackrabbits (*Lepus townsendi campanius* Hollister, 1915) collected from June, 1964, through February, 1965, were checked for ecto- and endoparasites. The following species were found: *Eimeria* sp., *Cittotaenia* sp., *Multiceps* sp., *Raillietina (Raillietina) loeweni, Taenia pisiformis,* filariid sp., *Cediopsylla inaequalis, Hoplopsyllus affinis, Pulex irritans,* and *Dermacentor andersoni*.

ACKNOWLEDGEMENTS

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REACTION OF ZIRCONIUM TETRACHLORIDE WITH SOME ORGANIC ACIDS AND ETHERS

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ABSTRACT

Recent interest in the zirconium salts of carboxylic acids and zirconium derivatives of ethers is related to the many possibilities of their use. These uses may include catalysts for processes of polymerization (1), esterification catalysts for the preparation of epoxy resins (2), fuel additives (3), and waterproofing agents (4), to name but a few.

A search of the literature indicates that there have been limited studies on the reaction of zirconium tetrachloride with organic acids (5, 6, 7) and an even lesser amount of work done on the reaction with ethers (8, 9). Very little agreement is to be found among the various researchers as to the nature of the products obtained in these reactions. The reaction of titanium tetrachloride with acids has already been studied in some detail in our laboratories (10, 11).

In the present investigation, zirconium tetrachloride was reacted with acetic, propionic, n-butyric, and iso-butyric acids to give various stubstitution products. Compounds of the type, $ZrCl_4 \cdot 2RCO_2H$ and $ZrCl_2(O_2CR)_2$ have been identified. Also studied was the reaction of zirconium tetrachloride with a variety of ethers which lead in some cases to the formation of solid molecular addition complexes of the type $ZrCl_4 \cdot 2R_2O$. Similar titanium complexes have been studied in our laboratories (12). The products of the reactions have been purfified and characterized in regard to their chemical composition, melting or decomposition temperatures, infrared absorption spectra and molecular weights.

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MECHANISM FOR DEACYLATION OF TRYPSIN AND CHYMOTRYPSIN CATALYZED HYDROLYSIS

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ABSTRACT

It has been indicated repeatedly that substrates without an acylated-α-amino group adjacent to the ester or amide linkage are hydrolyzed very slowly by trypsin and chymotrypsin catalysis. Goldenberg and Goldenberg (1) showed that the acylated and nonacylated substrates have different pH optima, and that when the rates of hydrolysis are compared at their respective pH optima they are of similar magnitude.

Lysine methyl ester (LYME), a nonacylated substrate, shows a pH profile similar to a double titration curve. The rate increases to pH 5.8, remains constant to pH 7.2, and then increases to an optimum at pH 8.0. The first increase in rate levels at pH 5.8 and is characteristic of nonacylated substrates, whereas the pH optimum, 8.0, is similar to that of acylated substrates.

From LYME it appears as though the hydrolytic mechanism of nonacylated substrates must be similar to that of the acylated substrates. The mechanism of acylated substrates was postulated (2) to interact initially with a serine and then with an imidazole.

Substrates with a nonacylated-α-amino group, which is usually positively charged below pH 8, should proceed *via* the same mechanism as the acylated substrates. However, in the shift of the acyl group from the serine to the imidazole, the unshared pair of electrons of the imidazole may attack either the ester carbonyl carbon or the protonated amine. Since interaction only results in hydrolysis when the carbonyl carbon is attacked, this competition accounts for the decrease in the rate of hydrolysis compared to that of the acylated substrate. LYME is an exception in that its amine group is not charged

at the pH optimum of acylated substrates. Hence it follows the same mechanism and gives the same optimum as acylated substrates.

If the pH is lower than 6.2, the imidazole will be protonated and the positively charged nonacylated-α-amino group will be repulsed preventing the shift from the serine. Since the rate of hydrolysis is pH independent between 5.8 and 7.2, it is proposed that a carboxyl group, adjacent to the serine in the enzyme (3), attacks the substrate carbonyl carbon and the hydrolysis in this pH range proceeds *via* an anhydride intermediate.

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SEPARATION AND IDENTIFICATION OF THE CAROTENOID PIGMENTS FROM FLAX RUST UREDOSPORES, *MELAMPSORA LINI (PERS.) LEV.*[1]

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INTRODUCTION

Few studies on the carotenoid pigments of rust uredospores have been reported (1, 2, 3). Irvine *et al.* (4), in a limited study of carotenoids from one sample of flax rust, reported *beta*-carotene, *gamma*-carotene and lycopene as the major carotene pigments. The carotenoid pigments have further been postulated to function as protective agents against photochemical reactions and as oxygen carriers in fungi and higher plants (5, 6, 7). This study was undertaken to isolate and identify the carotenoid pigments of the flax rust uredospores, *Melampsora Lini* (*Pers.*) *Lev.*, and to follow the relationships between the various carotenes during germination.

MATERIALS AND METHODS

Flax rust uredospores, Race I, freshly harvested from flax plants grown in the greenhouse, were used in all determinations. Carotenoid identifications were carried out on both germinated and nongerminated spores. Three-tenths gram samples of spores were germinated from 2 to 8 hours on demineralized water. The germinated spores were filtered on a vacuum filter, transferred to a Wig-L-Bug homogenizer, and ground for 8 minutes with a mixture of 0.3 gm of glass beads

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and 2.0 milliliters of 2N methanolic NaOH using steel balls to rupture the cells. When ungerminated spores were extracted, 0.3 gm of fresh spores were ground by the same method taking care not to warm the mixture above room temperature. The isomerization of carotenes is reported to be caused by heat, light and oxygen (3, 4). Consequently, attempts were made to keep the isomerization of flax rust carotenes to a minimum by storage of samples under nitrogen in a cool, dark place while extraction and identification was being performed. After the ground mixture was transferred to a large mouth test tube, 0.5 ml of water and 10 ml of ethanol were added, and the mixture was allowed to saponify at room temperature overnight.

The lipoidal materials were extracted and fractionated by the method of Rothblat, Ellis and Kritchevsky (8) (figure 1). The unsaponifiable fraction was dried over anhydrous Na_2SO_4 and evapor-

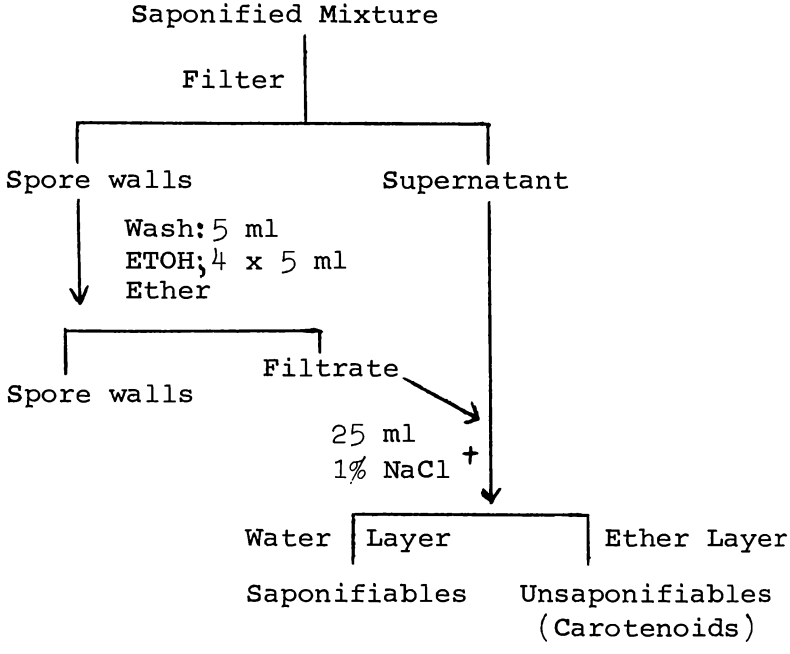

FIGURE 1—Extraction of Carotenoids and other unsaponifiables (Ref. 8).

ated to dryness under nitrogen. This fraction was taken up in petroleum ether (b. p. 40-60° C), and a white fluff was separated by centrifugation. The resultant supernatant was decanted and chromatographed by thin-layer chromatography (TLC) on aluminum oxide G:Silica gel G (1:1 wt/wt). A petroleum ether:benzene (70:30) solvent system was used for most separations. Known reference

samples shown in Table I were *beta*-carotene obtained from Sigma Chemical Co. and lycopene extracted from tomatoes. R_f values of the separated carotenoid pigments from the flax rust were compared with R_f values of the reference samples. The sections of the adsorbent containing the separated carotenoids were scraped from the thin-layer plates, the carotenoids eluted with ether: acetone (1:1) and their visible spectra determined in petroleum ether and other solvents (Table II).

RESULTS AND DISCUSSION

Results showed that the major carotenoids in both germinated and nongerminated flax spores were *beta*- and *gamma*-carotene, with *gamma*-carotene being approximately nine times more abundant than *beta*-carotene (Table I). An unidentified pink pigment was also found which moved slightly slower than the *gamma*-carotene on TLC. Several polar carotenoids were observed to be present in small amounts, but no lycopene was found in any of the flax rust samples examined.

Table I lists the flax rust carotenoids in order of decreasing R_f values on thin-layer plates when developed with the petroleum ether:benzene solvent systems. Sample number 1 had R_f values similar to known *beta*-carotene. Generally *gamma*-carotene and lycopene follow *beta*-carotene on TLC, whereas *alpha*-carotene precedes *beta*-carotene. Samples 2 and 3 could possibly be *gamma*-carotene and lycopene, respectively.

Final identification of the carotenoids was made by comparison of the visible spectra of the flax rust carotenoids with the known carotenoids and by comparison of literature value for the absorption maxima (Table II). *Beta*-carotene from flax rust spores absorbed light at the same wavelengths as known *beta*-carotene. Literature values for *gamma*-carotene correlated with the observed absorption maxima of sample number 2; thus, sample number 2 from the flax rust was tentatively identified as *gamma*-carotene. Sample 3 showed absorption maxima which were 12 to 14 millimicrons longer wavelengths than lycopene extracted from tomatoes. However, the absorption maxima of this pink carotenoid closely resembled a pink methoxylated carotenoid reported by C. R. Benedict (9).

Several authors have reported the metabolic conversion of carotenes to oxygenated carotenoids (5, 6, 7). One of the pathways proposed for this oxygenation occurs as *gamma*-carotene is cyclized to *beta*-carotene, which is oxidized to *beta*-cryptoxanthin (7). It was found in this study that the ratio of *gamma*-carotene to *beta*-carotene remained constant up to 8 hours after the initiation of germination, or, over the entire period of germination studied. This may indicate either that the oxidation of carotenoids had not begun during 8 hours of germination, or that a steady state system (i.e., precursor→carotene→oxygenated carotene) had been established early in the

TABLE I

THIN-LAYER CHROMATOGRAPHY OF CAROTENOIDS FROM FLAX RUST UREDOSPORES

Compound	Appearance	Source of Carotene	Pet. Ether:Benzene[a] R_f values in 70:30	80.20	Abundance % dry wt. of Spores
1. *Beta*-carotene	yellow	Flax Rust	0.8	0.55	.0097
Beta-carotene	yellow	Sigma Chem.	0.8	0.53	.089
2. *Gamma*-carotene	orange	Flax Rust	0.72	0.52	
3. Unknown 'a'	pink	Flax Rust	0.21	0.04	

[a]Aluminum oxide G:Silica gel G (1:1) thin-layer plates

TABLE II

ABSORPTION MAXIMA (mμ) OF FLAX RUST CAROTENOIDS

Compound	Source	Absorption Maxima Pet. Ether	$CHCl_3$	CS_2
1. *Beta*-carotene	Flax Rust	422,447,475	435,460,486	450,473,500
Beta-carotene	Sigma Chemical	425,447,475		
Beta-carotene	Literature (3)	425,450,482		
2. *Gamma*-carotene	Flax Rust	435,457,488	446,471,501	465,493,526
Gamma-carotene	Literature (11)	435,460,492		
3. Unknown 'a'	Flax Rust	454,482,512	468,499,532	
Pink Unkn.	Benedict (9)	454,482,516		
Lycopene	Literature (11)	440,470,500		

germination of flax rust. It is also possible that the oxidative process does not proceed through this pathway.

SUMMARY

1. The major carotenes of flax rust uredospores are *beta-* and *gamma*-carotenes.
2. No lycopene was found in the flax rust uredospores examined.
3. An unidentified pink pigment, possibly a methoxylated carotenoid, was detected.
4. The ratio of *beta-* to *gamma*-carotene remained constant over the germination period studied.

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FLORISTIC COMPOSITION OF THE SAND PRAIRIES OF SOUTHEASTERN NORTH DAKOTA[1]

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Throughout the more humid portions of the central North American grasslands, the tall grasses that stretched almost unbroken across the prairie peninsula have largely been obliterated to expose a foundation of soils rich enough and deep enough to support a corn belt economy. In Illinois, Wisconsin, and Iowa, and into Minnesota, the Dakotas, Nebraska, and Missouri, the level or gently undulating lands where six feet of rich black earth once supported grass six feet tall now grows domestic grass whose tassels reach even higher. In this region, in widely scattered localities, accidents of topography or soil formation have worked through the years of modern agriculture to prevent total destruction of the original ecosystem. These areas are the "sand prairies", places in which the soils were too poor, too dry, or too unstable to ever withstand the onslaught of the plow and its associates, and hence many have remained in virtually unchanged condition. They represent some of our last storehouses of scientific information about the natural community that since the last Pleistocene ice retreat has built the most fertile agricultural soil the world has ever known.

In recent years the Sandhills Region of southeastern North Dakota has received an increasing amount of ecological study. In particular, the Sheyenne delta, built when the river emptied into Glacial Lake Agassiz, is now undergoing rigorous examination of its forests, savannas, and grasslands, as well as its history, geology, geomorphology, and soils. In connection with this composite effort, 30 stands of sandhill prairie were studied during the summer of 1964. The stands were scattered through an area of approximately 125,000 acres of uncultivated grassland, including over 71,000 acres of the Sheyenne National Grassland, in Richland and Ransom Counties, North Dakota. These stands ranged from those that were virtually undisturbed through some that had been grazed or mowed for hay to varying degrees. A combination of quadrat and line-intercept methods was used to obtain estimates of certain vegetational attributes and extensive collections of vascular plants were made in the region. This paper concerns the floristic composition and its relationship among the major areas of studied sand prairie in North America. Nomenclature

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follows Hitchcock and Chase (1950) for the family Gramineae, and Stevens (1963) for all other vascular plants.

A total of 156 species of vascular plants was encountered in the sand prairies, representing 97 genera in 33 families (Table I). The majority of species are perennial and herbaceous, 27 species of annuals and nine of biennials accompanying 120 species of perennials, ten of which are woody. Based on presence percentages, ten grasses and a single sedge (*Carex pennsylvanica*) dominate the graminaceous vegetation. These grasses range from *Stipa comata, Poa pratensis, Andropogan hallii, Koeleria cristata* (%P = 100) through *Sporobolus cryptandrus, Elymus canadensis, Calamovilfa longifolia, Panicum leibergii,* to *Andropogon scoparius* and *Andropogon gerardi* (%P = 73). *Koeleria, Poa* and *Andropogon scoparius* are common throughout the North American grassland; *Elymus, Panicum,* and *Andropogon gerardi* are components of the tall grass prairie that ranges southeastward from North Dakota, while *Stipa comata* is probably the most widespread dominant of the mixed grass or mid-grass prairies straddling the 100th meridian. *Andropogon hallii, Sporobolus cryptandrus,* and *Calamovilfa longifolia* are characteristic arenicolous species, rarely encountered on heavier soils, but still relatively widespread in North America.

The principal shrubs and forbs, ranked according to percent presence (ranging from 100 to 80 percent) include *Artemisia ludoviciana, Lithospermum incisum, Ambrosia psilostachya, Solidago missouriensis, Tradescantia occidentalis, Erysimum asperum, Chenopodium leptophyllum, Physlis virginiana, Rosa arkansana,* and *Amorpha canescens.* Unlike the grasses which appear to have more cosmopolitan affinities, the leading forbs and shrubs are predominantly western. With the exceptions of *Amorpha* and *Solidago* the remaining prevalents are plants whose ranges extend through the Great Plains and Rocky Mountain region, rather than toward the prairie peninsula and deciduous forest to the east.

The predominant families represented are the Gramineae, with 24.2 percent of the total flora, the Compositae (21.0%), Cyperaceae (7.6%), Leguminosae (6.4%), and Rosaceae (5.7%). Thus, 64.9 percent of all species encountered were members of just five families. The grasses, sedges, sunflowers, legumes and roses are all large families well represented in temperate regions, and dominant in most grasslands in North America.

Through the years many studies have been made in various sand prairie areas in North America. In an effort to evaluate the floristic equivalence of the North Dakota sand prairies with other regions, comparisons were made with published species lists for sand prairies in nine states and two Canadian provinces.

Moyer (1910) and Ewing (1924) have studied the prairies of Minnesota, but the list of species was compiled from Rosendahl (1926) and unpublished checklists in the files of the junior author.

TABLE I

PRESENCE PERCENTAGES OF VASCULAR SPECIES IN THE SAND PRAIRIES OF SOUTHEASTERN NORTH DAKOTA AND COMPARATIVE TABULATION OF REPRESENTATIVE SAND PRAIRIE FLORAS IN NINE STATES AND TWO CANADIAN PROVINCES.

SPECIES	PRESENCE N. D. (%)	REGION										
		Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
EQUISETACEAE												
Equisetum arvense	10		x					x	x			
Equisetum kansanum	50	x	x		x	x		x	x			
GRAMINEAE												
Agropyron cristatum	23											
Agropyron repens	17	x								x		
Agropyron smithii	23	x	x	x	x	x	x	x				
Agropyron subsecundum	3	x	x	x				x				
Agrostis scabra	17										x	
Andropogon gerardi	73	x	x					x	x	x	x	
Andropogon hallii	97		x		x	x	x					
Andropogon scoparius	73	x	x		x	x	x	x	x	x	x	x
Bouteloua curtipendula	20	x	x				x	x	x	x		
Bouteloua gracilis	63	x	x	x	x	x	x	x				
Bouteloua hirsuta	27	x	x			x		x		x		
Bromus inermis	13											
Calamovilfa longifolia	80		x		x	x	x			x		
*Cenchrus longispinus	33				x	x		x	x	x		
*Echinochloa crusgalli	3							x		x		
Elymus canadensis	83	x	x		x			x	x	x	x	
*Festuca octoflora	10		x		x		x	x			x	

TABLE I (Continued)

SPECIES	PRESENCE	REGION										
GRAMINEAE (Continued)	N. D. (%)	Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
Koeleria cristata	97	x	x	x	x	x		x	x	x	x	
Muhlenbergia racemosa	20	x	x					x				
*Panicum capillare	10				x		x			x		
Panicum leibergii	77	x	x						x	x		
Panicum scribnerianum	60	x	x		x			x	x	x		x
Panicum virgatum	43	x	x		x	x		x	x	x	x	
Panicum wilcoxianum	27		x									
Poa compressa	3	x	x	x				x		x	x	x
Poa pratensis	100	x		x	x			x		x	x	
Redfieldia flexuosa	23				x	x	x					
*Setaria lutescens	7										x	
*Setaria viridis	13						x	x		x		
Sorghastrum nutans	10	x	x					x	x	x	x	
Spartina pectinata	10	x							x		x	
Sphenopholis obtusata	3							x				
Sporobolus asper	3										x	
Sporobolus cryptandrus	90	x	x		x	x	x	x		x		
Sporobolus heterolepis	37	x	x					x	x	x	x	
Stipa comata	100	x	x	x	x	x	x					
Stipa spartea	57	x	x	x		x		x	x	x		
*Triplasis purpurea	3					x		x				
CYPERACEAE												
Carex aurea	3											
Carex brevior	47		x								x	
Carex eleocharis	10			x	x		x					
Carex filifolia	10		x	x			x					

TABLE I (Continued)

SPECIES	PRESENCE	REGION									
Cyperaceae (Continued)	N. D. (%)	Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
Carex lanuginosa	10									x		
Carex pennsylvanica	100	x	x	x				x		x		x
Carex praegracilis	60											
Carex obtusata	3		x									
Carex sprengelii	3											
Carex tetanica	3											
Cyperus schweinitzii	63	x	x		x	x		x	x	x		
Eleocharis compressa	7											
COMMELINACEAE												
Tradescantia occidentalis	90	x	x		x	x	x	x				
JUNCACEAE												
Juncus balticus	33	x								x		
Juncus interior	7				x							
LILIACEAE												
Allium stellatum	10	x	x					x				
Lilium philadelphicum	3	x	x							x		
Smilacina stellata	3		x					x				
IRIDACEAE												
Sisyrinchium angustifolium	7		x							x		
SALICACEAE												
***Salix interior	10	x										
POLYGONACEAE												
Polygonum amphibium	7									x		
*Polygonum aviculare	3										x	
*Polygonum convolvulus	37							x				
*Polygonum ramosissimum	20							x				

TABLE I (Continued)

SPECIES	PRESENCE N. D. (%)	REGION Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
CHENOPODIACEAE												
*Chenopodium leptophyllum	90		x			x	x	x			x	
*Corispermum villosum	3					x						
*Cycloloma atriplicifolium	7		x			x	x	x		x		
*Salsola kali	73		x		x	x	x	x				
NYCTAGINACEAE												
Allionia hirsuta	50		x									
CARYOPHYLLACEAE												
Cerastium arvense	10		x	x								
Lychnis drummondii	3		x	x								
RANUNCULACEAE												
Anemone canadensis	23	x	x					x		x		
Anemone cylindrica	23	x	x	x				x	x	x		
CRUCIFERAE												
**Erysimum asperum	90		x									
*Lepidium densiflorum	73		x		x		x					
*Sisymbrium altissimum	3											
CAPPARIDACEAE												
*Cleome serrulata	3		x		x							
ROSACEAE												
Fragaria virginiana	7	x	x	x				x	x	x	x	x
Geum triflorum	3	x	x							x		
Potentilla norvegica	10		x	x						x		
**Potentilla pennsylvanica	40		x	x						x		
***Prunus americana	3	x						x				
***Prunus pumila	47		x									

TABLE I (Continued)

SPECIES	PRESENCE	REGION									
ROSACEAE (Continued)	N. D. (%)	Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
***Prunus virginiana	7	x						x				
***Rosa arkansana	80	x	x	x		x	x					
***Spiraea alba	30	x	x						x		x	
LEGUMINOSAE												
***Amorpha canescens	80	x	x			x	x	x	x	x	x	
**Melilotus alba	47				x			x		x	x	
**Melilotus officinalis	3				x			x		x		
Oxytropis lamberti	10	x	x	x						x		
Petalostemum candidum	27	x	x					x		x	x	
Petalostemum purpureum	53	x	x	x		x		x	x	x	x	
Petalostemum villosum	67	x	x	x	x	x						
*Strophostyles leiosperma	3											
Trifolium repens	3		x					x			x	
Vicia sparsifolia	3		x		x							
LINACEAE												
*Linum rigidum	37	x	x	x								
*Linum sulcatum	20	x	x	x				x		x	x	
OXALIDACEAE												
Oxalis violacea	10							x		x	x	
EUPHORBIACEAE												
Euphorbia esula	7		x									
*Euphorbia serpyllifolia	50		x					x				
ANACARDIACEAE												
***Rhus glabra	3							x	x		x	x
***Rhus radicans	20	x	x			x		x				x

TABLE I (Continued)

SPECIES	PRESENCE N. D. (%)	REGION Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
VIOLACEAE												
Viola pedatifida	37	x	x					x		x		
ONAGRACEAE												
Oenothera nuttallii	60	x	x			x		x		x		
**Oenothera strigosa	37		x									
UMBELLIFERAE												
Zizia aptera	10	x	x	x								
PRIMULACEAE												
*Androsace occidentalis	13		x					x			x	
ASCLEPIADACEAE												
Acerates viridiflora	47	x	x		x			x		x	x	
Asclepias ovalifolia	23	x	x									
Asclepias syriaca	7							x	x	x		x
Asclepias verticillata	20		x					x	x	x	x	x
BORAGINACEAE												
*Lappula redowski	7		x					x				
Lithospermum canescens	50	x	x		x			x	x	x		
Lithospermum incisum	97	x	x					x	x			
Onosmodium occidentale	20		x					x				
VERBENACEAE												
Verbena stricta	3							x		x		
LABIATAE												
*Hedeoma hispida	10							x		x		
SOLANACEAE												
Physalis virginiana	83		x			x			x	x		

TABLE I (Continued)

SPECIES	PRESENCE	REGION									
SCROPHULARIACEAE	N. D. (%)	Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
Pedicylaris canadensis	7	x	x							x		
Penstemon gracilis	3	x	x					x				
Penstemon grandiflorus	33	x						x				
CAPRIFOLIACEAE												
***Symphoricarpos occidentalis	43		x	x				x				
COMPOSITAE												
Achillea lanulosa	7		x	x				x	x	x	x	
Agoseris glauca	7	x	x	x								
Ambrosia psilostachya	97	x	x				x	x	x	x		
Antennaria neglecta	13		x	x				x		x	x	x
**Artemisia caudata	60	x	x			x	x	x	x	x		x
Artemisia frigida	63	x	x	x	x		x					
Artemisia glauca	73		x									
Artemisia ludoviciana	100		x					x				
Aster ericoides	73	x	x				x		x	x	x	
Brauneria angustifolia	3		x									
Chrysopsis villosa	77	x	x	x	x	x	x		x	x		x
Cirsium undulatum	40		x									
*Erigeron candadensis	77		x					x			x	
**Erigeron strigosus	17	x	x					x	x	x		
Helianthus maximiliani	3	x	x									
*Helianthus petiolaris	73	x			x	x	x	x		x		
Helianthus rigidus	60		x			x			x			
Kuhnia eupatorioides	3							x	x	x		
Lactuca pulchella	30	x	x					x				
Liatris aspera	13								x			

TABLE I (Continued)

SPECIES	PRESENCE	REGION										
COMPOSITAE (Continued)	N. D. (%)	Minn.	Man.	Alta.	Colo.	Nebr.	S. D.	Iowa	Wis.	Ill.	Mo.	Conn.
Liatris punctata	50	x	x	x			x	x				
Laitris pycnostachya	3	x						x	x		x	
Lygodesmia juncea	50	x	x	x	x	x	x					
*Lygodesmia rostrata	20		x			x		x				
Ratibida columnifera	3	x	x	x								
**Rudbeckia hirta	7		x					x	x	x		
Senecio plattensis	20		x			x		x				
Solidago canadensis	10	x	x		x			x		x		
Solidago graminifolia	10		x					x	x	x	x	x
Solidago missouriensis	97	x	x	x				x	x	x		
Solidago nemoralis	63	x	x	x				x	x	x	x	x
Solidago rigida	10	x	x					x	x	x	x	
**Tragopogon dubius	37											

(*annual; **bienniel; ***shrub.)

Shimek (1917, 1925) gives very complete lists for Iowa and Manitoba, and the Manitoba list is augmented by Scoggan (1957). The Illinois listing is also complete and explicit (Sampson 1921). The Colorado, Missouri and Alberta works are vegetation studies, but include good species lists (Drew 1947; Moss 1944; Ramaley 1939). Lists for Nebraska and South Dakota areas (Tolstead 1941, 1942) are incomplete, the less common species being omitted. In addition, while both studies are based on sand prairie vegetation, the samples are of very small areas and perhaps not necessarily representative of the states. The comparison with Wisconsin is based on a combination of the list given by Curtis and Green (1949) plus additional "Sand Barren" species listed in Curtis (1959). The former includes only those species with a presence value of 50 percent of greater in at least one of the four recognized prairie types. Connecticut (Olmstead 1937) was included only to test the effect of wide separation on floristic similarity, and the species list is also incomplete. In summary, the floras of the sand prairies of Manitoba, Iowa, Minnesota, Missouri and Colorado are probably quite accurately reported. Illinois and Alberta are perhaps over-represented, the available lists including species not necessarily found on sandy prairies, while Nebraska, South Dakota, and Wisconsin are under-represented. Connecticut is a matter of conjecture; total sand prairie flora could be less, greater, or equivalent to the given figure.

The number of taxa reported here for North Dakota compares favorably with the figures for most other regions. Family, generic, and specific representation is similar to Minnesota, Manitoba, and Colorado. Alberta, on the northwestern fringe of the great central grasslands, has a somewhat smaller total flora, while the more humid regions of Iowa, Illinois and Missouri to the southeast have a correspondingly richer composition (Table II).

It is well known that linear distance decreases the similarities between regional floras. As environmental differences become greater, the probabilities of overlapping ecological amplitudes among the component species decrease, and hence the species tend to sort themselves into gradient communities throughout the areal extent of the sand prairie habitat. No areas, even on a small scale, possess complete identity in their floristic composition, and as distance between areas increases, the similarity goes down. Nevertheless, the sand prairies of North America have evolved certain components that tend to bind them together. *Andropogon scoparius* and *Koeleria cristata,* for example, can be expected throughout the grasslands, along with *Amorpha canescens, Artemisia caudata* and *Chrysopsis villosa.* Thus, even areas as widely separated as Alberta and Connecticut share a low degree of floristic similarity due to the presence of these widespread species, many of which may be represented in these various regions by ecotypic populations.

TABLE II

NUMBERS OF VASULAR FAMILIES, GENERA, AND SPECIES REPORTED IN THE SAND PRAIRIES OF SOME REPRESENTATIVE AREAS IN NORTH AMERICA

Region	No. Families	No. Genera	No. Species
North Dakota	33	97	156
Minnesota	37	105	182
Manitoba	45	134	237
Alberta	29	81	132
Colorado	35	109	155
Nebraska[1]	21	57	73
South Dakota[1]	16	37	45
Iowa	65	198	397
Wisconsin[1]	52	91	117
Illinois	64	203	406
Missouri	41	128	186
Connecticut[1]	25	62	80

[1]Literature reports are based on dominants, prevalents, or most imporant components only.

Table III illustrates the relationsthip between the flora of the North Dakota sand prairies and those of 11 stations from the literature. Similarity has has been expressed in two ways; the number of species shared with North Dakota as a percentage of the total flora for each area and by use of the "Index of Similarity" (2w/a+b) (Curtis 1959). Differences in the two expressions are minor except in the cases of South Dakota and Nebraska, where the percentages of shared species are considerably higher than the index of similarity.

TABLE III

SOME COMPARISONS OF THE FLORA OF THE SAND PRAIRIES OF SOUTHEASTERN NORTH DAKOTA WITH OTHER NORTH AMERICAN REGIONS

Region	No. of Species Shared with North Dakota	Shared Species as % of flora	Index of Similarity (%)
Minnesota	74	40.6	44
Manitoba	109	46.0	55
Alberta	35	26.5	24
Colorado	36	23.2	24
Nebraska	35	47.9	31
South Dakota	28	62.2	28
Iowa	87	21.9	31
Wisconsin	42	35.9	31
Illinois	68	16.7	24
Missouri	38	20.4	22
Connecticut	14	17.5	12

This is due to the underrepresentation of the flora (as given by Tolstead 1941, 1942), coupled with the fact that the major species listed for those states are widespread dominants or subdominants that show up in the North Dakota sandhills. The Index of Similarity drops due to the inclusion in the index calculation of the relatively larger North Dakota flora. The reverse is true for Iowa and Illinois, where their larger floras lead to a lower shared percentage than might be expected.

Because of the inherent unity of sand prairie flora, complete dissimilarity is probably never reached between two widely disjunct areas. At the other extreme, regions of close proximity exhibit high degrees of congruence. A resulting curve of floristic correspondence as a function of distance (figure 1) is a logarithmic one, becoming asymptotic with the abscissa on a normal plot. Data for Kansas, Oklahoma, Texas, and Indiana, although not analyzed, are available in the literature, and should produce points that would fall on (or near) this curve. This illustrates that while sand prairies exhibit a low level of internal homogeneity, they are still characterized by evolutionary products whose environmental tolerances of the sandy habitat transcend regional divergences in macroclimate.

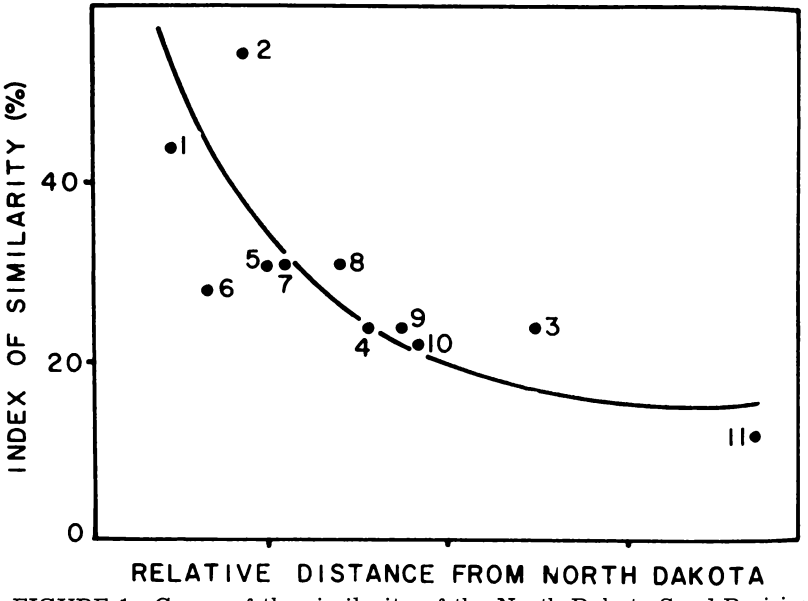

FIGURE 1—Curve of the similarity of the North Dakota Sand Prairies to those in other areas, illustrating a decreasing relationship with increasing distance. Points are (1) Minnesota, (2) Manitoba, (3) Alberta, (4) Colorado, (5) Nebraska, (6) South Dakota, (7) Iowa, (8) Wisconsin, (9) Illinois, (10) Missouri, (11) Connecticut.

In summary, the sand prairies of southeastern North Dakota are composed of varying assemblages of 156 species of vascular plants, representing 97 genera and 33 families, dominated by grasses, composites, sedges, legumes and roses. Major grasses show relationships with tall and mxied grass prairie areas with a strong admixture of species more or less restricted to sand environments. The forbs are predominantly of western derivation, showing affinities with the Great Plains and Rocky Mountain floristic regions. The North Dakota sand prairies have a high degree of similarity with those of Manitoba, Minnesota, and South Dakota, less with Colorado, Nebraska, Iowa, Illinois, Wisconsin, and Missouri, and still less with Alberta and Connecticut, although even these widely separated points have species in common.

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ANALYSIS OF SOME GENETIC TRAITS OF HUTTERITES IN NORTHEASTERN NORTH DAKOTA

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In this study the phenotype frequencies of a small, isolated population were compared with the corresponding phenotype frequencies of a large population which occupied essentially the same environment. The small population contributing to this study was the Forest River Hutterite Colony, located about 50 miles northwest of Grand Forks, North Dakota. The colony consisted of 41 individuals who belong to the religious faith known as the Hutterian Brethern. These people are direct descendents of a group forced to flee from their homeland in southern Austria in 1528 because of their beliefs in communal living, pacifism, and Anabaptist Protestantism. The group has retained its identity though they have been forced to emmigrate many times. The groups which are now located in the Dakotas settled this region in 1874.

Related work in this area has been reported by Glass (1952, 1953), Birdsell (1950), and Boyd (1950), along with others who have worked on the problem of genetic drift and orgin of races. Glass (1953) conducted a study of a Dunker isolate in Pennsylvania. Two of the traits which he investigated were used in our study: ear lobe attachment and double-jointedness of thumbs. He found that significant differences existed between the phenotype frequencies of the Dunkers and the Baltimore whites which were used as a base population.

METHODS

The phenotype frequencies of the sample groups were estimated quantitatively by observing the expressed phenotypes of certain gentically-determined traits. The traits chosen for study were limited as far as possible to those in which inheritance has been clearly established and in which alternative phenotypes are clear-cut, stable and non-adaptive. The five traits used in the present study are described as follows. (1) The ability to taste the chemical, phenylthiocarbamide (PTC), is inherited as a simple dominant characteristic (Montagu, 1959). Test strips for PTC taste ability were obtained from the General Biological Supply House, Inc., Chicago, and from Carolina Biological Supply Company, Burlington, North Carolina.

Unfortunately preliminary tests showed that the PTC test strips from Carolina Biological Supply Company were weaker and gave inconsistent results on individuals who were positive tasters. Members of the Hutterite Colony and the Midway School students were sampled with the stronger test paper which had given consistent results in preliminary tests. The University students were sampled with the weaker taste strips and the results obtained may have been influenced by this factor. (2) Free ear lobe is determined by a simple dominant gene (Glass, 1953), (3) The ability to roll the tongue; *i.e.*, to fold the sides of the tongue over the top, is also inherited as a simple dominant trait (Montagu, 1959). (4) Lack of doublejointedness (no distal hyperextensibility) is inherited as a simple dominant (Glass, 1953). (5) The inheritance of eye color is not completely understood. Researchers have used as many as 294 different categories for classification of this trait (Glass, 1953). We recognized that eye color inheritance is not simple and is likely influenced by several alleles or modifier genes, and even by metabolic disturbances during emryonic development. However, for purposes of analysis, the major genetic determinant of blue or brown eye color was assumed to be one pair of alleles, B and b. For this study we considered only pure blue eyes as the homozygous recessive condition, bb. All other shades were regarded as various expressions of a brown genotype, BB or Bb.

We compared the phenotype frequencies of these five traits in the Hutterite Colony with those of the general population of northeastern North Dakota. The general population was sampled by using freshmen biology students at the University of North Dakota, Grand Forks, as representatives of this area. In addition, the students at the Midway High School, Inkster, North Dakota, were used to sample these phenotype frequencies in the population within 10 to 15 miles of the Forest River Colony. The Midway School students served as a second basis for comparison.

RESULTS

Phenotype frequencies.—A summary of the sample data which shows both the numbers and the proportions of individuals of a particular phenotype in each group is presented in Table I. A cursory inspection of the data indicated similar proportions for all samples with regard to most of the traits; however, there were some exceptions which are of interest. The Hutterite Colony seemed to be different from the other samples for phenotype frequencies in ear lobe and eye color traits. Chi-square tests were applied to the data in order to evaluate the presence of statistical differences among all combinations of sample groups for each of the five traits. The chi-square values for these tests are presented in Table II. High chi-square values (greater than 7.88) indicated statistically significant ($P \leqq .005$) differences among the sample groups with regard to a particular trait. The statistical tests demonstrated a significant dif-

TABLE I

PHENOTYPE FREQUENCIES OF FIVE TRAITS FOR THREE SAMPLES FROM NORTHEASTERN NORTH DAKOTA

	PTC tasters		Ear lobe		Tongue roll		Thumb joint		Eye color	
	D	R	D	R	D	R	D	R	D	R
Hutterite Colony	.17	.29	.17	.83	.76	.24	.15	.85	.78	.22
(Forest River)	(29) +	(12)	(7) +	(34)	(31) +	(10)	(6) +	(35)	(32) +	(9)
University of	.59	.41	.48	.52	.70	.30	.14	.86	.51	.49
North Dakota	(95) +	(67)	(78) +	(84)	(114) +	(48)	(23) +	(139)	(83) +	(80)
Midway High	.72	.28	.57	.43	.72	.28	.11	.89	.49	.51
School (Inkster)	(171) +	(65)	(134) +	(102)	(170) +	(66)	(27) +	(209)	(115) +	(120)

D = dominant form of trait, R = recessive form of trait.
Numbers of individuals of D and R types are included in parentheses below each trait.

TABLE II

CHI-SQUARE VALUES OBTAINED FROM TESTS FOR HOMOGENEITY BETWEEN SAMPLES

	PTC tasters	Ear lobe	Tongue roll	Thumb joint	Eye color
Hutterites and University	2.0	18.0**	0.514	0.005	9.8**
Hutterites and Midway School	0.056	21.8**	0.217	0.340	10.0**
University and Midway School	8.2**	3.05	0.210	0.425	0.147

**P = .005

ference between the Hutterite Colony and the two samples of students from the University and Midway High School for proportions of ear lob and eye color characters. Another significant chi-square value was obtained between the University and Midway samples for PTC taste ability. The Hutterite Colony and Midway School proportions were very similar to proportions reported for other samples of Caucasian people (Parr, 1934). We felt that the abnormally low proportion for PTC taste ability which was observed for the University sample was due to the fact that weaker PTC test strips had been used exclusively in sampling this group.

Linkage analysis.—According to Neel and Schull (1954), some of the traits included in the present study are determined by genes which are located on the same chromosome; *i.e.*, they are linked. These traits are PTC taste ability, ear lobe attachment, tongue-rolling ability and eye color. We applied a statistical test which was designed by Penrose (1935) for linkage analysis to the data of the Hutterite Colony. This test utilizes intra-sibship comparisons for any two genetic traits. The test for linkage between PTC taste ability and ear lobe attachment gave a chi-square value of 1.53. The test for linkage between PTC taste ability and eye color gave a chi-square value of 1.60. And a test for linkage between eye color and ear lobe attachment gave a chi-square of 1.66. In order for the data to demonstrate significant evidence of linkage, the chi-square values would have to be greater than 6.63 at the one percent level. Our findings therefore did not give significant cause to assume linkage. However, the low chi-square values observed for this small population may be related to the fact that only five intra-sibship groups were available for comparison in the colony.

DISCUSSION

We have compared the phenotype frequencies of certain genetic traits of an isolated population with the corresponding frequencies sampled from a base population which occupied the same geographical area. It was shown that for two of the five traits examined, there were significant differences among the sample groups; *i.e.*, the small, isolated population (Hutterites) was significantly different from the University and Midway School student samples. According to the Hardy-Weinberg Law, autosomal genes will reach and maintain equilibrium frequency in any large, panmictic population after one generation (Stern, 1943). Therefore if the three samples of this study had been drawn from the same population we would expect them to have been similar in all phenotype frequencies. The University sample and the Midway School sample could not be shown by a valid statistical test to be significantly different. We can conclude that these two samples represent phenotype frequencies of a single panmictic population. However, since the Hutterite Colony exhibited significant differences in phenotype frequencies, we have concluded

that it was not a part of the panmictic population of northeastern North Dakota, and that it is maintaining itself as a separate genetic entity from the surrounding population.

SUMMARY

1. Analysis of five genetically-determined traits was carried out for members of the Forest River Hutterite Colony and for samples of students from the University of North Dakota and the Midway High School, Inkster.
2. The five traits used in the present study included PTC taste ability, ear lobe attachment, double-jointedness, tongue-rolling ability and eye color.
3. Significant differences were observed between the Hutterite Colony and the sample groups of students in the traits of ear lobe attachment and eye color.
4. Intra-sibship tests for linkage of PTC taste, ear lobe attachment and eye color were not statistically significant. However, this may have been due to the fact that only five sibships were available for analysis in the Forest River Colony.
5. It was concluded that members of the Forest River Hutterite Colony were genetically distinct from the general population of northeastern North Dakota.

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THE EDINBURG MORAINE OF NORTHEASTERN NORTH DAKOTA

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INTRODUCTION

General.—The final pulsation of the waning Pleistocene ice sheets in continental United States, the Valders (Thwaites, 1943, p. 136), occurred about 9,000 years ago, (Falconer, 1965, p. 609). However, the extent of its advance is controversial. Some geologists feel that the Valders halted in southern Canada (Elson, 1957, p. 1001); others believe it advanced far enough to form the Edinburg moraine in northeastern North Dakota (figure 1). Thus, the age determination of the Edinburg moraine is very important in the interpretation of the late Pleistocene history of the midwest.

A field study was begun in the summer of 1964 to learn more about the Edinburg moraine. The investigation was to define and evaluate the moraine through examination of its physical characteristics and its position with respect to surrounding features. This consisted of: 1. constructing a preliminary map of the area, 2. correcting and confirming it by field investigation, and 3. undertaking detailed studies of smaller selected areas for better identification and correlation of the moraine. The detailed studies included till fabric analyses, boulder and pebble counts, and complete particle size analyses.

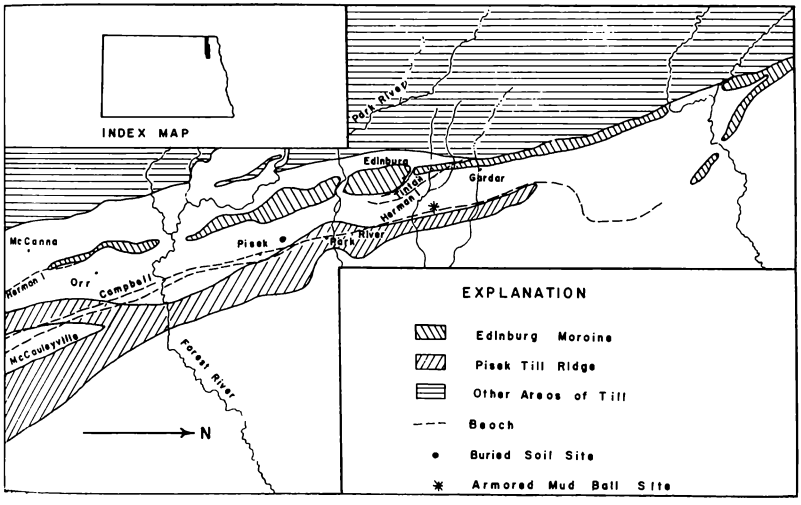

FIGURE 1—Geologic map of the Edinburg moraine.

Description of the moraine.—The Edinburg moraine (figure 1) extends from one mile North of McCanna, North Dakota to the Canadian border, where it merges with Darlingford moraine (Elson, 1954, p. 227a). The moraine is a very pronounced ridge between Edinburg, North Dakota, and the Forest River 20 miles to the south. At its widest point it is over 2 miles across; its height varies from more than 70 feet to less than 5. The most prominent part of the moraine begins near Edinburg, where the moraine rises steeply above the glacial Lake Agassiz plain. It widens slightly between Park River and Pisek and then tapers down to its arbitrarily defined southern end at the Forest River.

Just north of Edinburg, North Dakota, two beaches cross the moraine: 1. the Lower Herman, (Upham, 1896) right at the town, and 2. the Tintah, a few miles farther north. The moraine becomes more indistinct, but continues northward until it merges with the Pembina Escarpment. The sourthern end of the moraine merges with the Lower Herman beach near Orr, North Dakota, and disappears completely a few miles farther south. Many springs appear along the eastern edge of the moraine indicating that the Lower Herman beach follows along this edge up to Edinburg.

Previous Work.—Several researchers have contributed to the present understanding of the area of the Edinburg moraine. The earliest of these was Upham (1896) who published a definitive monograph on glacial Lake Agassiz. His work still serves as the basis for all present research. Another researcher who has contributed much to the understanding of this area is Elson (1954) who concentrated on the area immediately north in Manitoba, Canada. Unfortunately, most of his research is still unpublished.

More recently, Bliss and Laird (1963), Bell (1964) and Freers and Carlson (1964) have added to the information that was available up to the time of the writing of this report. A paper on Glacial Lake Agassiz by Dr. Wilson M. Laird is in press and will be available shortly.

ACKNOWLEDGMENTS

This research was carried out under a National Science Foundation Undergraduate Research Participation grant (GE4035) to the University of North Dakota, under the directorship of Dr. Wilson M. Laird. I would like to thank Dr. J. R. Reid who proposed the project, supervised the field work and advised me in the laboratory studies. I would also like to thank Mr. Helzler and Mr. Dahl, Soil Scientists for the Department of Agriculture, Grafton, North Dakota, for making more recent soils data aavilable to me. I also express appreciation to Chester Royce, Samuel Harrison, Dr. A. M. Cvancara, and to the numerous other individuals of the Department of Geology who assisted me in the various phases of this research.

METHODS OF INVESTIGATION

Mapping.—Aerial photographs (United States Department of Agriculture, Commodity Stabilization Service, 1952), and preliminary soil maps of the area (Department of Soils, North Dakota State University, 1964), were used to construct a working map of the Edinburg moraine. The preliminary Glacial Map of North Dakota (Colton, Lemke, and Lindvall, 1963) was found particularly helpful during this phase of the investigation. The working map was taken into the field where lithologic contacts and topographic relief were used to complete and verify it. In many places the topographic changes were so subtle it was necessary to walk out the moraine to trace it. River cuts and other exposures were examined wherever found, both to relate the moraine to the surrounding features and to determine variations within the moraine. Where the till had been modified by washing, concentrations of boulders were used to delineate the extent of underlying deposit. The study of the moraine was greatly aided by a series of 70-foot pits and associated 3-foot trenches excavated for the Minute Man missile system in the area.

Till Fabric Analyses.—Till fabric analyses, statistical studies of the orientation of particles in a till, are often used by glacial geologists to indicate direction of ice movement. The elongate pebbles in a till formed beneath a glacier (lodgement till) will generally have a major preferred orientation parallel to and a minor preferred orientation normal to the direction of ice movement (Holmes, 1941, p. 1350); ablation till, till accumulated on the melting ice surface, may show a preferred orientation normal to ice movement, but generally will show a random orientation of the particles. A preferred orientation normal to ice movement is characteristic of ablation till in end moraines, however.

Sites for fabric analyses were selected on the Edinburg moraine and an adjacent till ridge at a suficient depth to avoid the zone of active frost heaving. At all sites selected, a clean surface was cut in the till. Then, each pebble was removed and replaced by a tooth pick parallel to the long axis of the pebble. Pebbles showing no significant elongation were noted for completeness. The orientation of each tooth pick was measured with a brunton compass. A minimum of 100 pebbles was measured at each site. The data were plotted on Rose diagrams according to standard techniques (Holmes, 1941, p. 1308). The data are presented on figure 2.

A total of 1354 pebbles was measured from 12 selected sites. Eleven analyses were from the moraine; the twelfth was from a buried till ridge paralleling the moraine.

The till fabric analyses from the moraine suggest that the depositing ice came from a general northeast direction. However, the fabric of the till in the buried ridge shows an entirely different orientation (see figure 2). As only one sample was taken from the latter site, no conclusions are attempted.

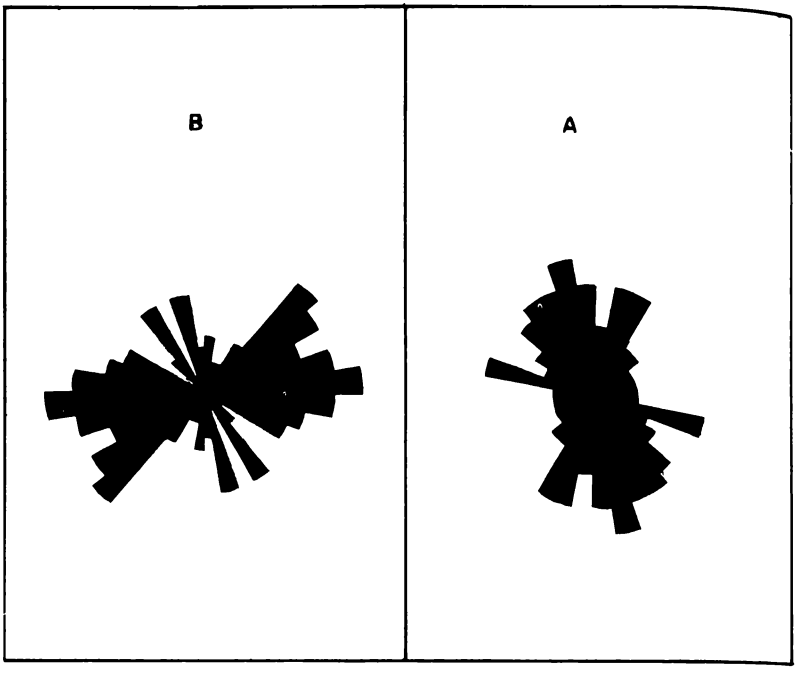

FIGURE 2—Typical till fabric analyses from (A) the Edinburg moraine and (B) the Pisek Till Ridge.

Pebble and Boulder Composition.—Pebble and boulder counting is a technique in which the composition of particles from a given site are identified and recorded to determine the average composition of that size particle. This analysis was made on both the moraine and the till ridge paralleling it (figure 1), to try and differeniate the two, so that the extension of the features outside of this area might be possible.

Boulder counts were made at one-mile intervals along the prominent part of the moraine between Edinburg, North Dakota, and Forest River, as well as in the section of the till ridge parallel to the moraine. At each site an area was selected and all cobbles and boulders over six inches in diameter wer identified. A total of 2924 boulders was involved in this aspect of the study, including at least 100 from each of 22 selected sites.

Pebble counts were taken in conjunction with each till fabric analysis and at additional random locations along the moraine. From the selected areas all pebbles greater than 1/8-inch in diameter were identified. These totalled 3266 pebbles from 26 sites, each site having at least 100 pebbles. The average composition of the pebbles and

boulders counted is given in Table I. It was found that the lateral variation of pebble and boulder composition within the moraine was greater than variations between the till ridge and the moraine. Thus, this type of analysis can not be used to distinguish the till of the Edinburg moraine from that of the associated till ridge.

TABLE I
AVERAGE PERCENT COMPOSITION OF PARTICLES FROM PEBBLE AND BOULDER COUNTS

Type	Boulders %	Pebbles %
Carbonates	7.8	50.6
Shale	——	30.7
Granitic	73.1	11.5
Basic Igneous	7.8	4.4
Others	3.9	2.8

Sediment Size Analyses.—To describe the Edinburg moraine in detail a complete particle size analysis was made on the till. Seventy-six representative samples were collected by an auger and on a one-mile grid pattern. In the laboratory the gravel-sand fraction was sieved from the samples. Then, the silt and clay fraction was further segregated by standard hydrometer analysis (Soils Manual, 1963, p. 186-202). The data from these analyses are to be programmed later this year on the University of North Dakota IBM 1620 computer to obtain statistical parameters to describe the till better.

The results of the particle size analysis are shown in figure 3. They show that both the till and associated fluvial sediments tend to be fairly coarse, having an abundance of sand and very little clay.

DETAILS OF SPECIFIC FEATURES

Edinburg moraine.—A thin boulder pavement is found within the moraine in a cut along the Park River (figure 4). This pavement separates the moraine into a lower, highly jointed compact till and an upper, less compact till. However, the till fabric and pebble counts of the two tills are essentially identical. It is suggested that the ice deposited the lower till, retreated slightly, possibly less than a mile, and then advanced again to deposit the upper till. The boulder pavement was formed between the two advances of the ice front.

Both undisturbed stratified lake sediments overlying the upper till at this same cut, and distorted lake sediment east of the moraine indicate that the Edinburg moraine may have once been almost submerged by glacial Lake Agassiz.

Lake and Surrounding Features.—The Edinburg moraine lies along the margin of former glacial Lake Agassiz, and is closely associated with it. At least two strandlines of this lake cross the moraine and wave erosion from the lake may be responsible for the indistinct nature of the moraine north of the town of Edinburg.

The Pembina Escarpment roughly parallels the western edge of

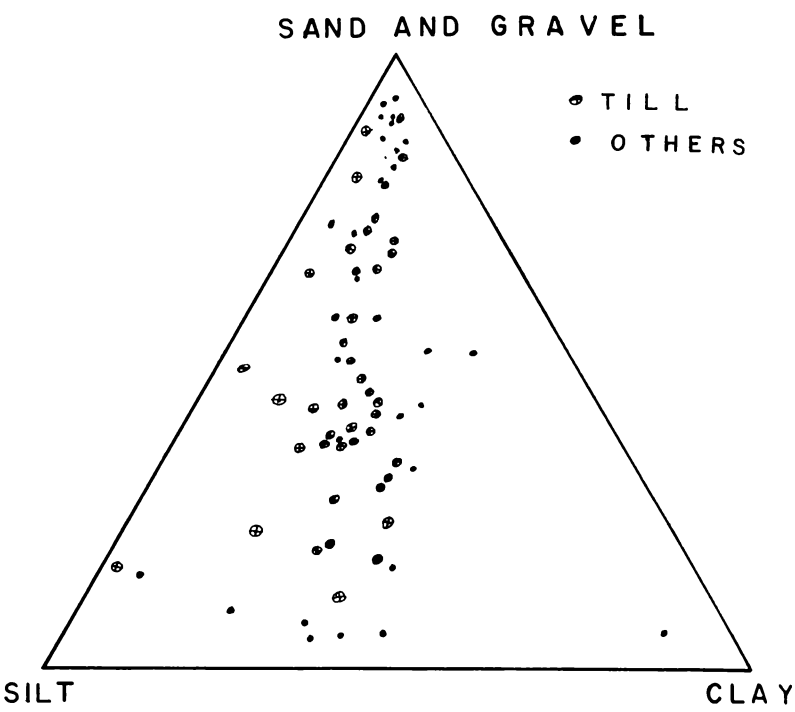

FIGURE 3—Triangular diagram showing the percentage of sand and gravel, silt, and clay in samples from the Edinburg moraine and surrounding area.

the Edinburg moraine. Between the escarpment and the moraine are thick sand and gravel deposits of the Elk River Valley; gravel pits in this area are up to 40 feet deep. Near Gardar, North Dakota the former Elk River cut through the moraine and the moraine merges with and becomes indistinguishable from the escarpment.

Pisek Till Ridge and Buried Soil.—A buried till ridge, here informally called the Pisek Till Ridge, lies parallel to and a few miles east of the Edinburg moraine. It begins near Mountain, North Dakota, and continues to 20 miles south of Grand Forks, North Dakota. Because of its low relief this ridge is recognized often only by a lag concentration of boulders at the surface. The Pisek Till Ridge is what is apparently mapped as the Edinburg moraine south of Highway 2 (Colton, Lemke and Lindvall, 1963).

No acceptable method was found to distinguish between the tills of the Edinburg moraine and the Pisek Till Ridge. However, one of the Minute Man missile excavations near Pisek revealed a buried soil between the two features. Where first observed, the organic-

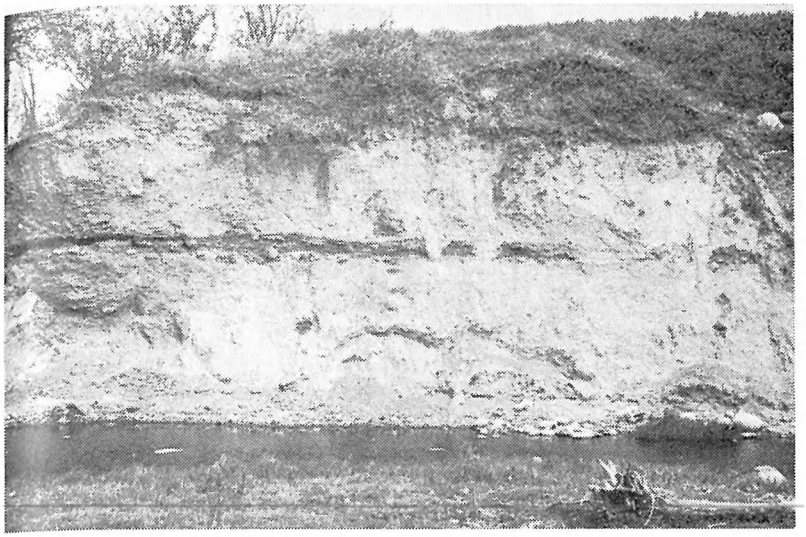

FIGURE 4—Boulder pavement in the Edinburg moraine. This occurs in a cut along the Park River. (SW ¼ Sec. 15, T. 157 N., R. 56 W.).

rich horizon of this soil is 6 inches thick and about 6 feet beneath the surface. It was traced for about one mile east; there it merges with the present soil and can no longer be disinguished. The buried soil was found nowhere else close to the moraine, but is present at additional sites in the Lake Agassiz plain (John Bluemle, oral communication, North Dakota Geological Survey). This soil horizon may pass under the Edinburg moraine, separating it from the till ridge, thereby making the till ridge significantly older than the moraine. Thus, care should be taken to differentiate the extentions of these features.

Armored Mud Balls.—Another Minute Man excavation site revealed an armored mud ball concentration (figure 5). This is a layer containing a concentration of sub-spherical balls of clay averaging one foot in diameter and coated with an armor of sand and gravel. This layer is found between an overlying coarse deltaic sediment and an underlying compact till. Crossbedding in the upper unit indicates that the mudballs may have come from the northeast. This would mean that the mud balls were not washed either from the Edinburg moraine or the escarpment, but were deposited by the retreating ice.

Homme Reservoir Site.—A sharp contact occurs between till and lake sediments in the east bank of Homme Reservoir (Sec. 24, 2. 157 N., R. 56 W.), an area geographically situated between the Edinburg

FIGURE 5—Armored mud ball layer in pit excavated for the Minute Man missile system.

moraine and the Pisek Till Ridge. In this area till, presumably related to the moraine, rests directly on extensive organic-rich lake sediments. Higher up, light brown lake sediments seem to be pushed into and abutt against the till. The unusual relationship of this upper lake sediment to the till is probably due to slumping. It is assumed that the lower lake sediment is from an early stage of Lake Agassiz. Till was deposited over this during the Edinburg stand of the ice. As the ice front retreated northward, the site was again inundated by waters of glacial Lake Agassiz I.

Boulder Pavements.—Concentrations of boulders cover the surface along the Pembina Escarpment near Edinburg, North Dakota. This represents a lag deposit formed by water, either from Lake Agassiz or surface drainage along the face of the escarpment. Because the boulders are around 25 feet above the level of the highest recognized strandline (Upham, 1896), it appears that the boulders were left upon removal of the fine particles by surface drainage.

CONCLUSIONS

1. The Edinburg moraine is physically distinct from the Pisek Till Ridge which lies several miles to the east. Thus, the moraine is much less extensive than previously mapped (Colton, Lemke, and Lindvall, 1963). It extends from the Canadian border to near Mc-

Canna, North Dakota, and does not include the boulder concentrations south of there.

2. Because the moraine is crossed by both the lower Herman and Tintah strand lines of Lake Agassiz I, it must be older than these features. The moraine, therefore, was not formed during Valders time but probably prior to or during early Lake Agassiz I. The moraine was probably constructed during late Mankato time (Dr. John R. Reid, oral communication).

3. Since the Edinburg moraine is not Valders in age, it is concluded that the Valders ice never advanced into North Dakota.

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KINETICS OF THE CHLORINATION-DEGRADATION OF URIC ACID IN AQUEOUS SOLUTIONS IN THE 10^{-4} TO 10^{-5} MOLAR RANGE

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ABSTRACT

Chlorine is used extensively in the purification of water. Numerous studies of the chlorination of water and nitrogenous materials in water have been carried out by various investigators.

The present paper is a report of a study on the kinetics of the reactions between chlorine and uric acid. The first step in these reactions is probably the formation of mono-N-chlorouric acid:

$C_5H_4O_3N_4 + Cl_2 \rightarrow C_5H_3O_3N_4Cl + HCl$.

The complete oxidation-degradation reaction is given by:

$C_5H_4O_3N_4 + 9Cl_2 + 7H_2O \rightarrow 5CO_2 + 2N_2 + 18\ HCl$

Uric acid of 0.5 to 1.0 x 10^{-4} M was reacted with chlorine solutions of 2.5 to 5.0 x 10^{-5} molar.

The log of residual chlorine was plotted vs. the time. A straight line relationship was obtained. For excess uric acid, the reaction is first order with respect to chlorine.

$$-\frac{-d[Cl_2]}{dt} = k[Cl_2]$$

The reaction constant k depends strongly on the pH of the solution.

Future studies will be carried out using higher concentrations of chlorine, up to excess for complete degradation.

IMMUNOLOGY: ANTIGENS AND ANTIBODIES

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INTRODUCTION

Man has always been curious about himself, yet for thousands of years he possessed little true knowledge of the functions of his body. He feared disease because it meant sickness and often death; yet, he was unable to explain it. Time saw him stumbling in the un-

certainty of fear and ignorance, and he finally came to the conclusion that disease was caused by evil spirits. One cannot blame men of old for using this basis of explanation, for science had not yet filled the gap in man's life.

The curiosity and ingenuity instilled in every member of the race caused man to seek out and attempt to solve the puzzles which plagued him. Mankind gained morsel after morsel of knowledge, yet each progressive step led to another, opening to men the whole world of engimas which surrounds them. They learned to recognize certain factors that were signs of harm within a body. Loss of blood, coughing, choking, dizziness, and cramps.

As early as 1616, a young scientist-lecturer, William Harvey, stated his observations on the circulation of blood. He was the first person to note this movement as he examined the tail of a living fish.

Anton Von Leeuwenhoek had perfected a few precious glass lenses in the year 1665 which were the first true microscopes. From that time on man's interest in the tiny world which is hidden from his sight, yet which plays such a tremendous role in the lives of all, greatly increased.

During the eighteenth and nineteenth centuries much of Europe was infested with smallpox and other dreadfully infectious diseases. In the midst of these plagues a young physician, Edward Jenner, became interested in the tale which a young milkmaid related to him. She told him that she had had cowpox, and therefore could not and would not contract smallpox.

Fortunately for the rest of the world, Jenner listened, and from his experiments and works the first vaccine was produced and proved successful. That was 1796. Thus the science of immunology was truly born.

Though immunology covers and includes many fields, there are two terms which describe the basis around which immunology is built—the antigen and the antibody. An antigen is a substance, which when introduced into an organism to which it is foreign, stimulates the production of antibodies. It follows, then, that an antibody is a substance produced in an organism in response to and reacting with an antigen to render it harmless or totally destroy it. Antigens are protein or polysaccharide in nature, and antibodies belong to the class of proteins known as gamma-globulins.

Every existing species has certain antigens common to each member of that species. Such antigens are called "species-specific" antigens.

Within a species certain particular members may have a certain antigen in common that other members of the species do not possess. These antigens are commonly known as "iso-specific" antigens.

"Hetero-specific antigens" are those which are common to **all** members of **all** species.

One might ask why man really needs to know why such minute substances exist, and what functions they perform. The query could almost be answered with other questions such as:

Why can't organs be transplanted without careful preparation and study, and then still be unsuccessful? Why can't whole blood from the nearest person be transfused without delay of investigation into a person in need of blood? Why does an expectant mother sometimes lose a second or third child because her first baby was a different blood factor than herself? How can a few small injections of a bacterium or a virus which ordinarily causes disease form an immunity in an organism?

Such questions are almost without number. They present a simple fact, yet deeply complex in nature. Some substance must be present in the tissues, blood, or bacteria which is foreign to the recipient and causes that body to reject it. But this raises the question of why some transplants and transfusions "take", or are successful. The answer, though basically complex, is "antigens and antibodies."

ABO BLOOD ANTIGENS OF MAN

When Landsteiner discovered the four blood groups in 1900, he revealed the fact that the method of identifying an antigen lay in placing it in contact with its opposing antibody. The converse, that is, the identification of an antibody by placing it in association with its adversary antigen, is also true.

Landsteiner found, as he worked with human sera and cells, that sera from some bloods always agglutinated the cells, of certain others, and that this method led to four definite patterns—the blood types.

Further research to the realization that the identifying antibodies which were present in the sera bloods were not truly **natural** antibodies. These antibodies are not present in a newly born child's blood stream but appear later on. Whether these antibodies are formed from the intake of certain food elements, or basic chemical changes and additions, is not known. Until this is more fully understood they will be refered to by scientists as "natural" antibodies.

Within the cell membrane of the human erythrocyte are found the antigens which determine blood type.

A person with type "B" antigen has what is known as "Anti-A" antibody in his plasma. This means that type "B" blood is not compatible with any of the other blood types which contain an "A" antigen. The converse is true with type "A" blood which contains an antibody against "B" antigens.

The name of the blood type refers to the antigen, or, in the case of type "AB", the **antigens,** which are present. Type "O" blood contains no antigens, that is , neither "A" nor "B", of the ABO System.

Below is an explanatory chart of the ABO Blood Group System in man. (Table I).

TABLE I

EXPLANATORY CHART OF THE ABO BLOOD GROUP SYSTEM IN MAN

Antigen	Antibody	Type
A	Anti-B	"A"
B	Anti-A	"B"
A and B	Neither	"AB"
Neither	Anti-A and Anti-B antibodies	"O"

Type "O" blood is known as the universal donor, and type "AB" blood as the universal recipient. Other systems of factors and antigens found in the human blood since Landsteiner's discovery include:

RH-HR SYSTEM
KELL SYSTEM
DUFFY SYSTEM
LEWIS SYSTEM
LUTHERAN SYSTEM
MNSs SYSTEM
KIDD SYSTEM
P-Tja SYSTEM

In typing a person's blood the two main factors which are usually always checked are the blood group (AB) and Rh factors.

Discovering the Rh factor was not easy, because no natural antibodies exist for it in the blood stream until the body has received a foreign Rh antigen. This explains why a pregnant mother may lose her second or third child if her first bady was of a different blood factor than herself. A leak in the placenta may have caused some of the first child's blood to be mixed with hers. An antibody was formed in the bother's body, and if a second or later child should also have an adversary Rh factor, destruction of the red cells in the child's body would occur, probably causing death.

This Rh factor is known to exist in 85% of the people. The knowledge of its presence has proved beneficial in saving many lives. The other blood systems recently discovered in man were also revealed because the body produced an antibody which was not present prior to invasions by opposing antigens. A succeeding child with Rh positive blood could be killed by the antibody present in the Mother's blood if another mixing of the two bloods was to occur.

BLOOD TYPE INHERITANCE PATTERNS

The inheritance patterns in man for blood types are in accordance with the inheritance laws set down by Mendel. A gene for type "O" blood is recessive, whereas genes "A" and "B" are dominant. Table II is a chart showing the gene combinations which are possible in each blood type.

TABLE II

POSSIBLE GENE COMBINATIONS FOR EACH BLOOD TYPE

BLOOD TYPE	GENE COMBINATIONS
"A"	AA or Ao
"B"	BB or Bo
"AB"	A and B only
"O"	oo only

The inheritance pattern has proven beneficial in tracing or proving parentage. The following problem is an example of how the inheritance pattern could be of use.

Problem.—Mr. "AB" claims he is the father of child "O". Mrs. "B" claims he is not.

Question.—Is it possible for child "O" to be the offspring of Mr. "AB" and Mrs. "B"?

Answer.—No.

Proof.—Possible gene combinations of type "AB" blood are A and B only. Possible gene combinations of type "B" blood are BB or Bo.

TABLE III

POSSIBLE GENE COMBINATIONS OF MALE AB AND FEMALES BB or Bo

GENES Male	Female—B/B Combinations	GENES Male	Female—B/o Combinations
A	AB	A	AB
	BB		Ao
B	AB	B	BB
	BB		Bo

In neither case could two recessive "O" genes be brought together (Table III).

Conclusion.—"AB" is not the father of child "O".

IMMUNITY

Immunity takes two main forms—active and passive. Active immunity involves the introduction of antigens into an organism. The antibodies are produced by that organism itself in response to these antigens. There are two types of active immunity. Naturally acquired active immunity—which means that a body has or contracts a disease, conquers it and thus becomes immune. Artificially acquired active immunity takes the form of vaccines or other injections of antigens.

Passive immunity deals with the introduction of antibodies, which cause temporary immunity. The recipient is passive, that is, he does nothing, but rather is acted upon by the antibodies. Naturally acquired passive immunity is transmitted from mother to fetus through the placenta. Artificially acquired passive immunity is given

through injections of antibodies, such as gamma globulins, immune sera, antitoxin, or other antisera.

SEEKING ISO-SPECIFIC ANTIGENS IN CHICKEN BLOOD

Literature states that there are seven separately inherited blood systems in chickens. This was concluded because there are several different alleles for blood systems inherited in the chicken. Thus it should be possible to identify chicken blood groups.

It is hoped that from the previous discussion of the ABO SYSTEM of blood groups in man, as well as the explanations and charts listing the antigen and antibody factors involved, that the reader is now aware of what must be done to identify chicken blood types, since it is stated that they do exist.

Several white Leghorn chickens were obtained for the following experiments. The chicken has proven to a great exent to be an easy animal with which to work in the laboratory. It was hoped that the chickens obtained do not have the same blood types. This was considered unlikely, since the animals were chosen at random from large numbers of chickens from various farms and ranches delivered to the hatcheries.

Since an antigen is identified by its opposing antibody, various cross-injections between the chickens should produce antibodies if and where foreign antigens are present. Such antibodies in the serum will agglutinate the red cells in vitro. Before any experiments like those mentioned above were performed, the chickens were bled, and it was concluded that there were no natural antibodies present.

PREPARATION OF MATERIALS

Preparation of materials.—Normal Physiological Salined was prepared by adding 0.9 gram of sodium chloride (NaCl) to 100 cc of distilled water. The 2% cell suspensions were prepared by adding 1 drop of red cells to 38 drops of Normal Physiological Saline. The 25% cell suspensions were made by adding I part red cells to three parts normal physiological saline or by adding ¼ test tube of red cells to ¾ test tube normal physiological saline. The serum was prepared by pouring it from the coagulated blood. The plasma was removed from centrifuged whole blood. Syringes, test tubes, bottles, and all other equipment were sterilized and covered after each use. Normal physiological saline is an iso-tonic solution in which red cells were placed to prevent any unnecessary hemolysis or shrinking of the red cells once removed from the organism.

EXPERIMENTAL

Experiment 1a.—Four chickens were bled and the blood was allowed to coagulate. The sera were removed and refrigerated. The red cells were removed from the clots by washings with Normal Saline, and these were placed in refrigerated compartments in the laboratory. Sera and cells were tested for natural antibodies—none existed.

Chickens C1 and C2, and, chickens C3 and C4, injected with one another's red cells in 25% suspension. The injections were repeated on three different dates. A space of seven days passed in which the chickens were given no further injections, and the antibodies were given time to develop. At the end of this time the chickens were bled again. The sera and cells were tested together in vitro for antibodies. None appeared. Again the chickens were given another set of injections, but this time Freund's Complete Adjuvant was added to each of the cell suspensions. At the end of the required space of time the chickens were again bled and the bloods tested for antibodies. Still none appeared. These experiments indicated that the chickens mentioned above had compatible blood types.

Experiment 1b.—The four chickens were again bled, and the sera and cells were removed and placed under refrigeration. It was hoped that a different pattern of cross-injections would produce antibodies to identify different blood types present. Chickens C3 and C1, and C2 and C4, were given injections of each other's red cells in 50% suspensions on three different dates. The necessary space of time was observed, and the chickens were bled again and checked for antibody production in the blood. None appeared. It was concluded that these chickens also had compatible bloods.

Experiment 1c.—Another pattern of cross-injections was perform. Chickens C_1 and C_1, and, C_2 and C_3, were bled and given injections of each other's red cells in 25% suspension. After seven days they were again bled and the bloods checked for antibodies—none existed. The total length of time involved from the first injection until the last bleeding of any set of injections and patterns is anywhere from fifteen to seventeen days. These experiments led to the conclusions that the above chickens had compatible blood types.

Experiment 1d.—Two more chickens of different breeds were acquired from the hatcheries. These chickens were bled along with others. Chickens C5 and C6, were given injections of each other's red cells in 25% suspension on three different occasions. Chickens C1 and C2 were also given injections of red cells from chickens C5 and C6 respectively. In a week the chickens were again bled and the bloods checked for antibodies. It was naturally suspected that if different chicken blood types did exist, antibodies would certainly be produced from injections of red cells from one breed to another, but this did not occur. It was therefore decided that the above chickens were of compatible blood types.

HETEROLOGOUS ANTIBODY PRODUCTION

Heterologous antibody production is the term used to describe the production of antibodies in an organism by means of injections of an antigen from another species. For the following experiments a gallon of fresh whole beef blood was obtained from a nearby meat packing company. This blood was allowed to coagulate in open con-

tainers, and the serum was removed and centrifuged. The fresh serum was placed under refrigeration, and three bottles were placed in the freezing compartment to preserve them for some length of time, and to prevent bacterial growth in them.

It was hoped that by injecting beef red cells into several chickens, and beef serum into several others, that these chickens would produce heterologous antibodies against beef red cells, and a precipitin against beef serum. (A precipitin is a substance which makes the serum of one animal incompatible with another.) The length of time involved in each of these experiments was about fifteen days. It is to be noted that the sera and cells were tested prior to the experiments to prove that there were no natural antibodies already present.

Experiment 2.—Chicken C3 was injected on three different dates with 2 cc's of beef serum. Chicken C_2 was injected on three different dates with 1.5 cc's of beef red cells. After the space of seven days both chickens were again bled. The sera and cells were tested together in vitro, and the sera from both the beef and chicken were tested in vitro in capillary test tubes. Chicken C3 had produced a precipitin against beef serum. Chicken C2 had produced an antibody against beef red cells.

Experiment 3.—The question arose as to whether or not the serum from chicken C2 containing an antibody (from the above injections) against beef red cells would agglutinate human blood. If this were to occur it would indicate an antigen on the human red cell (erythrocyte) which is also found on the beef red cell.

Twenty two blood specimens (human) were obtained from the hospital. Each of the four blood types were among these samples, and typing plasma was used to type each one. Each of these human bloods was mixed in vitro with serum from C2. All of the human bloods were agglutinated by the antibody in the serum. This indicates that there is present on the human erythrocyte an antigen also found on the beef red cell.

A STUDY OF PLANT SUCCESSION IN THE SANDHILLS OF SOUTHEASTERN NORTH DAKOTA[1]

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INTRODUCTION

Since the pioneering work of Henry Chandler Cowles (1899) at the turn of the century, and the contemporary exhaustive efforts of Frederick Clements, culminating in his classical treatise (Clements 1916), the concept of ecological succession involving changing assemblages of plants and animals has been both a touchstone and a millstone to the science. On one hand it has been the underlying principle guiding all workers in dynamic ecology, and has led to recent brilliant interpretations of community function in terms of entropy levels and thermodynamic laws (Odum 1956; Odum and Pinkerton 1955). This in turn has produced the feedback information requisite to an understanding of energy flow, environmental constituent cycles, and trophic level organization. Conversely, many times ecologists have looked long and hard for evidence of successional trends, with the result that a great deal of effort has resulted in ambiguity and subsequent misunderstanding. The science of ecology is still young, and perhaps insufficient time has elapsed since its birth for us to have accumulated, analyzed, and interpreted the necessary quantitative data concerning fluctuation in community structure and composition through time to document this seral phenomenon we label "succession." All these things notwithstanding, it is imperative that ecologists make every effort to collect data on natural communities so that someday in the future someone will be able to synthesize these informational bits into a unified concept of ecosystem dynamics.

Essentially two techniques are available for studying community development. In the sense of Preston (1960), these two merge into one. For example, the well-known filling in of a kettle lake in the glaciated northeastern United States through the various sedge mat and bog stages ending in a terminal lowland forest may be observed *in situ* by sitting in a boat in the middle of the lake for 10,000 years. For obvious reasons, this is not practical. We may also study this bog lake maturation process by finding, within the geographical

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limits of the causative land form and general macroclimate, enough examples of stages of varying degrees of maturity to be able to place these examples in a meaningful order. It is then possible to trace 10,000 years of vegetation development by analyzing the rise and fall of individual components in response to, or in correlation with, the temporal sequence, and indeed this has been done many times (Tolstead 1941; Whitman, Hanson, and Loder 1943; Tomanek, Albertson and Riegel 1955). For shorter successional periods, both the space and the time approaches have been used. On abandoned fields in the North Carolina Piedmont, on the ground exposed by glacial retreat in Alaska, and on the drought-devastated grasslands of midcontinent North America, it has been possible to study a single area for a short span of time, or to study several areas representing differing magnitudes of community development (Judd and Jackson 1939; Costello 1944; Mentzer 1951).

Despite all of our theoretical philosophy, data on community succession in general are scant. Grasslands, in particular, are poorly understood, perhaps because grasslands present a vastly different kind of instability and subsequent development than other types of vegetation. Fragmentary information is available, however, for portions of the North American prairie, particularly with respect to recovery after plowing and abandonment, grazing, and drought. It behooves each scientist to examine areas of particular interest, and perhaps to elucidate, through the medium of quantitative, reproducible data, objectively obtained, clearly presented, and logically explained, this fundamental aspect of community establishment, development, and stability.

DESCRIPTION OF THE AREA

The sandhills of southeastern North Dakota lie principally in Richland and Ransom counties, coincident with a major portion of the delta of the Sheyenne River. The delta was originally formed when the Sheyenne discharged into Glacial Lake Agassiz during late Wisconsin time (*ca.* 12,000 B.P.). The deltaic deposits are largely sand, and subsequent erosion, deposition and aeolian movement have resulted in an area of large dunes scattered in a matrix of level to rolling sandy plain. Due to soil instability, much of the region has never been cultivated, and in many places, grazing has been slight. The great drought of the 1930's denuded many portions of the sandhill region, and blowing and drifting sand was common throughout southeastern North Dakota, and even today, despite almost thirty years of recovery time, strong winds still cause blowouts to form and sand to move.

Most of eastern North Dakota is subject to a subhumid continental climate, with short, hot summers and long, often bitter cold winters. Precipitation averages from about 18 to 21 inches, most of this as rain. During the growing season, evapotranspiration is high,

resulting in a water deficit through the region, producing dormancy in many of the prairie species. Dry conditions in August are often followed by some moisture in late August and early September, resulting in a resumption of growth among many species, particularly the cool-season grasses.

The soils are characteristically sands or sandy loams, relatively sterile, often deep and excessively drained, poorly structured and immature. Buried profiles are frequently seen on exposed dune faces. Consequently, vegetation development tends to be slow, subject to sudden catastrophic destruction where high winds undermine dune slopes, strip away the vegetative veneer, and leave characteristic blowouts.

The development of vegetation in the area, from a community of hardy initial species of wide tolerance that colonizes the blowouts to the mature tall grass sand prairie provides opportunity for interpreting species behavior as conditions ameliorate. Time, coupled with the reaction of plants on the habitat, are the major extraneous agents involved, always operating under the limiting conditions imposed by the regional climate, and always subject to the vagaries of local weather conditions.

To investigate species behavior, a study was made in a sandhill area near the Sheyenne River south of Leonard, North Dakota, in the summer of 1964. The area (almost 100 acres in size) consists of a few large, partially stabile dunes with intervening swales and rolling ground (figure 1). Blowouts average about 20 feet in diameter,

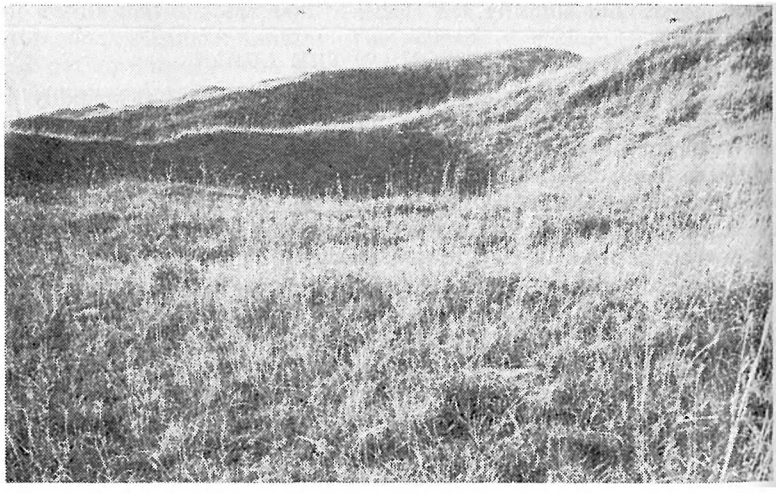

FIGURE 1. General aspect of the study area, showing dune slopes, partially revegetated blowouts, and cover of prairie vegetation. The area has been completely free of human disturbance since 1932.

usually elongated parallel to the direction of the prevailing winds, but may range from four to 50 feet. They are present at a rate of about one per acre, although local areas may contain several blow-outs in each acre. These are most common in windward dune slopes (SW, W, NW), but are occasionally found on level areas.

Virtually denuded by drought and wind by 1932, the area has been undisturbed since that time, and now represents over thirty years of developmental history. Undisturbed areas of this size are rare in the sandhills, rarer still in North Dakota, and for teaching, study and research purposes, every effort must be made to preserve these remnants for the future.

Nomenclature used in this study follows Hitchcock and Chase (1950) for the family Gramineae, and Stevens (1963) for all other vascular plants.

METHODS

After initial reconnaissance to determine general stand characteristics and compile a species list, the area was sampled using 60 quadrats, one square meter in size. These were placed 20 meters apart along three east-west compass lines 100 meters apart. Twenty quadrats were placed on each line.

In all quadrats, each species present was recorded, and crown foliage cover per species was estimated. Total percent cover in each quadrat was estimated first and then species values were recorded. Random quadrats were checked for accuracy by visually plotting the species distribution on mm graph paper and computing percentage cover. It was found that the estimates in general were reasonably accurate, and that the initial estimate of total cover decreased the marked tendency to overestimate the cover of individual species.

When field data were tabulated, it was found that 17 quadrats had a total cover of 20 percent or less, 26 quadrats ranged from 21 to 40 percent cover, and 17 had a total cover greater than 40 percent (figure 2). Frequency percentage and average cover per quadrat were then calculated for each species based on sample sizes of 17, 26, and 17 quadrats, respectively.

Relative percent cover for each species in each quadrat grouping was then determined. This is the percent cover of each species divided by the total cover for all species in each group of quadrats. It thus expresses the relative amount of cover contributed by each species, and is dependent on the numbers and sizes of associated species and the total average cover of the quadrat group. The sum of vegetation in an area of any reasonable size will be dependent upon the interaction of biotic potential and environmental resistance. The biotic potential includes floristic availability, propagule dissemination, and amplitudes of tolerance in all stages of the life cycles of the component species. As vegetation takes hold, environmental conditions impose selective pressures on the plants and plant popula-

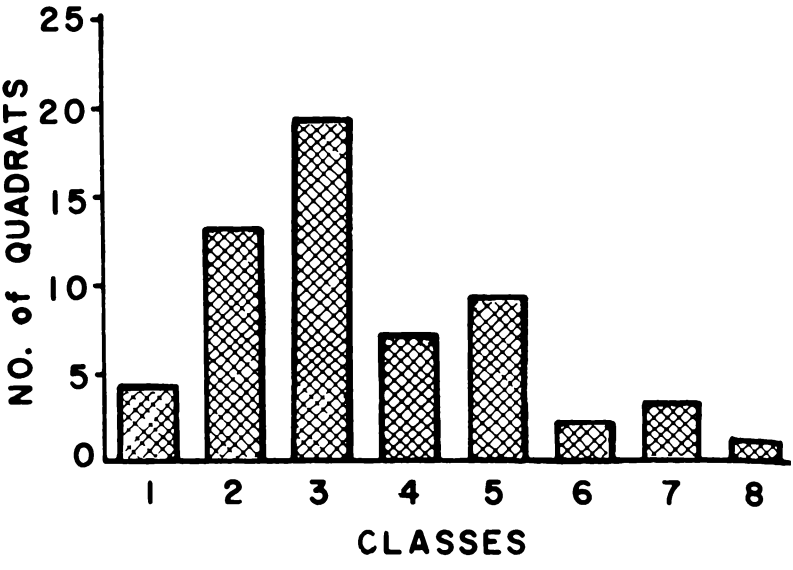

FIGURE 2. Frequency distribution of quadrat cover by classes (1—0-10%; 2—11-20%; 3—21-30%; 4—31-40%; 5—41-50%; 6—51-60%; 7—61-70%; 8—over 70%).

tions. As more individuals (and more species) become established, ranges of many environmental factors are decreased through shading, reduced superficial evaporation, addition of organic matter to the soil, and so on, thereby altering conditions so that other species of narrower amplitudes (stenoecic species) may become established. Gradually, the area approaches optimum conditions for maximum plant growth in the region. Many individuals of the early colonizers may still persist, and their aggregate cover or biomass in the later stages may far exceed that of the initial stages, but they are no longer a major component of the areal vegetation. They have relinquished their early dominant status to species better adapted to the changed conditions of the site. Thus it is not only possible, but highly probable, that while a species may increase in absolute density, cover, basal area, biomass, or any other quantitative measure of size or number, its relative position within the total community may decrease. It is important to understand this relative relationship. For example, in the last 15 years, food prices in this country have doubled (analagous to the increased cover or density of a pioneer species). Concomitantly, wages and salaries have more than doubled in this period (analagous to the increased number of species and increased cover of the vegetation). We are thus in a situation where despite the increase in food prices, our expenditure for food repre-

sents a smaller fraction of our spendable dollar than it did before. In other words, on a relative basis, food prices have actually declined. When comparing behavioral aspects of plant species in a community undergoing change, relative values represent the most valid measures. For these reasons, then, it was imperative that relative cover values be determined for each species in each quadrat group.

An index of relative importance was then constructed by multiplying the percent frequency by relative cover for each species. The product is thus weighted in favor of those species that are either widely distributed (high frequency) or large (high cover), or both, and it is believed that an integration of these characters constitutes an accurate estimate of species importance within the community.

RESULTS AND DISCUSSION

Forty species of vascular plants were found, including 13 species of grasses, 2 sedges, and 25 forbs (Table I.). These ranged from widespread and abundant species such as Junegrass (*Koeleria cristata*) to rare species encountered only once (*Artemisia ludoviciana, Penstemon grandiflorus*). Twenty-nine species were sufficiently important (as determined by the importance index, frequency times relative cover), to undergo further analysis. When the index was calculated for each species in each quadrat grouping, characteristic behavioral trends appeared. Certain species were restricted to the quadrats of low total cover, others to high cover areas. Most of the important species ranged throughout all classes of quadrats, but quantitative differences in cover and distribution were evident. Certain species (both grasses and forbs) showed a greater degree of success in quadrats characterized by certain magnitudes of cover. Thus, an explanation of continuous autogenic succession can be illustrated by a series of behavioral curves for the species components. As the prairie community matures, with concomitant increases in standing crop, species diversity, and niche utilization, a series of plant (and animal) species gradually replace one another in importance, as the preceding vegetation prepares a suitable microenvironment for succeeding vegetation.

Areas of low total cover included several quadrats situated in sand blowouts. This community is dominated by Sandhills bluestem (*Andropogon hallii*), prairie dropseed (*Sporobolus heterolepis*), blowout grass (*Redfieldia flexuosa*), sandbur (*Cenchrus longispinus*), hairy prairie clover (*Petalostemum villosum*), sand sunflower (*Helianthus petiolaris*), grooved flax (*Linum sulcatum*), and bugseed (*Corispermum villosum*) (figures 3 and 4). *Redfieldia, Andropogon,* and *Petalostemum* have all been reported in the early successional stages in Colorado, northern Nebraska, and South Dakota (Ramaley 1939; Tolstead 1941, 1942), and Pool (1914) listed *Redfieldia* as the most important pioneer species in the Nebraska sandhills. These species are joined by a number of subordinates including *Chrysopsis villosa, Erigeron canadensis, Lithospermum canescens,* and *Cycloloma*

TABLE I.

SPECIES FREQUENCY AND COVER PERCENTAGES IN AN AREA OF SANDHILL PRAIRIE IN SOUTHEASTERN NORTH DAKOTA, ARRANGED ACCORDING TO MODAL INDEX VALUES IN PIONEER, TRANSITIONAL, OR CLIMAX AREAS

SPECIES	PIONEER		TRANSITIONAL		CLIMAX	
	F	C	F	C	F	C
Andropogon hallii	58.8	2.65	50.0	3.77	29.4	5.41
Artemisia ludoviciana	5.9	.06	0	0	0	0
Cenchrus longispinus	17.6	.18	3.8	.12	5.9	.06
Chrysopsis villosa	5.9	.06	0	0	0	0
Corispermum villosum	23.5	.29	7.7	.04	5.9	.06
Cycloloma atriplicifolium	5.9	.06	3.8	.04	0	0
Erigeron canadensis	11.8	.18	3.8	.04	0	0
Helianthus petiolaris	17.6	.12	11.5	.23	11.8	.35
Linum sulcatum	23.5	.24	19.2	.04	5.9	.06
Lithospermum canescens	11.8	.18	11.5	.04	5.9	.12
Petalostemum villosum	58.8	1.71	57.7	2.88	58.8	5.18
Redfieldia flexuosa	5.9	.77	0	0	0	0
Sporobolus heterolepis	23.5	.59	19.2	.46	17.6	.41
Andropogon scoparius	0	0	3.8	.08	0	0
Artemisia caudata	0	0	7.7	.12	0	0
Artemisia frigida	0	0	4.8	.12	0	0
Aster ericoides	17.6	.24	38.5	1.23	47.1	1.06
Carex pennsylvanica	0	0	7.7	.08	0	0
Elymus canadensis	5.9	.06	15.4	.31	5.9	.18
Erigeron strigosus	0	0	11.5	.04	5.9	.12
Koeleria cristata	76.5	3.94	88.5	9.38	88.2	12.18
Melilotus alba	0	0	11.5	.38	5.9	.06
Setaria lutescens	0	0	3.8	.08	0	0
Sporobolus cryptandrus	47.1	.65	42.3	2.08	29.4	1.24
Tradescantia occidentalis	5.9	.06	15.4	.08	11.8	.12
Ambrosia psilostachya	5.9	.12	15.4	.19	11.8	.71
Andropogon gerardi	23.5	.41	30.8	1.62	35.3	7.59
Artemisia glauca	5.9	.18	15.4	.50	17.6	2.06
Calamovilfa longifolia	11.8	.24	26.9	.65	23.5	4.47
Chenopodium fremontii	5.9	.06	0	0	5.9	.18
Cyperus schweinitzii	47.1	.24	15.4	.12	29.4	1.29
Euphorbia serpyllifolia	29.4	.47	34.6	1.27	41.2	2.29
Lygodesmia juncea	11.8	.06	7.7	.08	5.9	.24
Oenothera nuttallii	0	0	0	0	5.9	.06
Panicum leibergii	17.6	.24	15.4	.27	47.1	1.53
Penstemon grandiflorus	0	0	0	0	5.9	.18
Physalis lanceolata	0	0	0	0	11.8	.18
Poa pratensis	0	0	3.8	.12	17.6	3.18
Salsola kali	0	0	0	0	5.9	.18
Solidago nemoralis	11.8	.18	26.9	1.00	41.2	2.41
TOTAL		14.24		27.46		53.16

atriplicifolium, forming a mixture of arenicolous dominants and assorted widespread weedy species that are often found in a variety of ruderal habitats throughout the upper midwest.

Weaver (1954) reported rapid spread of *Sporobolus heterolepis* into drought-denuded areas if enough moisture was present to insure

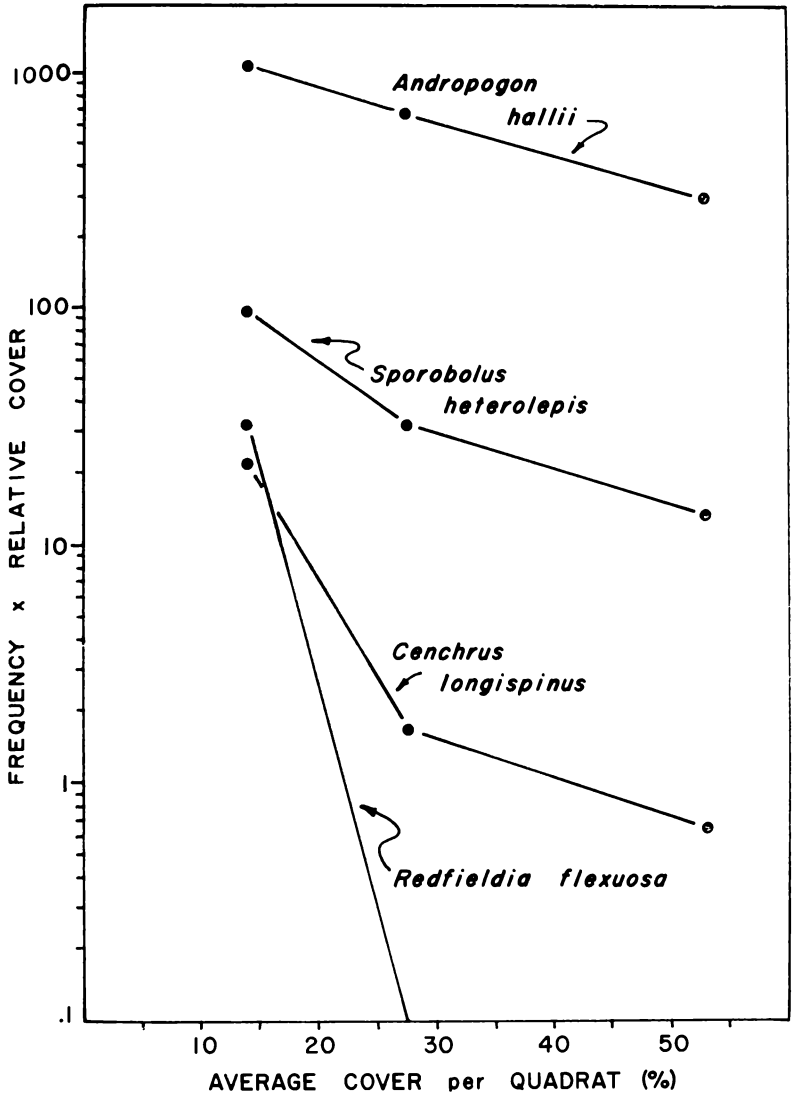

FIGURE 3. Behavioral curves of major pioneer grasses.

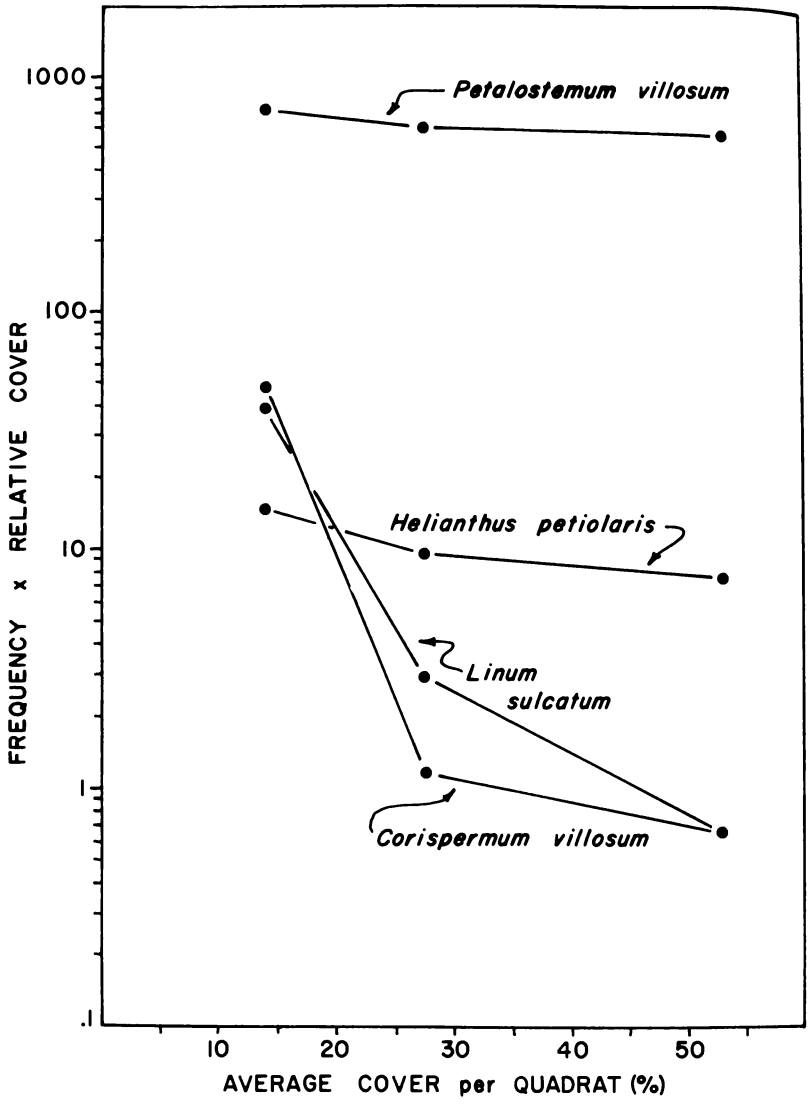

FIGURE 4. Behavioral curves of major pioneer forbs.

good seed production. In the eastern reaches of the prairie peninsula, prairie dropseed is a normal component of transitional and climax areas, although it tends to be more abundant in drier prairie communities (Curtis 1959). In eastern North Dakota, prairie dropseed

is near the northwestern limit of ts range, and perhaps peripheral selectivity may account for its presence in the pioneer habitats, where it may be less able to compete with better-adapted, more western species. In 1946, eight or ten years after the great drought, communities of *Sporobolus heterolepis* were invaded and eventually replaced by big bluestem (*Andropogon gerardi*) in Nebraska and Iowa (Weaver 1954). This implies less than climax status for these dropseed communities in the western prairies.

Tolstead (1914b) found that seeds of *Redfieldia flexuosa, Andropogon hallii,* and *Corispermum villosum* germinated readily upon release from the parent plant. Other species in sand prairie needed a cold treatment to germinate. The three species above showed higher percentages with treatment, but sufficiently good germinability without it to insure reasonable success in bare areas. The ability to germinate in late summer and early fall and then over winter as seedlings gives the species a decided advantage during the following growing season, as they can begin to photosynthesize immediately when temperature and moisture conditions become favorable.

Species that comprise the transitional stages of succession include Junegrass (*Koeleria cristata*), sand dropseed (*Sporobolus cryptandrus*), Canada wildrye (*Elymus canadensis*), heath aster (*Aster ericoides*), spiderwort (*Tradescantia occidentalis*), white sweet clover (*Melilotus alba*), and daisy fleabane (*Erigeron strigosus*) (figures 5 and 6). These are accompanied by two species of sage (*Artemisia caudata* and *A. frigida*), and little bluestem (*Andropogon scoparius*). Junegrass, whose index of importance is highest in the transitional stages, is dominant throughout the area, with high values in all three stages. Validity of the curve (figure 5), however, is supported by the fact that under grazing pressure, Junegrass acts as an "increaser species" in North Dakota, and its response to grazing would approximate a curve the reverse of that in Figure 5. Junegrass is very shallow-rooted. During the 1930's, the populations were decimated by the advent of drought. They came back rapidly, however, during brief respites, only to be killed once again by the return of dry years. Such behavior can easily be interpreted as one of a transitional species, tolerant enough to regenerate after experiencing adverse conditions, but intolerant to excessive swings of the environmental pendulum (Weaver 1954). While Junegrass is the major species in all subdivisions of the study area, it reaches its greatest degree of relative success in the transitional areas, where it far surpasses all other species.

It is suspected that sand dropseed, classified as an "invader" by Weaver (1954), is characteristically a transitional species in the sandhills of North Dakota. The short growing season and the generally unfavorable substrate would tend to favor species in intermediate stages of succession that might well be considered undesir-

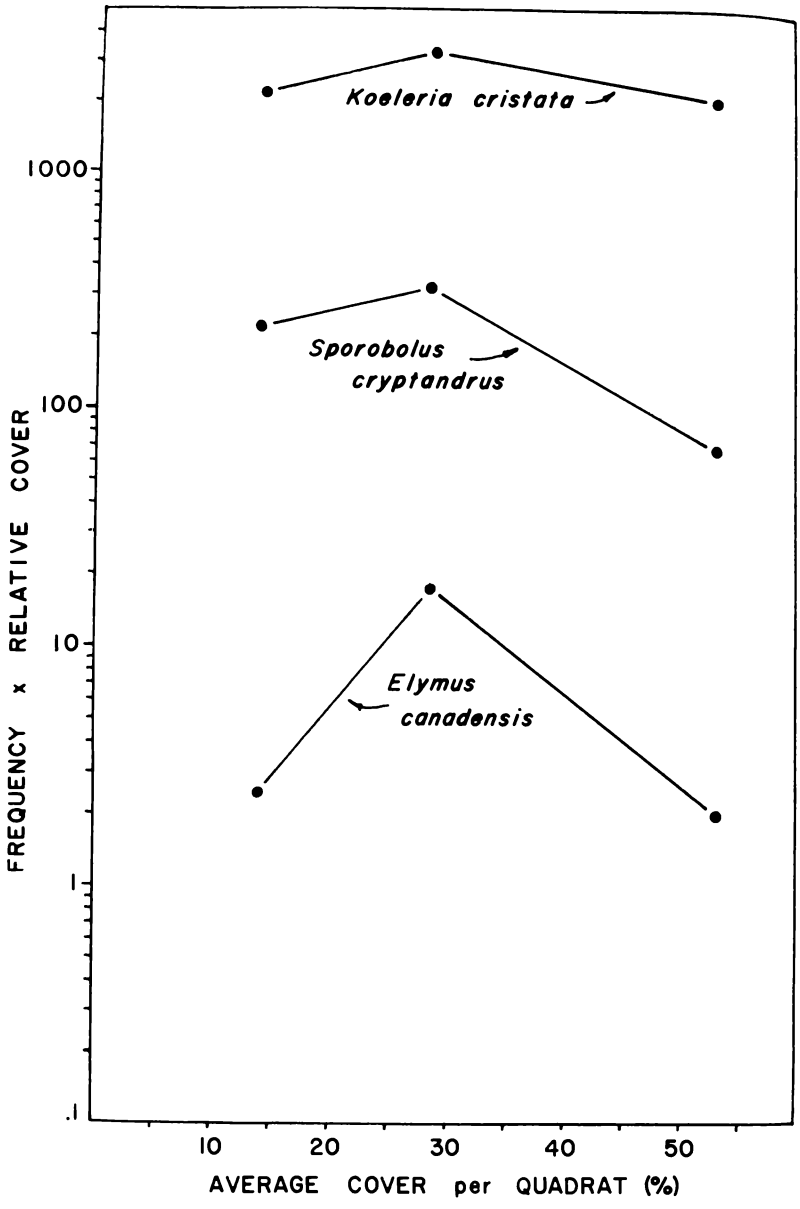

FIGURE 5. Behavioral curves of major transitional grasses.

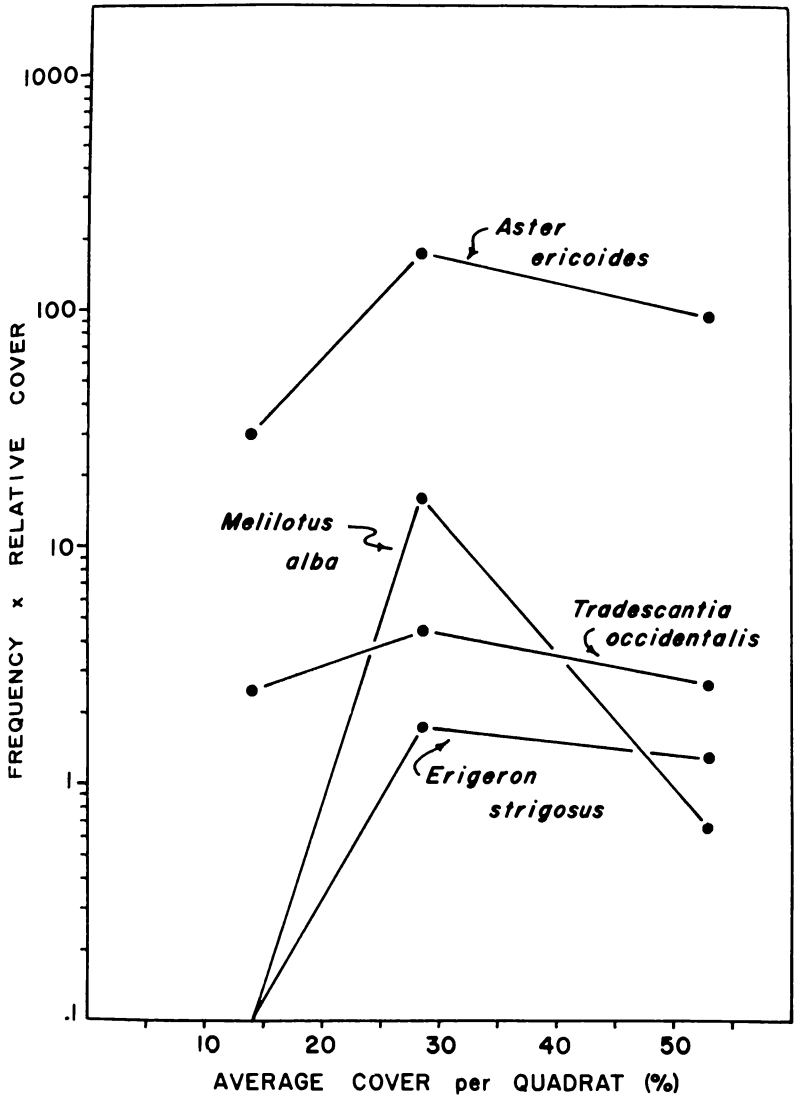

FIGURE 6. Behavioral curves of major transitional forbs.

able invaders in less rigorous portions of the North American grassland.

Elymus canadensis, generally a mesic or wet-mesic species, shows a behavioral optimum in the transitional areas. This tall grass

is found, however, in disturbed uplands, particularly microsites around animal burrows and on the rim areas of sand blowouts (Weaver 1954).

The genus *Tradescantia* is commonly found on sands throughout eastern and midwestern regions, and its importance here fits the generalized successional sequence. It has not the eurytopic abilities of many of the northern weeds, yet has sufficiently wide ecological tolerances to inhabit areas (such as sandhills and plains) that do not support dense vegetation, and hence can be considered open communities. As succession progresses, the relative position of this species declines, probably due to its inability to propagate vegetatively, a factor that would make it a better competitor in prairie communities (Tolstead 1942). The heath aster often dominates bare areas in the western portions of the tall grass prairie region (Weaver 1954), but has abilities to persist through the later stages. Evident inability to colonize the barren blowouts in southeastern North Dakota may be attributed again, to the short growing season, or perhaps cold winters or hot summers, either (or both) of which impose restrictions on species establishment not experienced elsewhere within the range of the species.

The most mature areas are characterized by big bluestem (*Andropogon gerardi*), sand reedgrass (*Calamovilfa longifolia*), Leiberg's panicgrass (*Panicum leibergii*) and Kentucky bluegrass (*Poa pratensis*) (figure 7). The U. S. Forest Service, administrator of the Sheyenne National Grassland a few miles from the study area, recognizes a "Bluegrass Type" in the southeastern sandhills. In this type, *Poa pratensis* is an unquestioned dominant in the climax grassland, while the same species is treated as an increaser or invader in other grassland types in the area. Perhaps an ecotypic explanation of this apparent anomaly is called for, but in this particular study area, *Poa* appears as a dominant only in the later stages of the proposed succession, and in fact is completely absent from the low cover, bare sand quadrats. Weaver (1954) states that *Poa pratensis* is everywhere in true prairie, and perhaps its general treatment as an undesirable and exotic invader is unwarranted. However, Mentzer (1951) reported that Kentucky bluegrass was almost eliminated, or at least greatly decreased, in the later successional stages of true prairie. This may not happen in the sand prairies, however, inasmuch as environmental conditions on the sands, even in terminal stages, are somewhat less favorable than on the deep loams in the main body of the tall grass prairie.

Sand reedgrass (*Calamovilfa longifolia*) and big bluestem (*Andropogon gerardi*) both need cold temperatures and stratification for good germination percentages (Tolstead 1941a). Delayed germination, particularly among grasses, may be correlated with successional behavior, as generally ameliorated environmental conditions should

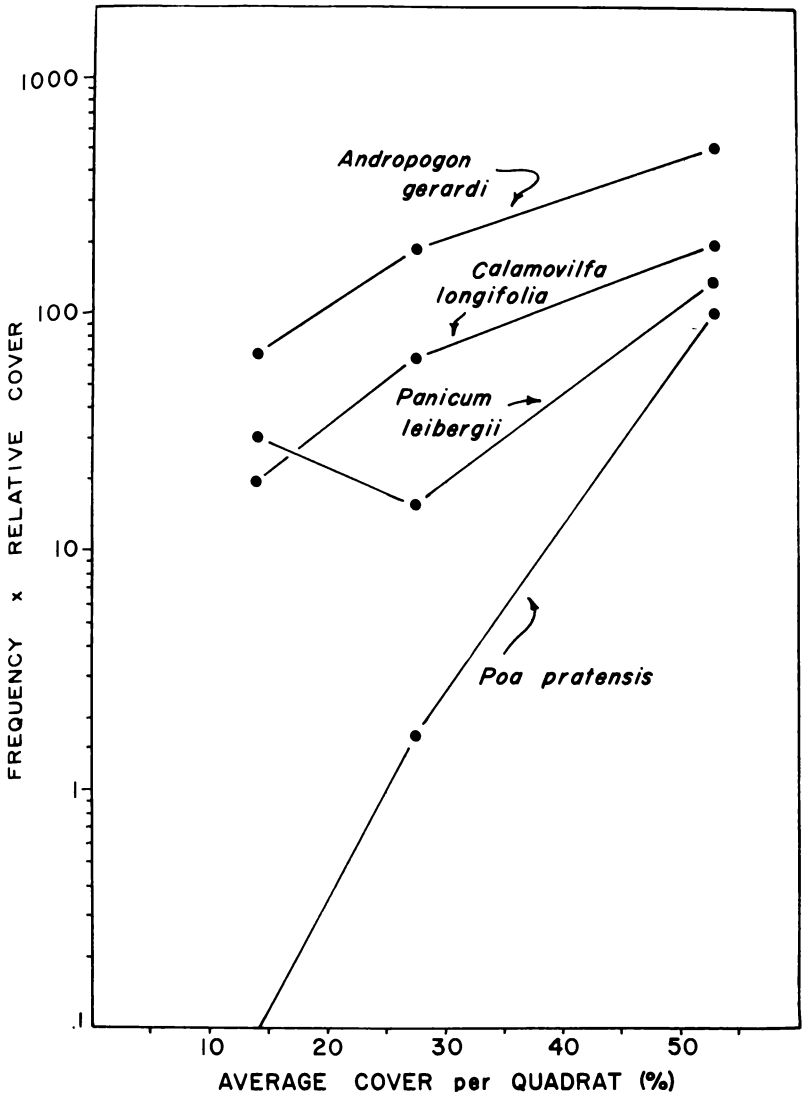

FIGURE 7. Behavioral curves of major climax grasses.

be more evident the following spring than during the current growing season.

Dominant forbs in the terminal stages are *Euphorbia serpyllifolia, Solidago nemoralis, Artemisia glauca* and *Ambrosia psilostach-*

ya (figure 8). They are joined by *Cyperus schweinitzii, Physalis lanceolata,* and *Penstemon grandiflorus. Cyperus schweinitzii* possesses high index values in both pioneer and climax areas, while dropping in transitional quadrats. Small sample size could, of course, account for this variation. It is possible, however, that *Cyperus* is

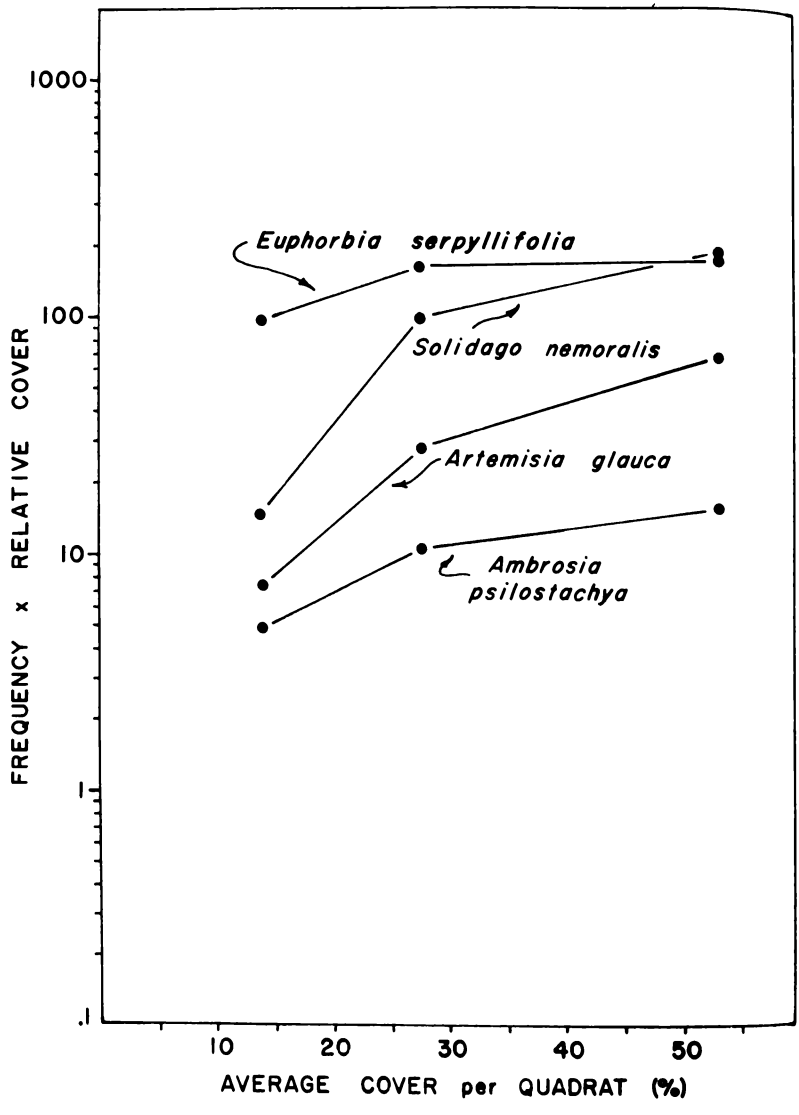

FIGURE 8. Behavioral curves of major climax forbs.

responding to an environmental component available in both pioneer and stabilized areas, but perhaps usurped by a better competitor in the transitional quadrats.

In general, the community developmental pattern on the dune sands of southeastern North Dakota correlates well with other studies of western sand prairies (Pool 1914; Ramaley 1939; Judd and Jackson 1939; Tolstead 1941, 1942; Costello 1944; Tomanek, Albertson and Riegel 1955). The mechanism of this development is believed to be a combination of "initial floristic composition" and "relay floristics," *sensu* Egler (1954). The first hypothesis states that propagules present in the area account for all species in all stages, with germination or growth essentially controlled by site maturation. Relay floristics, on the other hand, pictures a steady increment of disseminules from outside areas as succession proceeds. In the sandhills, there is little doubt that seeds and rhizomes of many species are present in the sand, but it is just as probable that relay floristics must function to provide additional species at various successional intervals. At the scale involved, where small areas are denuded and then revegetated, perhaps these divergent concepts of successional mechanics are really one and the same.

CONCLUSIONS

Barren sand dunes in southeastern North Dakota initially support a community of vascular plants dominated by several species of prairie grasses and forbs. Many of the latter are wide amplitude, weedy types. With maturation, a series of population replacements results in a continuous change in species composition as microenvironment is modified. Concomitant with these changes in species importance, there are proportional increases in total ground cover, standing crop, and ecosystem productivity. With these increases, greater dune stabilization is assured, as aerial shoots lessen raindrop impact and wind velocity, and root systems penetrate to bind the loose sand into a sod capable of withstanding erosive forces to a much greater degree.

Quadrat cover in the area studied ranged from about one percent to over 70 percent. When values for the major grasses and forbs are combined and placed on a relative basis, the community transition can be clearly demonstrated (figure 9). The grasses show that the three groupings (pioneer, transitional, and climax) are quite distinct. The pioneer grasses contribute little to the transitional and climax communities while the climax grasses show low relative values in the intermediate and pioneer areas. In contrast, the forbs show a greater tendency to range across the successional spectrum, except that again, the pioneers are quite distinct.

It is notable that, as in other studies, most genera with more than a single species represented are segregated in their successional roles. Thus, of three species of bluestem present in the area, *Andro-*

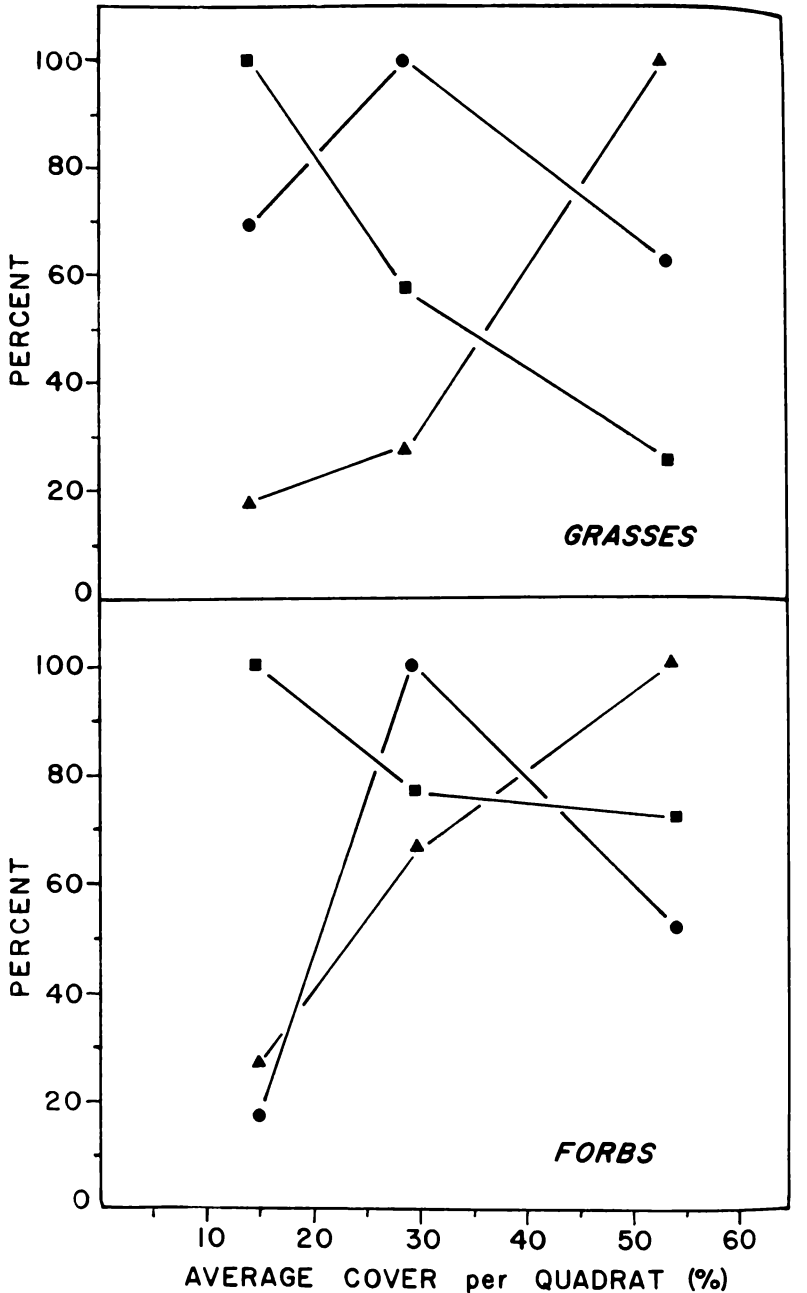

FIGURE 9. Relative behavioral curves for combined major species of grasses and forbs, each species group expressed as a percentage of the maximum value obtained in pioneer, transitional, and climax areas.

pogon hallii is a pioneer dominant, *A. scoparius* an intermediate accessory, and *A. gerardi* an important constituent of the climax community in the most highly stabilized areas. Similar patterns are exhibited in the genera *Artemisia* and *Sporobolus*.

It is believed that similar successional patterns are common in the sand prairies of southeastern North Dakota, where recovery from drought, wind erosion, grazing, and occasional cultivation is permitted to follow natural ecological processes. The transition from pioneer to climax conditions is thus accomplished through orderly changes in specific composition, coupled with microenvironmental changes in moisture, light, nutrient, and temperature regimes. Whatever regional differences are noted are probably due principally to geographic location and the correlated differences in available flora.

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A GENERAL METHOD FOR SOLVING ENGINEERING PROBLEMS

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When an engineer is in the process of obtaining his formal education, he is exposed to quite a number of different disciplines. These include: the basic properties of the physical world (Physics, Chemistry), some means of describing this world (Mathematics), and some tools for manipulating some portion of the aforementioned world (the various engineering courses). The problems he has been given as training exercises have been, of necessity, concise, limited, and with well-defined solutions. Unfortunately, when a young engineer goes out into the industrial world, the problems he encounters are not concise, nor are they limited in scope. There may be many solutions to any given problem, or there may be none.

Altogether too often when our young engineer is given an assignment he flounders. He makes a number of false starts, and does a great deal of unnecessary work simply because he does not know how to go about solving a complex problem. This paper will present a general method that will apply to all engineering type problems.

It does not guarantee an answer to everything, but it will at least give an individual a place to begin.

Engineering is defined as "the art or science of making practical application of the knowledge of pure science". A problem is defined as "anything requiring something to be done". By extension then, an engineering problem can be defined as "anything that needs to be done, using the sciences as a tool, that will terminate in a practical result." The nature of any problem will determine the nature of its solution, and in the case of an engineering problem, the solution will very often take the form of a process, a structure, or a device that has some practical value.

It is proposed that any engineering problem can be resolved by the following nine steps.

1. Identify the problem.
2. Identify an ideal solution concept.
3. Identify a number of possible solutions.
4. Reduce the possible solutions to practical solutions.
5. Make a decision as to which solution to develop.
6. Develop a block diagram for the solution chosen in step 5.
7. Obtain a solution for each block of step 6.
8. When all the blocks of step 6 have satisfactory solutions, review the total package.
9. Proceed with the detail design effort.

Let us examine each of these steps so as to gain insight as to their application.

The identification or description of a problem is perhaps the most difficult phase of finding its solution. It is far and above the most important. Just as a question must be understood before an answer can be given, so must an engineering problem be understood before a solution can be developed. There are some techniques that can be applied to a problem that will make it easier to understand. First, state the problem in terms of a single verb ("to measure", "to amplify", etc.), Second, tabulate the known characteristics of the problem. Third, develop a problem statement incorporating the verb, its principal modifiers, and its object (if required). An example would be "to accurately measure voltage". On occasion it may appear that a problem is too complex to describe with a statement such as this. The way out is simple, choose that portion that is felt to be the most difficult for the original problem statement and assume that the remaining parts have a solution. These can be found later. This may sound arbitrary, but as in drawing a circle, one must start somewhere. Sometimes a problem will be accompanied with such a mass of detail in the form of specifications, requirements, and conditions that one feels a problem statement is unnecessary. A concise statement that will bring the really difficiult portion out into the open is particularly valuable in a case like this.

Once the problem is defined, the ideal approach to a solution is often quite obvious. If not, set down your own concept of how the problem can be solved. If a great deal of difficulty is encountered in obtaining an ideal solution concept, perhaps the problem is not defined as well as it should be, and more effort should be expended in that direction.

Now set down a number of possible solutions; include those that use the concept found in the last step and those that do not. This is an area where an individual can, and indeed must, be truly creative. Don't limit the possible solutions to your own ideas, consult with others. Don't be afraid to consider ideas from individuals who work in other fields, and don't be afraid to set down an idea because it sounds ridiculous. Of course, a person can get so involved with finding possible solutions to any given problem that all else is forgotten, so where do you stop? There is no hard and fast answer, and a person's judgment must prevail.

This list of possible solutions must be reduced to solutions that are practical before anything more can be done. The factors that are pertinent include such items as: cost, availability, ease of operation, reliability, company policy, and many, many others. There may be many compromises between the various factors and again there is no rigid rule to follow. We may well find that none of the possible solutions are practical enough to warrant development. There are two alternatives, either the original problem must be modified, or the search for a possible solution must continue.

Somewhere along the line, a decision must be made as to which of the possible solutions will be developed. This step is second only to problem definition in importance. In many cases a decision is obvious, occasionally there will be a choice of several equally desirable alternatives. A decision can be reasoned out to the n'th significant figure, or it can be arbitrary, *but a decision must be made!*

Since a great majority of engineering problems ultimately result in a piece of equipment or a process, a block (or flow) diagram can be used to assist in the solution development. In general, each block is some common or easily obtained function that can be separated from the other functions in the equipment or process that is being described. We can also say that a block diagram breaks a large problem into a series of small ones. A block diagram is useful in two ways; it serves to tie all concepts into one cohesive package, and it serves as a convenient method of dividing the effort if the job is too large for one individual. A solution to each block can be obtained by applying the steps we are describing until all blocks have a satisfactory solution. At this time a review of the total package is in order. One comment on this, Finagle's well known law "a person cannot see his own mistakes" certainly applies here. The review should be done by someone other than the originator.

If the proposed solution stands the test of the review, now, and

only now, can the detail design effort start. Detail design is generally taken as that area of work for which solutions are available from textbooks, handbooks, or as the result of elementary calculations. For an electronic design, this would include such items as: calculation of the component values, choice of component types, the drafting effort, etc.

This method is not a substitute for intelligence or a sound education. It is simply an organized way of looking or going about a problem. The size or difficulty of the problem has no bearing on the use of this technique. The problem can be as difficult as that of designing a guidance system for an ICBM, or it can be as simple as that of determining the length of the power cord of an electrical appliance; this method will work.

THE OCCURRENCE OF DAMPED OSCILLATORY TRANSIENTS IN THE EARTH'S MAGNETOTELLURIC FIELD

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ABSTRACT

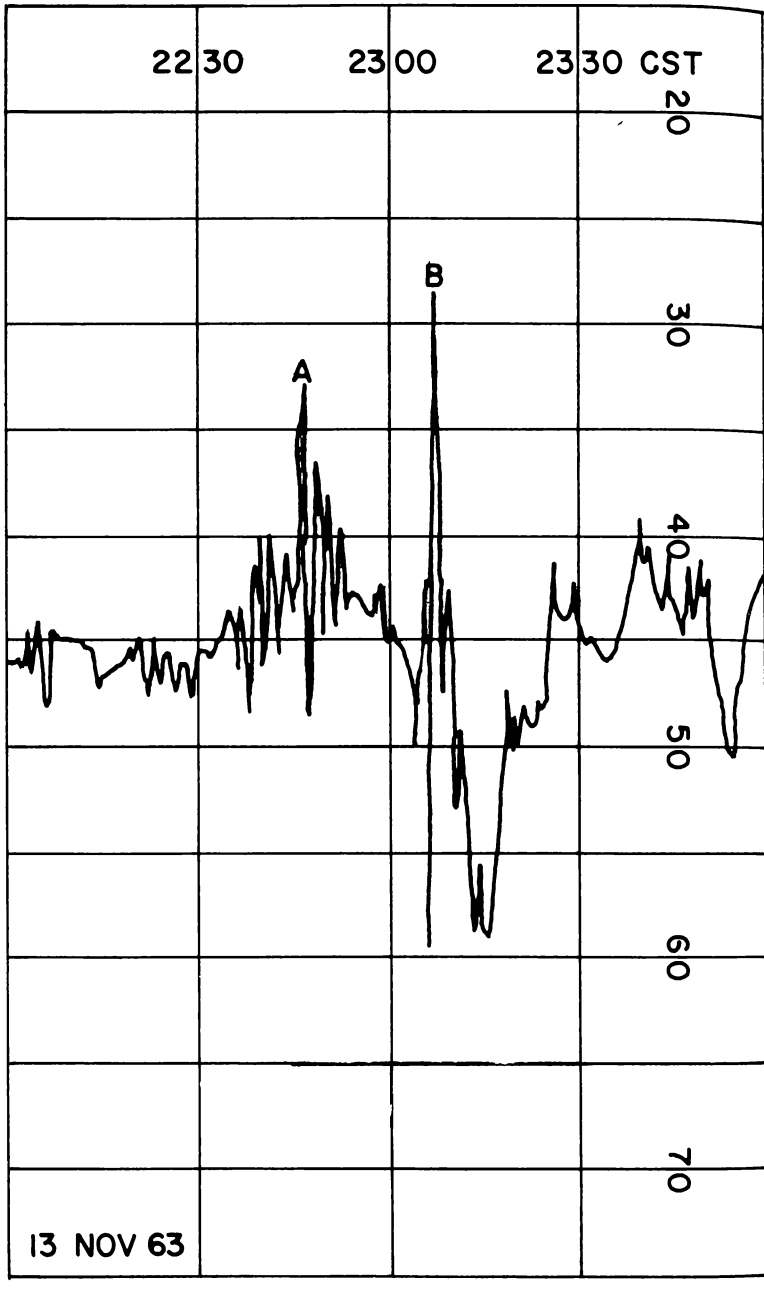
Damped oscillatory transients in the earth's magneto-telluric field occurring in the period from August 1963 through July 1964 are investigated. Their occurrence is found to have a local time dependency, the most probable time of occurrence being when the observation site is located in the night hemisphere, and to be independent of the day and season of the year. It is suggested that the cause of these transients is somehow determined by the daily relative position of the earth and sun.

EQUIPMENT

The measuring apparatus as constructed by VanBeek (1) consisted of a self balancing potentiometer recorder connected across two copper ground rods spaced 500 ft. apart on a North-South line. The recorder had a chart speed of 2 inches per hour and a balancing time of 10 seconds.

INTRODUCTION

Recordings of the earth's magneto-telluric field at Jamestown, North Dakota over a 12 month period from August 1963 through July 1964 reveal a number of damped oscillatory transients. This paper investigates the relationship between the occurrence of such a transient and the local time, day, and season of the year when it occurred.


22 30
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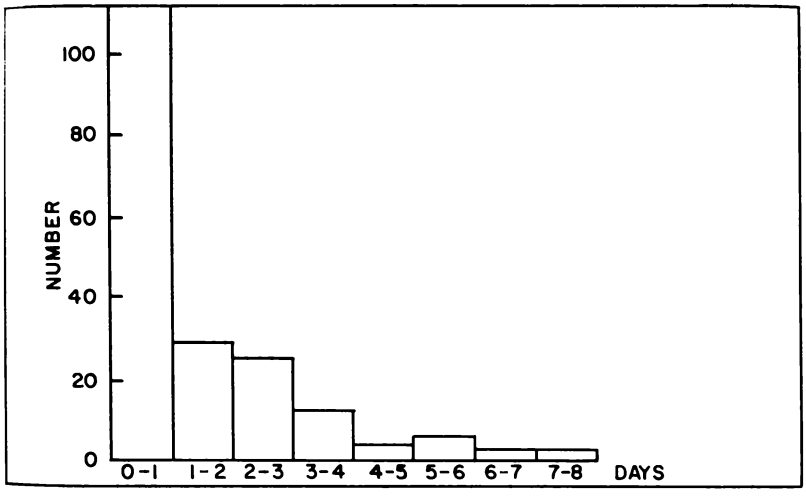
A
B
13 NOV 63

OBSERVATIONS

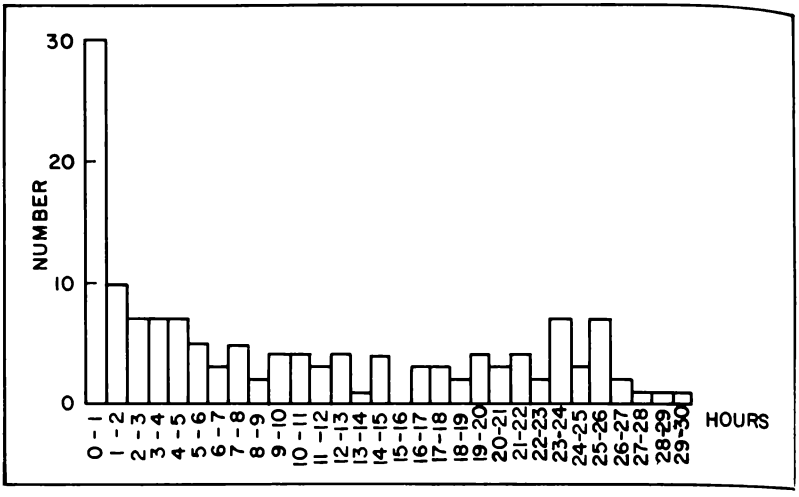
Transients being investigated.—Figure 1 gives two examples of the damped oscillatory transients whose occurrence was investigated. Transient B. is superimposed on some other variation, as was found to be the case most often, with clearly defined transients such as A. occurring less often. Using only clear-cut cases where the background variation wasn't large enough to seriously distort the general form, approximately 200 such transients were found occurring in the period from August 1963 through July 1964.

Time interval between successive occurrences of the transients.—To investigate the pattern of occurrence, the time interval between successive occurrences of the transients was considered. Two histograms were made, one plotting the number of occurrences of a given time interval between successive transients (in terms of days) against the time interval, and the other a breakdown of this, where the time interval is in terms of hours. The results are given in figure 2.

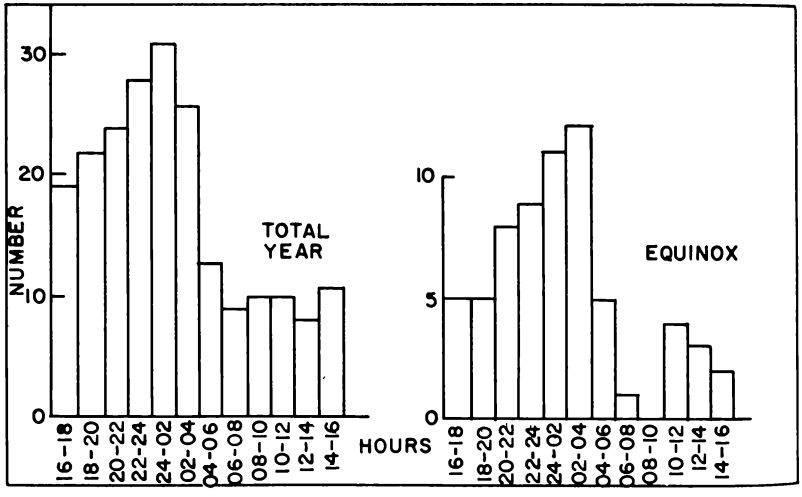
From figure 2A., the histogram appears similar in form to an exponential distribution, Ae^{-At} where A is a constant. If it is indeed true that the probability that time, t, elapses between successive occurrences of the transients is given by this function, then the transients are interpreted as being random events (2).

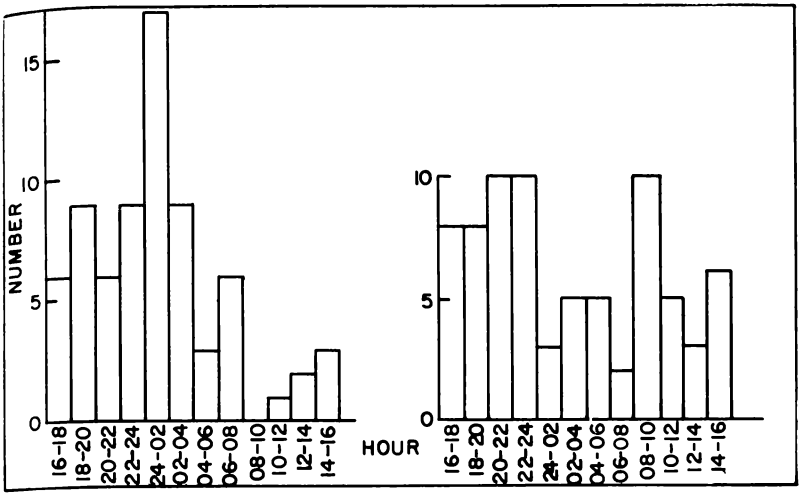


Considering figure 2B, however, there also appears to be a slight reoccurrence tendency at approximately a 24 hr. interval, which would indicate that the occurrence of such transients might have a local time dependency.



Local time dependency of frequency of occurrence.—In order to see if these occurrences have any local time dependency, the 24 hr. period was divided into 12 intervals of 2 hrs. each. Then a histogram was made, plotting the number of transients occurring in a given 2 hr. time interval against that time interval for, 1) The entire 12 month period considered; 2) June Solstice (May, June, July, August); 3) Equinox (March, April, September, October); 4) December Solstice (November, December, January, February). The results are given in figure 3.





As seen from figure 3, considering the entire 12 month period, the frequency of occurrence of these transients has a definite local time dependency, being a maximum between 00:00 and 02.00 hrs. local tim. The June Solstice shows a definite maximum between 00:00 and 02:00 hrs. and the Equinox shows one between 02:00 and 04:00 hrs. local time, but no definite dependency appears during the December solstice, rather it seems the occurrences are random with respect to local time. However, if this assumption is made, applying the Chi Suqare test to the data in the December Solstice, it is rejected as being false 99% of the time. Therefore, in all likelihood the frequency of occurrence of these transients in the December Solstice has a local time dependency also, but whether its maximum coincides with that shown by the June Solstice or the Equinox cannot be definitely determined here.

Seasonal variation of the number of transients occurring.—There was a total of 71 transients occurring in the June Solstice, 65 in the Equinox, and 75 in the December Solstice. In view of the total number of transients occurring in the entire 12 month period, the slight differences, in the number of transients occurring in each season are not significant. Therefore it is concluded that the occurrence of such a transient is independent of season of the year.

Daily variation of the number of transients occurring.—A histogram was made, plotting the number of days having a given number of transients occurring against that number of transients. The results are given in figure 4, where it is seen that the histogram appears to be a distribution of the form Ae^{-At} again, in which case the occurrence of such a transient would be random with respect to the

of the year. Making this assumption and applying the Chi Square test to the data of figure 4, the assumption is found to be true 99% of the time. Therefore it is concluded that the occurrence of these transients is independent of day of the year.

CONCLUSIONS

The frequency of occurrence of these damped oscillatory transients has a definite local time dependency, the most probable time of occurrence being when the observation site is located in the night hemisphere. This local time dependency does not appear to vary significantly with season. Also the occurrence of the transients themselves is independent of day and season of the year.

These conclusions seem to indicate that the cause of these damped oscillatory transients is related to something that varies at the observation site with local time, but does not vary from day to day or season to season. This suggests that the cause may somehow be determined by the daily relative position of the earth and sun, independent of the earth's particular orbital position about the sun.

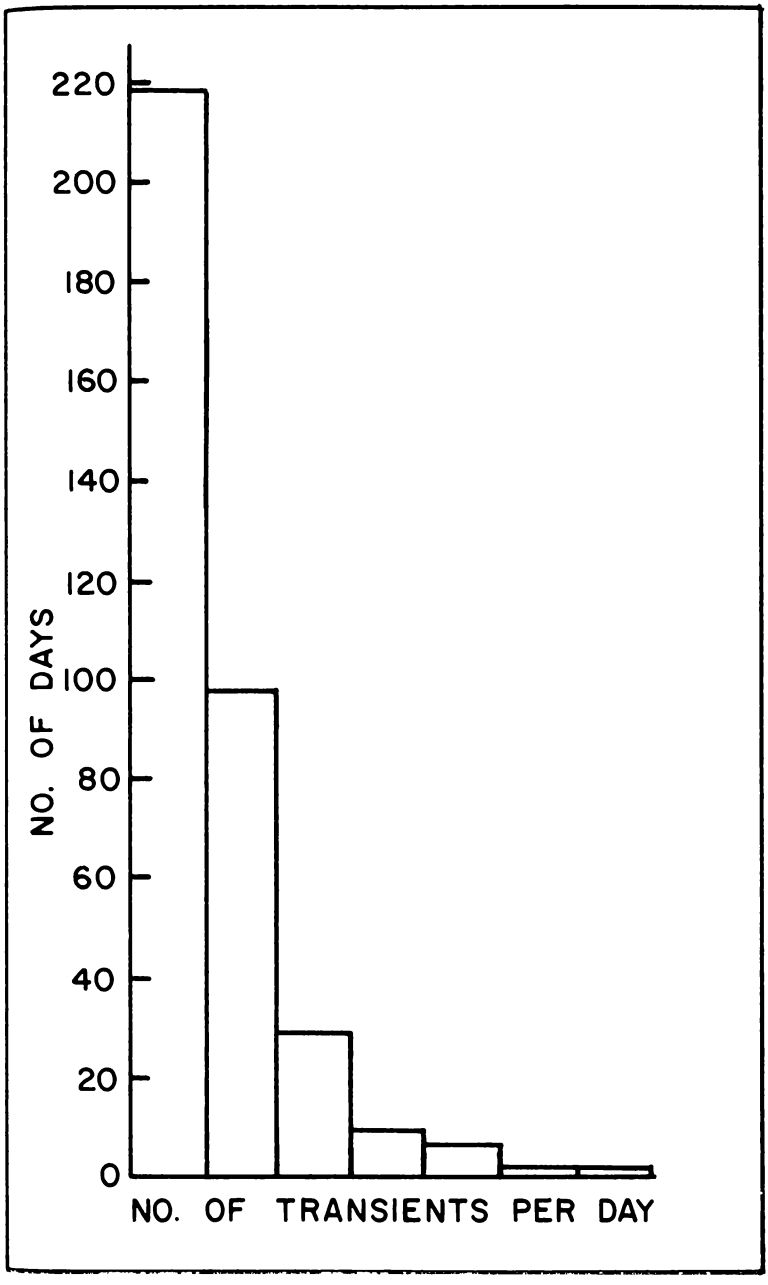
DISCUSSION

Troitskaya (3), investigating geoelectric field transients of similar form, found them to occur when the observation site was in the night hemisphere, to have a definite 24 hr. reoccurrence tendency, and a simultaneous occurrence at widely separated points of the earth. He then asserted that the cause of the transients is to be found in the charged corpuscular stream radiated by the sun and focused by the magnetic field of the Earth.

Hessler and Wescott (4), have shown that there is a strong correlation between disturbances in the geoelectric and geomagnetic fields.

Sugiura (5), investigated damped oscillatory transients in the geomagnetic field, but found them to occur at local sunrise and sunset, depending on the season, instead of in the night hemisphere. Also he found them to be elliptically polarized with the magnetic vector rotating counter-clockwise and therefore identified them as low frequency hydromagnetic waves propagated along the lines of magnetic force in the longitudinal mode, perhaps being caused by large-scale oscillations of gas in the exposphere at geocentric distances of several earth radii. Ginzberg (6), has mathematically shown such waves to be emitted by solar corpuscular streams interacting with the earth's magnetic field.

Kato (7), in classifying geomagnetic transients of this type occurring in the night hemisphere indicates that they have been closely correlated with x-ray bursts, possibly due to the precipitation of energetic electrons trapped in the exosphere. He has therefore suggested that the transients may be caused by the hydromagnetic compression of the local hot gas in the outer exosphere near the equatorial plane in the dark hemisphere, which results from the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200
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NO. OF DAYS
NO. OF TRANSIENTS PER DAY

precipitation of trapped energetic electrons from this local region to the auroral zone.

It is beyond the scope of this paper to accept or reject any of the preceding theories as to the cause of these damped oscillatory transients in the geoelectric and geomagnetic fields, there being insufficient observational data. However, it should be noted that the local time dependency found in this investigation is in agreement with most of the proposed causual theories.

ACKNOWLEDGMENTS

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I wish also to thank Professor Harry Mason for his invaluable help and advice in carrying out this study.

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STUDY OF WATER VAPOR SORPTION ON HUMIC ACID FROM LIGNITE[1]

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ABSTRACT

The adsorption and desorption isotherms for water vapor at 25° and 35° on completely vacuum-dried humic acid obtained from lignite are reported. The number of slopes in both sorption isotherms is established. The appearance of these slopes is attributed to the presence of different functional group-sites in humic acid.

The amounts of water vapor sorbed were evaluated by extrapolation of the upper slopes of the sorption isotherms to the saturation pressures of water vapor. It is suggested that these amounts of sorbed water vapor represent the total amounts needed for monolayer sorptions. The quantities found are 11.55 millimoles/gram at 25° and slightly lower at 35°.

The differential heats of adsorption and desorption, calculated by the Clausius-Clapeyron method, show noticeably higher values for desorption especially in the region of small amounts of sorption. As the amount of sorbed water vapor reaches monolayer coverage, the differential heats for both adsorption and desorption approach the value of the heat of condensation of water vapor.

INTRODUCTION

Humic acid (abbreviated H-Ac) (1) is an organic substance of unknown constitution which can be extracted as the appropriate alkali metal salt from lignite (or other decayed organic matter in soil) by an alkaline solution. It can be regenerated in its acidic form by acidification of the alkaline solutions.

Lignites are a type of 'brown-coal' formed from organic matter by a slow geological carbonization process. They contain H-Ac or its salts, humates. These are probably the only organic substances present in lignites. The amount of H-Ac found in lignite may be estimated up to about 70% by weight of the dry substance.

In the North Central States, including North Dakota, there are large deposits of lignite which provide a ready source of H-Ac. Much interest has centered on finding ways to utilize this resource, and many institutions in this country (2) and abroad, are involved in lignite research. To the present, this research has been mainly con-

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

cerned with the industrial utilization of lignite as a fuel, as a source of isotopes, and as a soil fertilizer. It is expected that an extension of the knowledge of the chemical and physical behavior of H-Ac from lignite will aid in the establishment of its structure, and thus foster a further utilization of lignite.

From the sparsely scattered literature data concerning the basic chemistry of H-Ac, it can be concluded that it is a very complex substance. However, it appears that the variety of functional groups present are appended to similar skeletal units. Detailed information about the nature of the functional groups, however, is lacking.

The present work is an effort toward this clarification which utilizes the interaction between the surfaces of H-Ac and water vapor. A high vacuum technique was employed for the gravimetric determination of sorption isotherms. From the data obtained the differential heats of sorptions were calculated, and thereby provide an estimate of the types of bonds established between the sorbed water vapor and the H-Ac surface.

EXPERIMENTAL

Measurements were made in a high vacuum system containing a sealed-in McBain quartz spiral balance with a sensitivity of 1.498 ± 0.002 mg/mm. and which was thermostated at $40.00 \pm 0.02°$ by a surrounding water jacket. From a sample[2], a specimen was weighed into a light-weight glass bucket and attached to the McBain balance by a glass thread extension. The specimen was suspended in a closed tube that was separately thermostated at the experimentally required temperature to a precision of $\pm 0.005°$. The extension or contraction of the balance spiral was measured by a traveling microscope with a precision of ± 0.001 mm. Pressures in the sorption system were read from a mercury manomenter by means of a cathetometer accurate to ± 0.005 mm. All pressures were corrected to the density of mercury at 0°. The low pressures in the system were measured by a McLeod gauge and were periodically checked by an electronic gauge.

A standard procedure of desorption was followed for each run on the specimen. This consisted of connecting the system to the vacuum pumps through a series of capillaries so that the speed of pumping in the system could be regulated to avoid spattering of the powdered sample on account of rapid changes in pressure. A mercury diffusion pump, with a cooling trap in series with a mechanical pump, was employed. After the pressure in the system reached 10^{-3} mm, the system was opened completely to the pumps until complete desorption was reached. Desorption was considered complete when the specimen came to constant weight. In cases where

[2]Provided by Boroid Division, National Lead Company, Houston, Texas.

sublimation was observed, the slope in the plot of weight loss versus time was established and the correction taken into account.

Isotherm data for adsorption and desorption of water vapor were established at 25° and 35°. Each measurement of vapor pressure in the system and of the amount of adsorption on the specimen was continued to a constant value which indicated that equilibrium had been established.

RESULTS AND DISCUSSION

A. *Vacuum Drying of Original H-Ac.*—Five specimens of the same batch were completely desorbed in the vacuum system according to the procedure previously described at a final pressure of 10^{-7} mm. All specimens behaved similarly. Typical results by desorption under conditions of increased vacuum and temperature indicated the loss of weight of specimen as a function of time and temperature. It showed four slopes of weight loss with marked dependence on time, and less definite dependence on temperature in the range investigated (25°-65°). See figure 1, curve I.

The completely dried-desorbed specimen of 182.74 mg still showed a weight loss of 0.005% per hour at 65°. When the temperature was lowered to 25° however, there was no longer an observable loss in weight. On this specimen water was absorbed up to 20%. Then desorption was repeated starting at 25°. Data of this desorption, presented as the content of water per 100 of dry H-Ac, was plotted as a function of the desorption time and shown in figure I, curve II. Although the second desorption was under slightly different tempera-

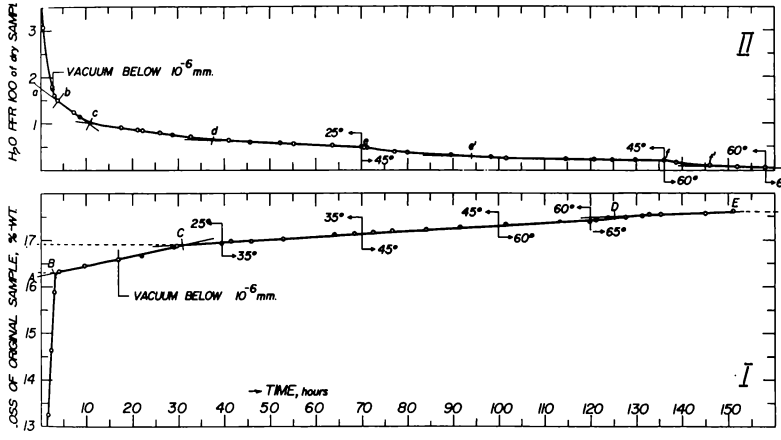

FIGURE 1—Desorption-drying of H-humates at high vacuum. I. First desorption of original sample, total loss in weight = 17.608%, final weight of dry sample = 182.74 mg. II. Second desorption of H_2O (after adsorption up to about 20% of dry weight), final weight of dry sample = 182.76 mg.

ture-time conditions, the loss of weight can be fitted to five slopes which indicates five stages of desorption.

From a comparison of the initial and the second desorption it is evident (figure 1) that the initial loss of weight during desorption is due to physically bonded H_2O (curve II down to point b) and probably some volatiles including gas from lignite or air in the original sample (curve I — up to B).

Further desorption, which requires higher vacuum, is caused principally by water which is held by stronger bonds. This means that water may be coupled with possibly three or four different functional groups (—COOH, -O - or others).

It is interesting to compare the loss of weight due to these strongly bonded water molecules in both runs. In run I, the total amount of strongly bonded H_2O was 1.37%; in run II it was 1.73%. The difference (0.36%) can be ascribed to volatiles in the original sample. After the volatiles were desorbed completely, more sites (functional groups) were exposed for H^2O readsorption in run II. This prably caused the appearance of the additional slope of desorption, b-c, curve II.

Even though there were no more volatiles present in the fully dried specimen, a slight loss of weight was observed at 65°. This latter loss (0.005%/hr) could possibly be cause by sublimation of H-Ac. A comparable occurrence has been noted with insulin-H, which has a molecular weight of 12,000 and which sublimes under high vacuum 37-47°[3]. In addition, partial dissociation of amino hydrochloride functionatalities, which originated by the use of HCl in the liberation of free humic acid from its salts, could be involved(3).

B. *Sorption of Water Vapor on H-Ac.*—All specimens (five), after complete desorption-drying at different conditions, were used for determination of adsorption and desorption isotherms. Many repeated adsorption-desorption runs were required to establish the limits of reproducibility of the data. As a result of such runs, the following were establsihed:

(a) The adsorption of water vapor on a completely vacuum-dried H-Ac specimen occurs rapidly at the start (up to 2 millimoles/gm), then slows down so much that at medium adsorption values (up to 14 millimoles/gm) it requires two to four days to reach equilibrium. Further adsorption, however, is characterized by marked diminishing of the time for equilibration.

(b) Reproducibility of the isothermic data was obtained at a specified temperature only if the specimen of H-Ac was pretreated at the same temperature. By a pretreatment is meant repeated adsorption of water vapor in amounts greater than that necessary for monolayer formation (above 25% by weight) with subsequent complete desorption at the same temperature.

[3]From author's observation. Results will be published.

This finding may possibly be explained in terms of the geometrical orientations assumed by the H-Ac molecules. Thus, the molecules that have adsorbed water in excess of the amount needed for monolayer formation may exist in a "loosened state", in which they assume geometrical orientations required thermodynamically by the spatial relationships of the functional groups. Naturally, with each particular molecular orientation, there would be associated a specific number of exposed sorption sites (functional groups or their combinations). Furthermore, the molecular spatial orientation must be temperature dependent. Therefore, it is necessary to pretreat the specimen at a particular temperature in order to obtain the appropriate orientation of the H-Ac molecules upon which the reproducibility of the isothermic data depends.

Rephrasing, the significance of the pretreatment lies in the fact that the molecule(s) of H-Ac can be oriented (or reoriented) only if they are in a "loosened" state due to the presence of excess water. Then upon desorbing, a particular orientation of H-Ac becomes fixed (hardened) even if the water is completely desorbed. This means that the same number of active sites appropriate to the temperature are exposed.

(c) An adsorption on a specimen pretreated at another temperature leads to non-reproducible data because of the reorientation process that occurs in functional groups of the H-Ac molecule(s), (i.e., reopening). It is interesting to note that a similar phenomenon of reproducible sorptions of water vapor was observed with proteins (4), when they are treated similarly.

Below are presented sorption data obtained on a typical specimen that was desorbed-dried as described. The amounts of water adsorbed (or held in desorption runs) in millimoles of water per gram of completely dried H-Ac versus pressure of water vapor in equilibrium with the specimen are plotted for 25° and 35° as shown in figure 2. In addition to the above general statement about the sorption, the following particular features of sorptions at different temperatures, can be seen from figure 2.

1. *Sorption at 25°.*—Curve 1 shows the reproducible adsorption data. It starts rapidly on the most active sites; however, when these sites are saturated the isotherm goes into a slope (extension of which to zero pressure is point A) of slower adsorption indicating saturation of less active sites.

Further adsorption produces a new, slightly different, slope (A'O'). This, then, changes into the final slope, which starts at an approximated point 0', and continues to the saturation pressure of water at 25° (=23.75mm). It is very difficult experimentally to continue measurements at an equilibrium pressure of a magnitude close to the saturation point, because of the labile state. It is, however, evident that the considerable length of the final slope justifies its approximation to saturation pressure which ordinate represents

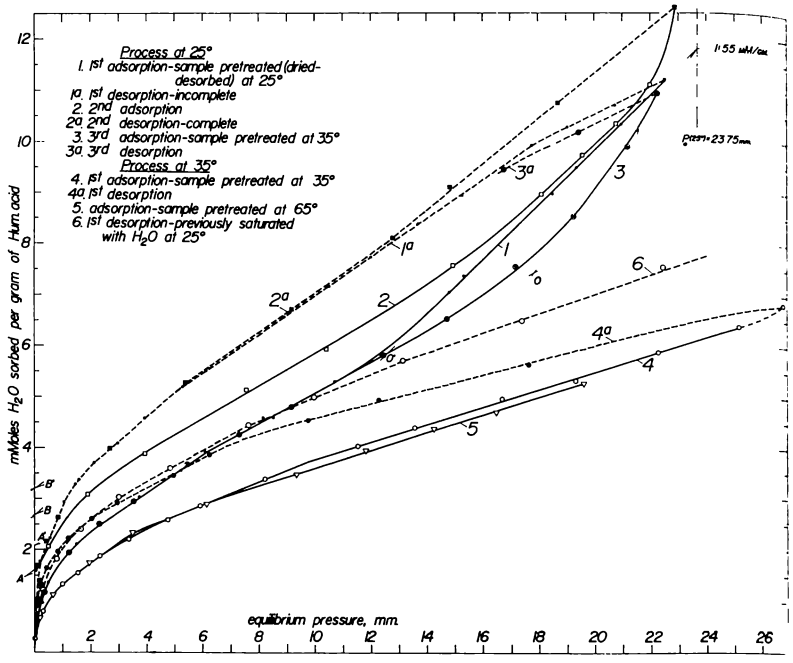

FIGURE 2—Sorption isotherms of water vapor on dry humic acid at 25° and 35°.

the total monolayer value. This monolayer value, which is considered to be a summation of monolayers on the particular active sites (functional groups), is found to be equal to 11.55 millimoles of water per gram of H-Ac.

It should be pointed out that the characteristics of a monolayer are used as suggested in the sorption on natural polymers (large molecular substances) (4, 5). This representation of monolayer sorption differs somewhat from that of the BET theory (6) which would require revision to apply to the present instance.

In this adsorption isotherm there can be seen three definite slopes and one initial curvature. The slopes indicate that there are three different kinds of sites (functional groups) involved in an interaction with the water molecules. That the initial adsorpion is characterized by a curvature needs some clarification. This initial adsorption is possibly caused by the sites of higher activities which may be closely related and which will be distinguished only on a sample of H-Ac with more highly developed surface. Experiments in this direction are in progress.

A desorption at this temperature is represented in curve 1a, which, as the corresponding adsorption, contains three slopes and a curvature. Also the upper slope of desorption (approximated to the saturation pressure), has only a slightly higher value for the monolayer. The desorption isotherm, however, shows higher values of amounts sorbed (held) on the specimen which can be characterized as a hysteresis.

A hysteresis in desorption on natural substances is a general but not definitely explained phenomenon in which an involved activation energy seems to be the main factor.

The influence of a pretreatment of specimen at a higher temperature (35°), on which then the adsorption was carried out at a lower temperature (25°), is presented in curve 3. These data show initial coincidence with curve 1 (reproducible data), but the uptake slows down at about one-half of the monolayer amount sorbed. This is a good indication that spatial reorientations of H-Ac are involved (reopening of structure). Its final slope, approximated from point O to the saturation pressure, seems to give the same value of the total monolayer sorption.

Some readsorptions were performed on specimens which were not completely desorbed. The adsorption isotherms of such runs demonstrated values intermediate between the adsorption and the desorption isotherm, as illustrated in curve 2.

2. *Sorption at 35°.*—Determinations of sorption isotherms were experimentally limited to the region below the equilibrium pressure of 26 mm which is far below the saturation point of water vapor at 35° (52.17 mm).

At this temperature it required less time for equilibration.

Curve 4 of figure 2 presents a reproducible adsorption isotherm in the range of the pressures investigated below 26 mm. Its shape can be approximated to three slopes and one initial curvature. These slopes are different from those obtained at 25°.

Curve 4a is a desorption isotherm at 35° which is not a typical one since the desorption was started below monolayer coverage.

Curve 5 shows the influence of pretreatment of the specimen at a higher temperature (65°) which demonstrated the same effect described above (see adsorption at 25°, also statement (c)).

Finally, curve 6 shows data which could be considered as a desorption isothem at this temperature, since it started from a multilayer of H_2O. The shape of this desorption isotherm again can be approximated to three slopes and a curvature at low pressure which corresponds quite closely to those of the desorption isotherm at 25°. Furthermore, an extension of its upper slope to the pressure of saturation (not shown in figure 2) gives a value which is only slightly lower than the total monolayer sorption approximated at 25°.

It should be noted here that an approximation of the upper

slope of adsorption curve 4 at 35°, relative to the saturation pressure, shows a value 12% lower than that of the desorption isotherm. Such a discrepancy should be clarified in further investigations; however, it is suggested that at this temperature additional kinds of active sites may be present. These sites may produce a further sorption (not reached because of limits in equilibrium pressure) demonstrated by a new slope, the extension of which to the point of saturation pressure may coincide with the total monolayer value.

C. *Differenial Heats of Water Sorption on H-Ac.*—The isothermic data (from figure 2) were used for calculations of differential heats of adsorption and desorption by use of the Clausius-Clapeyron method. These heat values, $-\Delta H$ in kcal/mole, were then plotted against the amounts of sorbed (or held) H_2O, expressed in millimoles per gram of H-Ac.

The resulting curves are shown in figure 3, where a horizontal dashed line represents the average heat of condensation of water between 25° and 35°.

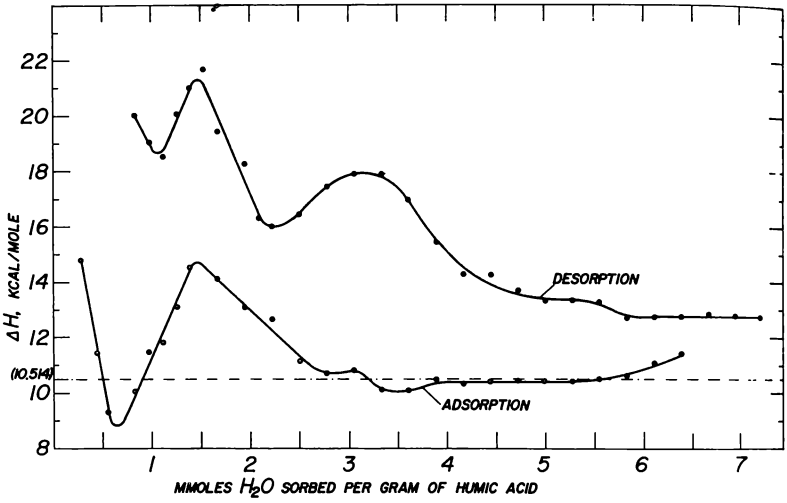

FIGURE 3—Differential sorption heats of H_2O-vapor on humic acid in the temperature range 25-35°.

From this plot it can be seen that the heats for adsorption are below those found by desorption. However, there is a relative similarity in the two curves.

In the case of adsorption, the initial heat measured at 0.40 millimoles/gm is around 15 kcal/mole, which may be extrapolated up to 20 kcal/mole at the start of adsorption. A relatively high value of

sorption heat indicates a strong bond and explains the rapid initial equilibration during an adsorption.

That a minimum appears for ΔH, which is even below the heat of condensattion for water may indicate a retardation in the adsorption process such as hydrophobic screening of the active sites. This seems to be overcome after an amount of water adsorption is "pushed forward" by increasing the pressure. It reaches a new maximum of 15 kcal/mole at an amount adsorbed equal to 1.5 millimoles/gm.

From this point on, the heats decrease linearly crossing the line of heat of condensation for water and reaching the second minimum value which is only slightly below. A further adsorption is characterized by heats around the value of water condensation. Finally, a rise in the curve cannot explained as yet.

A curve of differential heats of desorption contains two distinct maxima of ΔH between 16 and 22 kcal/mole covering an amount of sorption equal to approximately 4 millimoles/gm which must be associated with a strong chemical bond. The content of water above 5.7 millimoles/gm has a value of ΔH around 13.0 kcal/mole and can be described as very weakly bonded.

The comparison of adsorption and desorption heats shows large differences along the whole process below the total monolayer amount sorbed. These differences in differential heats apparently are caused by hysteresis in desorption.

If the hysteresis of desorption is dependent on the activation energy, as is suggested (4), it could be expected that this difference in ΔH of desorption and adsorption may be equal or at least proportional to the activation energy, q: $\Delta H_{des} - \Delta H_{ads} = Kq$. where "k" is a proportionality factor, whose value may be $\leqq 1$, if the activation is the only factor causing the hysteresis.

A clarification of this relation could be of specific value for structural studies, but it needs further experimental confirmation. It seems that the heats of sorption cannot be easily interpreted.

It is hoped that further investigations using these specially prepared samples, mentioned before, which have a very highly developed surface, will provide further clarification in sorptions as well as in heats of sorption.

CONCLUSION

The results presented here show that H-Ac from lignite is a molecular complex for which the method of high vacuum technique of investigation can be further applied.

Its sorption isotherms of water vapor have demonstrated the presence of three different sites (functional groups) and probably some smaller amounts of other very active sites. Further investigations of a more highly purified sample (with a highly developed surface) are needed to distinguished between these sites.

The surface studies using other substances for sorption as well as hydrogen⇆deuterium exchange are suggested as the next step in clarification of the physical and chemical behavior of H-Ac.

Also, investigations of acid-base behavior in liquid colloidal state interactions should be made for the purpose of analyzing functional groups.

ACKNOWLEDGMENT

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SYNTHESIS OF ETHYLMETHYLPHENOLS

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Tar acids extracted from the tar of lignitic coal carbonized at low temperature contain, among other phenolics, small amounts of ethylmethylphenols. To determine which ethylmethylphenols are present and in what quantity, these phenols were synthesized to obtain their infrared spectra for comparison with the spectra of the tar acid fractions.

Of the ten isomers of ethylmethylphenol, eight were synthesized according to procedures given in the literature (18). 3-Ethyl-4-methylphenol had been synthesized previously by Morgan and Pette (9), but it was found advantageous to prepare this isomer through a simpler route starting with 3-ethylphenol, forming the methyl ether and introducing an aldehyde group into the 4-position using the Gat-

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termann aldehyde synthesis (10). The aldehyde group is then reduced to the methyl group, and the desired phenol obtained after the methoxy group is demethylated.

The remaining isomer, 2-ethyl-3-methylphenol was prepared from 2, 3-dimethylphenol by oxidizing the 2-methyl group to the carboxylic acid, forming the acid chloride, then extending the carbon chain by reacting the acid chloride with dimethylcadmium. The resulting acetophenone is returned to the ethyl group and the product obtained after demethylation.[2]

EXPERIMENTAL[3]

3-Ethylanisole.—Dimethyl sulfate (63 g) was added dropwise to a well-stirred, cold solution of m-ethylphenol[4] (61 g) dissolved in a solution of sodium hydroxide (21 g) in 200 ml of water. Following the addition of dimethyl sulfate, the reaction mixture was refluxed with stirring for 2 hours, cooled, and 150 ml of water added. The product, separated from the mixture as the top layer, was washed once with water, twice with dilute H_2SO_4, then with water until the washings indicated that no acid was present. The product was dried over $CaCl_2$ and distilled; yield 52.3 g (77 percent).

2-Ethyl-4-methoxybenzaldehyde.—3-Ethylanisole (50 g), 100 ml of benzene (dried over sodium), and anhydrous $Zn(CN)_2$ (64.8 g), were placed in a flask equipped with a stirrer, condenser, and gas inlet. The mixture was cooled while dry HCl was bubbled through the stirred mass for 1 hour. The addition of HCl was then discontinued while anhydrous $AlCl_3$ (59 g) was added. After adding the $AlCl_3$, HCl was again bubbled through the mixture for 4 hours. The contents of the flask were then poured into a dilute aqueous HCl solution and refluxed for 1 hour. The product was extracted with benzene and distilled, giving 21.7 g (36 percent); b.p., 90-92° (3mm).

Anal. calcd. for $C_{10}H_{12}O_2$: C, 73.14; H, 7.37
Found: C, 72.66; H, 7.44

3-Ethyl-4-methylanisole.—2-Ethyl-4-methoxybenzaldehyde (21.2 g) was refluxed for 10 hours with amalgamated zinc (11) (50 g) in a solution of 100 ml of conc. HCl and 100 ml of H_20. The refluxed mixture was extracted with ether and the ether was dried over $CaCl_2$, and distilled to yield 14.5 g (74 percent) of product; b.p., 57-59° (2 mm).

Anal. calcd. for $C_{10}H_{14}0$: C, 79.95; H, 9.39.
Found: C, 79.82; H, 9.46.

3-Ethyl-4-methylphenol.—3-Ethyl-4-methylanisole (14.3 g) was refluxed for 15 hours in a solution of 50 ml of conc. HBr and 50 ml

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[2]In order to form the acid chloride it is necessary to replace the phenolic hydrogen with a methyl group.

[3]All melting points were determined on a Fisher-Johns melting point apparatus and are corrected. The boiling points are uncorrected.

[4]Eastman yellow-label reagent used as-received.

of glacial acetic acid. The reaction product was cooled, neutralized with $NaHCO_3$ and extracted with ether. The etheral solution was dried over $CaCl_2$ and the product distilled to yield 5.5 g (43 percent); b.p., 81-83° (2 mm); n_D^{20}, 1.5343; aryloxyacetic acid m.p., 136°.

Anal. calcd. for $C_9H_{12}0$: C, 79.37; H, 8.88
Found: C, 78.70; H, 8.87.

The product was further purified by gas-liquid chromatography and the I.R. spectra recorded (12).

2-Hydroxy-6-methylbenzoic acid.—Potassium hydroxide (120 g) was added to a nickel crucible containing 2,3-dimethylphenol[5] (20 g) while the temperature was raised slowly to 235° C. The melt was stirred rapidly under a slow stream of air for 20 hours, cooled, dissolved in water, and acidified. The precipitate was extracted with ether, and the product was removed from the etheral solution by several extractions with dilute $NaHCO_3$. The $NaHC0_3$ solution was acidified, and 2-hydroxy-6-methylbenzoic acid was precipitated. The product was recrystallized from hot water giving 4.9 g (20 percent); m.p., 169°; reported m.p., 168°(13).

Anal. calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30
Found: C, 62.88; H, 5.41.

2-Methoxy-6-methylbenzoic acid.—Dimethyl sulfate (32.8 g) was added dropwise to a well-stirred, cold solution of 2-hydroxy-6-methylbenoic acid (20 g) in 115 ml of 10 percent NaOH. The mixture was refluxed for 2 hours; then an additional 200 ml of 10-percent NaOH was added and refluxing continued until the product was completely dissolved. The solution was acidified and the product removed by filtering. The crude product was recrystallized from hot water to yield 18.5 g (85 percent); m.p., 139°; reported m.p., 139° (14).

Anal. calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07.
Found: C, 64.91; H, 6.08.

2-Methoxy-6-methylacetophenone.— 2-Methoxy-6-methylbenzoic acid (18.5 g) and thionyl chloride (21.3 g) were dissolved in 20 ml of benzene (dried over sodium) and refluxed for 2 hours. The unreacted thionyl chloride was then removed by distillation and 100 ml of dried benzene added to the product. The acid chloride was not isolated.

Dimethylcadmium was prepared by the reaction of methyl Grignard reagent with cadmium chloride. Methyl bromide (14.4 ml) in 200 ml of ether was reacted with magnesium (5.4 g). After the reaction had subsided, the flask was surrounded with an ice bath and anhydrous cadmium chloride (20.2 g) was added in small portions with rapid stirring. The ether was then replaced with dried benzene and the previously prepared acid chloride stirred into this solution. The mixture was refluxed for 2 hours with continuous stirring, then hydrolyzed with 250 ml of dilute HCl. The benzene

[5]Aldrich Chemical Company research grade reagent used as-received.

layer was recovered, washed with dilute NaOH solution, and dried over $CaCl_2$. The product was recovered by distillation to yield 12.0 g (67 percent); b.p., 80-82° (2 mm).

Anal. calcd. for $C_{10}H_{12}O_2$: C, 73.14; H, 7.37.
Found: C, 73.16; H, 7.50.

2 - Ethyl-3-methylanisole. — 2-Methoxy-6-methylacetophenone (11.8 g) was refluxed for 15 hours with 40 g of amalgamated zinc in 60 ml of conc. HCl and 60 ml of water. The product was extracted with ether, dried over $CaCl_2$, and distilled to yield 7.7 g (71 percent); b.p., 57° (2.4 mm).

Anal. calcd. for $C_{10}H_{14}O$: C, 79.95; H, 9.39.
Found: C, 80.25; H, 9.46.

2-Ethyl-3-methylphenol.—2-Ethyl-3-methylanisole (7.2 g) was refluxed for 15 hours with 25 ml of conc. HBr and 25 ml of glacial acetic acid. The refluxed material was extracted with ether and washed successfully several times with a sodium bicarbonate solution and water. The product was extracted in a 10-percent NaOH solution, neutralized, and dissolved in ether.

The etheral solution was dried over $C.Cl_2$ and the product obtained by distillation to yield 2.2 g (34 percent); b.p., 90° (6 mm); n_D^{20}, 1.5357; aryloxyacetic acid m.p., 134.5°.

Anal. calcd. for $C_9H_{12}O$: C, 79.37; H, 8.88.
Found: C, 78.38; H, 8.84.

The product was further purified by gas-liquid chromatography and the I.R. spectra recorded (12).

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INTERVALENCE COMPOUNDS OF VANADIUM

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ABSTRACT

Sarma (1) observed an intense green coloration when a solution of ascorbic acid was added to a solution of a metavanadate. Subsequent work showed that ascorbic acid reduced vanadium(V) to vanadium(IV) which then reacted with an excess of vanadium(V) producing the green coloration. The same coloration was observed when a solution of vanadyl sulfate, $VOSO_4$, was added to a solution of sodium metavanadate, $NaVO_3$, at room temperature. The product had a low solubility and was free from sulfate. Since there was no sodium in it, it was believed that the product was a complex polyvanadic acid. X-Ray powder patterns of this compound appeared to differ from those of the parent compounds. However, analysis by Job's continuous variation method in spectrophotometry, conductometry, and high frequency titration showed that the final composition of the product depended upon the concentrations of the reactants, time of reaction, temperature, and pH. Therefore, variations in one or more of these experimental conditions should permit the synthesis of several polyvanadic acids from vanadium(IV) and vanadium(V). Investigations are being conducted to study the compositions of some of these polyvanadic acids.

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NITRILE COMPLEXES WITH COPPER (I) HALIDES

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ABSTRACT

Recent work on metal olefin compounds involving copper(I) salts has led to a new method of synthesis which may be generally applicable to other similar complexes (1). The extension of this method to complexes involving organic nitriles and copper(I), halides (chloride and bromide) is now reported. Complexes of malononitrile, succinonitrile, glutaronitrile, adiponitrile, acetonitrile, benonitrile and acrylonitrile with $CuX(X = Cl$ or $Br)$ have been prepared and characterized. Copper(I) bromide complexes with methacrylonitrile, butryonitrile and propionitrile have also been prepared. The corresponding chloro complexes have not as yet been isolated. The acrylonitrile complexes and the CuBr(adiponitrile)

complex contain two moles of CuX per mole of nitrile (2:1 stoichiometry). All the other complexes so far isolated have a 1:1 stoichiometry. This is in contrast to nitrile copper(I) nitrate complexes which show a 1:2 stoichiometry (two moles of nitrile per mole of CuX) for the succinonitrile, glutaronitrile and adiponitrile cases and 1:4 stoichiometry for the acetonitrile complex.

Infrared data on the nitrile copper(I) halide complexes indicate that the nitriles are probably coordinated through the N atom rather than the π system of the nitrile group. This is in accord with the coordination found in the copper(I) nitrate complexes (2).

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COMPLEXES OF CEROUS AND FLUORIDE IONS[1]

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According to the literature, cerous ion produces one of the most insoluble fluorides. However, one of us (Sarma) observed that if a solution of cerous nitrate was carefully added to a solution of sodium fluoride, the precipitate of cerous fluoride which was initially formed, redissolved on the addition of an excess amount of cerous nitrate. It was believed that the precipitate reacted with cerous nitrate forming a complex ion. Therefore, an investigation was made to identify this complex ion using potentiometry, nephelometry, conductometry, and high frequency titration. In all of these methods, it was assumed that an inflection point in a graph relating moles of sodium fluoride added to a fixed number of moles of cerous nitrate indicated the formation of a new ionic or molecular species. In the potentiometric, conductometric, and nephelometric methods, the graphs showed three inflection points corresponding to the reactions between 1.00 and 0.94, 1.00 and 1.94, and 1.00 and 3.00 mole of cerous ion and moles of fluoride ion, respectively. Therefore, it was concluded that cerous and flouride ions reacted producing CeF^{++}, and CeF_2^+, and CeF_3. Also, with the potentiometric method, the addition of cerous nitrate

[1]This investigation was part of a project supported by the U. S. Public Health Service Grant DE-01918-01 from the National Institute of Dental Research.

to a suspension of cerous fluoride was found to produce two inflection points which corresponded to the formation of CeF_2+ and then CeF^{++}. Within the experimental conditions, high frequency titration indicated the formation of only CeF_3. Studies are being conducted to investigate similar complexes of other rare earths and halogens.

ACID-CATALYZED ANILIDE REARRANGEMENTS

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ABSTRACT

It has been shown that rearrangements of anilides occur under the conditions of the Fries reaction and under photochemical conditions. The products resulting from these reactions are o- and *p*-aminoaromatic ketones as well as cyclization products in some cases. The optimum conditions for the rearrangement of the anilides with Lewis acids is poorly developed in comparison to those of the Fries reaction.

The purpose of this study is to determine the optimum conditions (e.g., the proper Lewis acid, molar ratio, temperature and reaction time) using the model system, benzanilide. The absence of an active methylene in this molecule prevents the formation of cyclization products. In order to facilitate the running of large numbers of reactions and the determination of the yields of these reactions quickly, the following spectrophotometric technique is being employed.

For a given wavelength in the ultraviolet spectrum of a mixture of three compounds, the optical density of the mixture is equal to $\epsilon_o C_o + \epsilon_p C_p + \epsilon_b C_b$ (where ϵ = liters/mole and C = moles/liter). The concentrations of the three components can be determined by using the ϵ's at three different wavelengths on an obsorption maxima in a region where all three compounds absorb light. For the three different wavelengths, three equations are obtained containing three unknowns (which in this case are the concentrations of o-aminobenzophenone, (o); *p*-aminobenzophenone, (p); and the starting benzanilide, (b). The three equations were constructed and programmed to be solved on an IBM-1620 computer.

Preliminary experiments have shown that the reaction mixtures contain o-aminobenzophenone, *p*-aminobenzophenone and the starting benzanilide.

PHOTOISOMERIZATION OF SEMICARBAZONES

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ABSTRACT

Stenberg and Rao (1,2), in the investigation of the irradiation of Benzaldehyde and acetophenone semicarbazones, found the corresponding geometric isomers as products. From acid isomerization studies and the ultraviolet spectra of these semicarbazones, the investigators were able to assign *syn* configuration to the starting matrials and the *anti* configuration to the photoproducts. The object of this study included the determination of whether or not these reactions developed a photostationary equilibrium and, if so, the determination of the percent composition of the isomers at photoequilibrium.

The *syn* forms of benzaldehyde semicarbazone and acetophenone semicarbazone have ultraviolet maxima at 280 and 275 millimicrons, respectively, while the photoproducts have noticeably lower *epsilon* values in that range. By irradiating both isomers of each semicarbazone, the absorbance value of the reaction mixture at the equilibrium point could be found; and, from this, the relative concentrations of the isomers could be estimated.

The irradiation of acetophenone semicarbazone yielded the predicted pattern of *epsilon* changes. However, after reaching an equilibrium, the *epsilon* value continued to slowly decrease, indicating the presence of a slow secondary reaction. Assuming a negligible effect from this secondary reaction, the photoequilibrium of acetophenone semicarbazone results in an *anti-syn* mixture in about 75% and 25%, respectively.

In the irradiation of benzaldehyde semicarbazone, both the *anti* and the *syn* forms started with relatively large values of *epsilon* and dropped rapidly to a much smaller, but identical, *epsilon* value (after 1½ hours irradiation). The value remained constant with additional irradiation and in the absence of light. This indicates: (1) that there is no *syn-anti* equilibrium, but rather, both isomers are converted to a second photoproduct, or (2) that the less stable *anti* isomer partly isomerized to the *syn* form while standing. The latter possibility was ruled out on the basis of melting point comparisons and thin layer chromatography of an aged sample with the original photoisomer. Column chromatographic work-up of a benzaldehyde semicarbazone irradiation afforded a major portion of an oil and a minor portion of a white crystalline compound (m.p. 68-70°), neither of which have, as yet, been identified.

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PHOTOOXIDATION OF ALCOHOLS TO ALDEHYDES WITH TRINITROBENZENE

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ABSTRACT

The primary objective of this work was to explore the photooxidation of alcohols inthe presence of nitroaromatics. Various alcohols were irradiated in a solution of trinitrobenzene with diglyme as the solvent. These solutions in turn were analyzed by quantitative vapor phase chromatography analysis using the peak area method. The amount of alcohol converted was noted, and the per cent yield was calculated.

These alcohols were irradiated under two separate conditions. The first set of conditions, 24 hour irradiation in closed quartz cells under nitrogen atmosphere, gave the results as shown in Table I. Ethanol, propanol, and butanol were the only alcohols used. Significantly none of the corresponding acids were found.

Time studies were conducted with butanol in a closed quartz

TABLE I

APPROXIMATE PERCENTAGE BY VOLUME OF H_2 AND CO IN IRRADIATION OF ALCOHOL-DIGLYME-TNB MIXTURES IN SEALED PYREX VESSELS

Alcohol	$\%H_2$	%CO
Ethanol	0.025	0.2
Propanol	0.035	0.5
Butanol	0.045	1.0

TABLE II

IRRADIATION OF ALCOHOL-TNB-DIGLYME IN QUARTZ VESSELS(a) AND SEALED PYREX TUBES(b)

Alcohol	% Converted S. M.	% Yield[1] aldehyde	Other products
Ethanol (a)	47.1	7.9	
Ethanol (b)	—	—	
Propanol (a)	37.5	39.1	
Propanol (b)	20.4	15.4	
Butanol (a)	69.1	9.1	13.6 (n-hexane)
Butanol (b)	15.0	10.1	4.6(n-hexane)

vessel, and three graphs were plotted (figure 1). The appearance of n-hexane was due to the known photodecomposition of butraldehyde. Carbon monoxide and hydrogen are also produced in this reaction. The leveling of each curve can be explained either by the

[1]All yields are based on converted starting material.

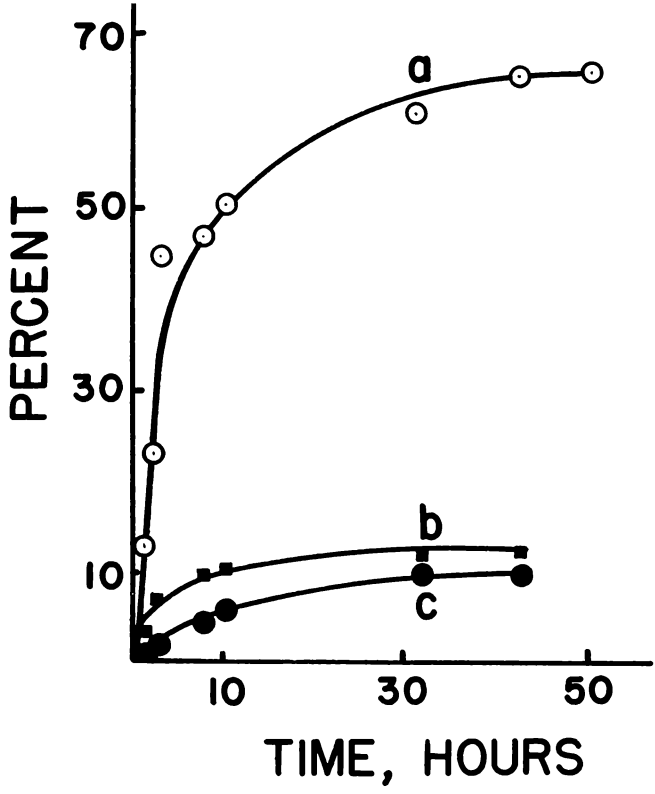

FIGURE 1—Rate Studies on a Butanol Irradiation
(a) per cent conversion of butanol
(b) per cent yield of n-hexane
(c) per cent yield of butraldehyde

filtering effect of the azoxy compounds formed in the reaction mixture or by a film of tar which begins to appear on the inner surface of the irradiation vessel after several hours of irradiation.

The photodecomposition of simple aldehydes is a major obstacle with these conditions. Since the amount of light absorbed by the aldehyde is greatest at wavelengths of 2500-3100 A, the irradiations were in sealed Pyrex tubes. The vapor phase chromatography results of the exhaust gases in Table I indicate that decomposition of the aldehydes still proceeds. Table II lists the condensable products and yields of these reactions.

The results can be explained by the proposition that a hydrogen

molecule is abstracted by an excited state nitro group. The lack of carboxylic acid products can also be explained *via* this mechanism. The secondary photodecomposition of the aldehydes formed is a significant side reaction which is not hindered by a Pyrex filter. The latter fact can be attributed to a triplet state energy transfer of energy from trinitrobenzene which acts as a photosensitizer.

PILOT SCALE EXTRACTION OF THE ANTIPYRIDOXINE FACTOR IN LINSEED MEAL[1]

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INTRODUCTION

The laboratory scale extraction of an antipyridoxine factor from oil-free flaxseed cotyledons has been reported (1). The presence of the factor was demonstrated by the poor growth which resulted when chicks were fed 70% alcoholic extracts of flaxseed cotyledons. This poor growth was readily counteracted by supplying 40 ppm. of pyridoxine to the ration containing the extracts. The relatively large amounts of extracts required to conduct meaningful assays which depended upon chick growth studies indicated the need for extracting a substantial quantity of either linseed or flaxseed cotyledons. Since flaxseed cotyledons are relatively difficult to prepare, a system based upon some form of commercial linseed meal appeared to be most desirable. Schlamb *et al.* (2) had shown that commercial linseed meal was much less toxic than laboratory extracted meal. This improvement in nutritional quality was probably the result of the heating and toasting process to which linseed meal is subjected in the final processing stages. The lack of toxicity of commercial linseed meal had been confirmed in this laboratory. Thus it was necessary to obtain a specially prepared linseed meal for this pilot scale extraction process.

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METHODS

A quantity of "spent linseed flakes" was supplied by the Minnesota Linseed Oil Company, Minneapolis, Minnesota for this extraction process. Spent linseed flakes are obtained from the commercial solvent extraction process, but have not been subjected to the toasting or other heating process that is usually used in the final processing steps. The hexane-saturated, oil-free linseed flakes were removed from the commercial extractor and the solvent was allowd to evaporate at room temperature.

The feasibility of using spent linseed flakes as a source of the antipyridoxine factor was demonstrated on a small scale, using the general procedures outlined earlier (1). This involved the adsorption of the factor on a cation exchange resin, Amberlite IR-120, buffered to pH 3 with 2M phosphate buffer, followed by elution with 1M ammonia solution. Preliminary studies showed that the factor could also be adsorbed by an anion exchange resin Amberlite IRA-400 (Acetate form). It was also found that 70% isopropyl alcohol could be substituted for the 70% ethyl alcohol without loss of efficiency.

Conversion of Amberlite IRA-400 (Cl^-) to the Acetate Form.

Ten kilograms of Amberlite IRA-400(Cl^-) (20-50 mesh) was packed into two columns, each 120 cm long and 10 cm in diameter. Sodium hydroxide (1M) was passed through the resin beds until a test for chlorides became nearly negative. An excess of 1M acetic acid was passed through the resin and the columns washed free of acid by distilled water. The columns were ready for use.

Extraction of the Antipyridoxine Factor.

The general extraction process is shown in figure 1. The spent linseed flakes were mechanically stirred in 70% isopropyl alcohol and the slurry centrifuged in a Sharples continuous flow centrifuge. The supernatant from the centrifuge was turbid and required further clarification in a continuous rotary vacuum filter. The clear filtrate was pumped through the two columns which were connected in series at a flow rate of 800 ml/min. After the extract from approximately 250 lbs. of linseed flakes had been passed through the ion exchange columns, the first of the two columns was eluted with 1N acetic acid to give the crude antipyridoxine factor preparation. After the elution was complete, the resin was rinsed with distilled water until free of acid and replaced in the system behind the column which had not been eluted. This process was repeated at regular intervals, whenever the extract from another 250 lbs. had been passed through the columns.

The crude preparation was concentrated under reduced pressure and stored frozen for assay and further fractionation studies.

Assay for the Toxicity of the Crude Preparation.

Week-old chicks that had been reared on a semisynthetic ration which was free of vitamin B_6 were used as the test animals. The crude preparations were diluted to various concentrations, and in-

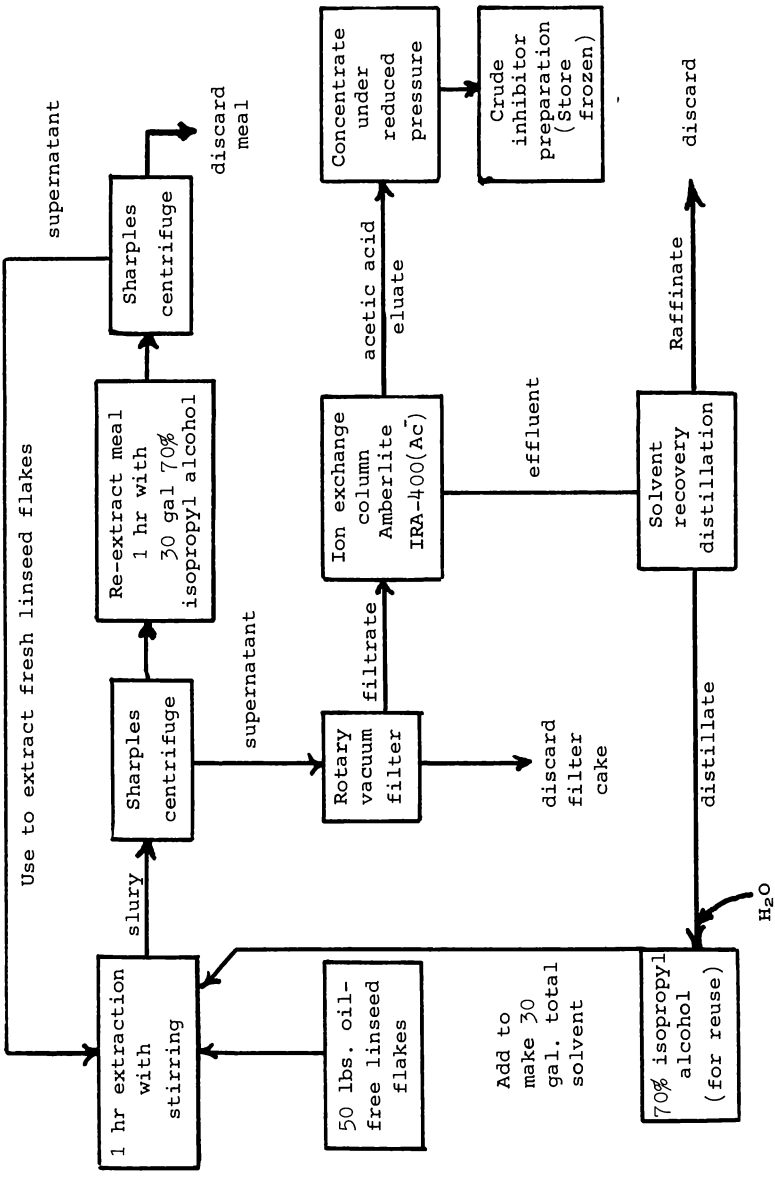

FIGURE 1.—Flow diagram for the pilot scale extraction of the anti-pyridoxine factor.

jected into the peritoneal cavity of the chicks. Control birds received either an equal volume of water or the crude preparation plus a quantity of pyridoxine hydrochloride. The time required for the development of typical acute vitamin B_6 deficiency symptoms was noted. At high levels the crude preparations were lethal.

RESULTS

Altogether 1500 lbs. of spent linseed flakes was extracted by the process outline in figure 1. The crude preparations were toxic but not as toxic as preparations obtained using small scale laboratory extraction processes. In general, the assay showed that the pilot scale preparations were lethal at the 50 gram-equivalent level, but not at the 20 gram-equivalent level. In contrast, laboratory prepared extracts were usually lethal at the 20-30 gram-equivalent level.

The assay chicks which received the lethal injections developed vitamin B_6 deficiency symptoms in 2-3 hrs. after injection. These were characterized successively by loud chirping, loss of equilibrium, erratic running, convulsions, seizures, and finally death. Post mortem examination of the birds showed the accumulation of mucous-like fluids in the epithelial tissues and digestive tract. Many of the birds appeared to succumb as a result of suffocation from fluids regurgitated from the crop and drawn into the respiratory tract.

Assay birds which received either injections of water or crude preparations plus 1 mg pyridoxine hydrochloride were normal in every respect.

The crude pilot scale preparations contained 5.8 of solid material per kg of linseed flakes extracted, or 0.58% of the total meal. Paper chromatography showed this to include a wide range of ninhydrin positive substances, carboxylic acids and phenols plus a number of substances which reduced periodic acid. There were no particularly unique substances indicated.

Characterization of the antipyridoxine factor will depend upon the development of more refined fractionation procedures and more sensitive assay techniques.

SUMMARY

A pilot scale process was developed for extraction of the antipyridoxine factor from spent linseed flakes, a form of linseed meal. The process utilized an aqueous isopropyl alcohol extraction followed by adsorption on an anion exchange resin, Amberlite IRA-400 (Ac^-). The acetic acid eluates of the resin were toxic to chicks, using an interperitoneal injection assay technique. The crude antipyridoxine factor preparations were suitable for further fractionation and purification procedures.

ACKNOWLEDGMENTS

The assistance of Mr. A. W. Dafoe and Mr. T. M. Farley in the development of the chick injection assay and Mr. Tony Hammerlik in

the execution of the extraction process is gratefully acknowledged. The vitamin B_{12}-free chick ration was developed by Mr. W. J. Lockhart.

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A NOVEL OXIDATION OF CYCLIC KETONES TO LACTONES WITH CHROMIC ACID

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ABSTRACT

Oxidation of 2,2,4,4-tetramethyl-1, 3-cyclobutanedione with chromic acid in 90% acetic acid yielded the gamma-lactone of 2,2,4-trimethyl-3-keto-4-hydroxypentanoic acid in 35-40% yield with complete destruction of starting material. The previously unreported lactone was a white solid, m.p. 39.5-40.0°; oxime m.p. 202.0-203.5°; 2.4-dinitrophenylhydrazone, m.p. 182.5-184.5°. The infra-red spectrum of the lactone indicated the strongest absorption at 1110 cm^{-1}, tentatively assigned as the gamma-lactone C-O stretching vibration, and carbonyl stretching absorptions at 1760 and 1790 cm^{-1}. The nuclear magnetic resonance spectrum indicated the presence of methyl protons only, with singlet resonance peaks of equal area at 79 and 91 cps. Hydrolysis of the lactone with a variety of strong and weak bases in water and in ethanol invariably yielded 2,4-dimethyl-2-hydroxy-3-pentanone. This reaction was analogous to the ketonic cleavage of acetoacetic ester.

Oxidation of 2,2,4,4-tetramethylcyclobutanone yielded the gamma-lactone of 2,2,4-trimethyl-4-hydroxypentanoic acid with properties identical to those reported by Wilcox and Nealy (1).

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1. Department of Chemistry, Minot State College, Minot, North Dakota. Support of a National Science Foundation Cooperative Graduate Fellowship at the University of Delaware is gratefully acknowledged.

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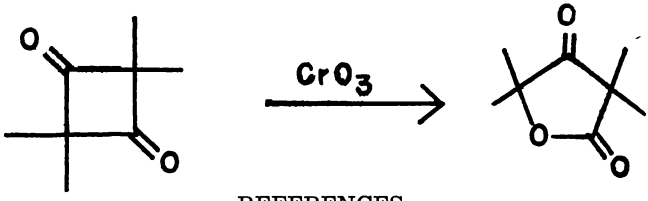
A mechanism is proposed in which the reaction proceeds via the chromate ester of the ketone hydrate (2).



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PURIFICATION OF BARLEY STRIPE MOSAIC VIRUS USING DIFFERENTIAL FILTRATION

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ABSTRACT

Frozen barley plants infected with barley stripe mosaic virus were ground while frozen and suspended in an equal volume of 0.005 M phosphate buffer and 0.1 M ascorbic acid at pH 6.8. The plant extract was clarified by centrifugation at 6000 x g and the supernatant was filtered successively through millipore membranes of 2000 and 1000 A average pore diameter. The precipitates on the two filter membranes were suspended in the phosphate-ascorbic acid buffer at pH 6.8 in the same volume as the original plant sap. This suspension was centrifuged at 6000 x g, the pellet was discarded and the virus collected from the supernatant by centrifugation at 105,000 x g for one hour. The virus-containing pellet was resuspended in phosphate buffer and the suspension centrifuged at 1500 x g. The pellet was discarded and the supernatant was used for infectivity and electron microscope studies.

GARRISON RESERVOIR GAME MANAGEMENT AREAS

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INTRODUCTION

Garrison Reservoir began filling in June, 1953. Since that time, North Dakota has lost many acres of excellent wildlife habitat to the reservoir. Flooding has occurred not only on the river bottoms, but on adjacent uplands as well. Big game and upland game species have suffered the greatest loss of habitat. Waterfowl have gained habitat.

On Garrison Reservoir areas where sufficient land remains above the high-water mark, 1850 feet above sea level, and the Corps of Engineers take-line, we have worked closely with the Corps of Engineers and the Bureau of Sport Fisheries and Wildlife in managing these areas to partially compensate habitat losses to flooding. I say "partially compensate" because I do not believe it is possible to replace the habitat which has been lost to flooding, no matter how intensively we manage lands which remain above water.

Work on Garrison Reservoir game management areas began in 1956. Eleven of these areas are managed through a Control License with the U. S. Army Corps of Engineers. A twelfth area, the Snake Creek Game Management Area, is operated under a Cooperative Agreement betwen the Corps of Engineers, th Fish and Wildlife Service, and the North Dakota Game and Fish Department. Agreements with the two federal agencies grant the Department permission to enter on and manage designated Corps lands for the improvement of wildlife habitat and outdoor recreation.

DESCRIPTION OF AREAS

The original Control License and Cooperative Agreement permitted Department management of 12,485 acres of Corps lands. Douglas Creek, Riverdale, Snake Creek game management areas and 130 small wildlife cover plantings distributed along Garrison Reservoir shorelines were included in these original agreementts; (see figure 1). Amendments to the original license have increased the reservoir area under Department management to 51,669 acres, and have added Antelope Creek, Beaver Creek, Deepwater Creek, de-Trobriand, Hille, Hofflund, Tobacco Garden Creek, Van Hook, and Wolf Creek units to our system of Garrison Reservoir game management areas.

Cultivated lands account for about one-third of the area under Department management. Remaining acreages consist of grasslands, marshes, wooded coulees and open water. Amount of land under our management decreases as Garrison Reservoir pool size increases.

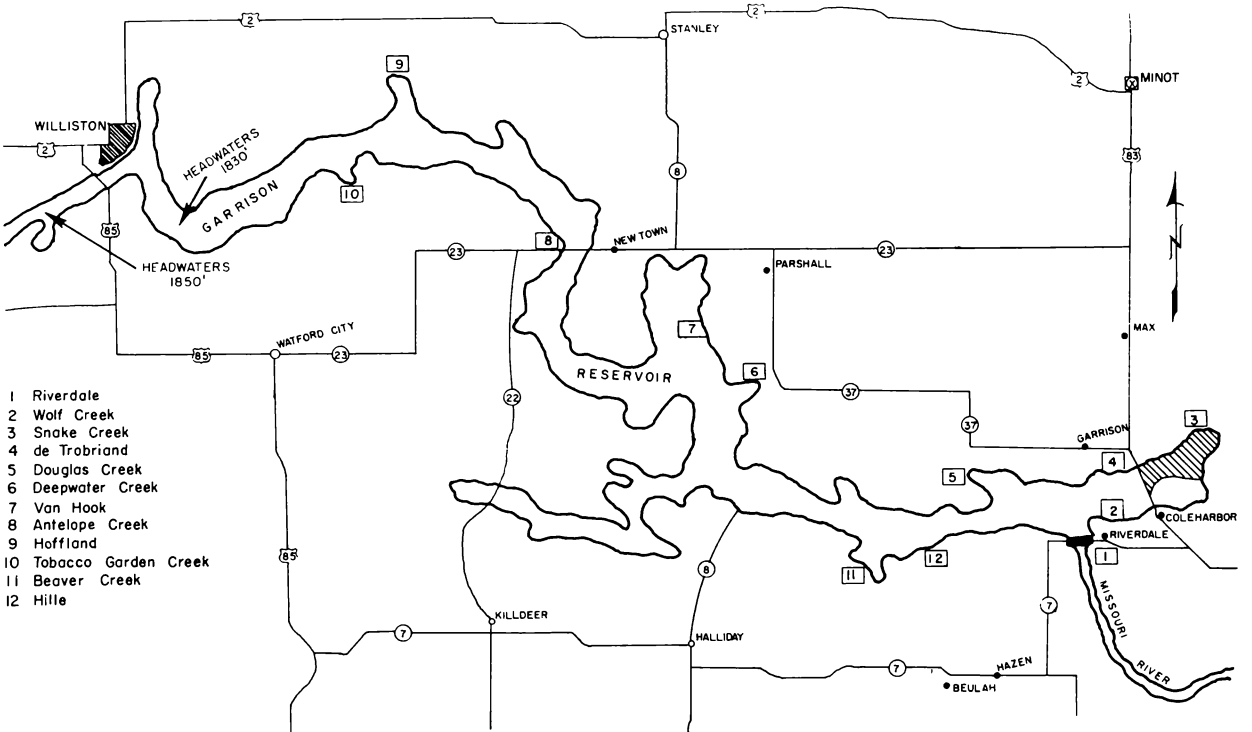

FIGURE 1—Garrison Reservoir game management areas.

MANAGEMENT TECHNIQUES

Cultivated lands are managed through Service Contracts with local farmers. Our cooperators furnish all necessary equipment and materials and harvest three-fourths of the crop. The one-fourth Department crop-share is left unharvested for wildlife food, used to reimburse cooperators for services rendered, or, in years of above-normal crop yields, sold to our cooperators to avoid undesirable waste. Grasslands are grazed or cut for hay as needed to improve wildlife habitat. Income realized from management areas is returned to them in the form of development and improvement projects.

Waterfowl.—Duck use has increased as Garrison Reservoir has filled (1). Summer duck use has increased to the extent that duck depredation on adjacent private lands can be a major problem during the small grain harvest season. Duck use during fall migration provides excellent hunting and has resulted in some depredation on grazed corn fields in the Garrison Reservoir locality. Open water in the Missouri River below Garrison Dam encourages some ducks to winter in North Dakota. Depending on winter weather, this flock has varied in size from several hundred to 60,000 ducks. During mild winters these ducks present depredation problem on grazed corn fields in the Beulah-Hazen area of Mercer County.

In an effort to alleviate small grain depredation, our cooperators are required to swathe and stack small grains in small, shock-sized piles. We also require our cooperators to seed small grains below expected high water levels for the current year. Both of these practices create excellent late summer and early fall waterfowl feeding areas. Garrison Reservoir ducks have shown a preference for barley and durum wheat.

To provide late season field shooting and to lessen duck depredation on private corn fields, our cooperators are permitted to graze cattle in designated management area corn fields from October 1 to November 1.

In recent years, the Department has constructed a series of sub-impoundments and access embankments on the Wolf Creek and Snake Creek game management areas. When completed, this project will provide us with a number of managed marshes on which we will be able to maintain water levels at desired elevations to improve and maintain waterfowl production and feeding areas. We rely on personnel of the Soil Conservation to design and supervise construction of these embankments. Department personnel have also created a number of small wildlife waterholes by blasting with ammonium nitrate. Use of this blasting agent and wildlife use of resulting watering areas are still being evaluated. Results to date have been encouraging.

Upland game.—Approximately 1200 acres of winter cover plantings were established in cooperation with the Corps of Engineers in 1956 and 1957. Field studies since then have indicated a need for

additional winter cover plantings and two-row travel lanes. Department personnel have planted additional trees and shrubs since 1957. This work will be continued for the foreseeable future.

Our cooperators are required to stack unharvested grain in all winter cover plantings. Five-acre corn fields are planted and left unharvested near winter cover plantings. Selected cultivated lands are left idle or seeded to sweetclover to provide needed herbaceous cover. Upland game will also benefit from the Wolf Creek and Snake Creek sub-impoundment work.

Wild-trapped pheasants have been released on Douglas Creek, Riverdale, Snake Creek, and Van Hook game management areas. Success of these releases has varied.

Big game.—Very little has been done specifically to benefit big game species. However, deer have benefited from cover plantings, food patches and food stacks provided for other game species. We do have a small timber stand improvement project on the Riverdale area which will improve deer habitat on that area.

Public use.—Public use of our management areas has been somewhat of a dilemma. We can increase use of an area by surveying, fencing and posting its boundaries. Use may also be increased by publicizing an area through news media or public tours. However, we have had conflicts between different types of public use. This was particularly true on the Snake Creek Game Management Area during the 1964 hunting season.

Excellent fishing provided by Snake Creek Reservoir resulted in heavy shoreline use by fishermen and constant traffic over most of the area. Consequently, waterfowl use and feeding patterns were disrupted. Waterfowl hunting deteriorated.

In 1965, a portion of the Snake Creek area was closed to fishing. About one-third of the shoreline has been fenced off and closed to the use of vehicles. We hope these practices will improve waterfowl use and lessen conflicts between hunters and fishermen. Otherwise, more restrictive measures will have to be instituted if we are to realize the potential offered by these areas.

DISCUSSION

Originally, the Department assigned one biologist to part-time work on Garrison Reservoir. As field work progressed and management opportunities became apparent, the work load on Garrison Reservoir became more than one man could handle. Game Management personnel assigned to Garrison Reservoir now include a biologist, assistant biologist, one full-time maintenance and development man and, beginning in 1965, one seasonal maintenance and development man. Our total expenses on Garrison Reservoir have increased from part-time salary and traveling expenses for one biologist in 1955 to approximately $70,000 in 1964.

Results of our management techniques have been encouraging.

Efforts to improve known techniques and to discover new management practices are being continued. Our goal in managing these areas is to improve and maintain wildlife habitat and provide recreation.

SUMMARY

1. Garrison Reservoir began filling in June, 1953.
2. Many acres of excellent wildlife habitat have been lost to the reservoir.
3. Flooding has not only occurred on river bottoms but on adjacent uplands as well.
4. Work on Garrison Reservoir game management areas began in 1956.
5. The Game and Fish Department manages 51,669 acres of Corps of Engineers lands as 12 game management areas.
6. These areas are managed under a Control License with the Corps of Engineers and a Cooperative Agreement between th Corps of Enginers, the Fish and Wildlife Service, and the Game and Fish Department.
7. Cultivated lands are operated through Service Contracts with local farmers on a crop share basis.
8. Lands operated by the Department are managed to create wildlife feeding areas and to improve wildlife habitat.
9. Public use has been a dilemma, but practices are being tried to reduce conflicts between fishmen, hunters and wildlife use.
10. Results of our management program have been encouraging.
11. Expenses on Garrison Reservoir have increased from part-time salary and expenses for one biologist in 1956 to $70,000 in 1964.
12. The Department's goal in managing these areas is to maintain and improve wildlife habitat and to provide public recreation.
13. Department work on Garrison Reservoir is being continued.

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FISHES OF THE SHEYENNE RIVER OF NORTH DAKOTA

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Fishes from the Red River and North Dakota tributaries to the river system have been studied sporadically. Check lists of North Dakota fishes have been published by Woolman (1895), Hankinson (1929), Carufel (1958) and Dotson (1964). These studies

were broad in scope and neglected a detailed survey of the tributaries to the Red River.

Fishes in the headwaters of the Red River have been studied by Bailey and Allum (1962). Eddy and Surber (1947) and Underhill (1957) surveyed the fish fauna in the Minnesota tributaries to the river system. Some revisions and additions to the Minnesota check lists have been made by Eddy and Underhill (1959), and Nordlie, Underhill and Eddy (1961). Red River fishes found in Manitoba have been surveyed by Hinks, Keleher and Kooyman (1957).

North Dakota tributaries to the Red River system have only recently received any attention from ichthyologists. Feldman (1962) examined the fishes of the Forest River and Hoffman (1953) surveyed the fish parasites in the Turtle River.

Fishes in the upper portion of the Sheyenne River were studied by Wilson (1950) prior to the impoundment of the river by the Baldhill dam, near Valley City, North Dakota. In an effort to eliminate rough fish population in Lake Ashtabula, the North Dakota Game and Fish Department poisoned the headwaters of the Sheyenne River at the time of impoundment. The present study was undertaken to evaluate the success of the rough fish removal program and to find the distribution of fish in the Sheyenne River.

The help of the North Dakota Game and Fish Department is gratefully acknowledged. The study was supported in part by N.S.F. undergraduate research participation grant G. E. 2846.

PHYSICAL DESCRIPTION

The Sheyenne River originates in north central North Dakota and flows eastward as an intermittent stream. In Nelson County, the stream makes a slow sweeping bend southward and becomes a permanent stream. Many small low water dams are located in the upper portion of the stream.

Baldhill Dam is the largest impoundment on the Sheyenne River. Below the dam the stream flows southeast until it reaches the southeastern portion of the state. At this point the stream reverses directions and flows northeast across the lacustrine plain of glacial Lake Agassiz to intersect the Red River.

PROCEDURES

Fish were collected during June and July 1964. Twenty-five stations were located at accessible points along the stream. Fish were taken with gill nets, seines, traps and selective poisoning. Rains and high waters made collecting difficult, but we attempted to sample each type of stream habitat.

Chlorides, alkalinity and dissolved oxygen content of the water were measured at 10 stations. Standard methods recommended by A.P.H.A. (1960) were used for all chemical measurements.

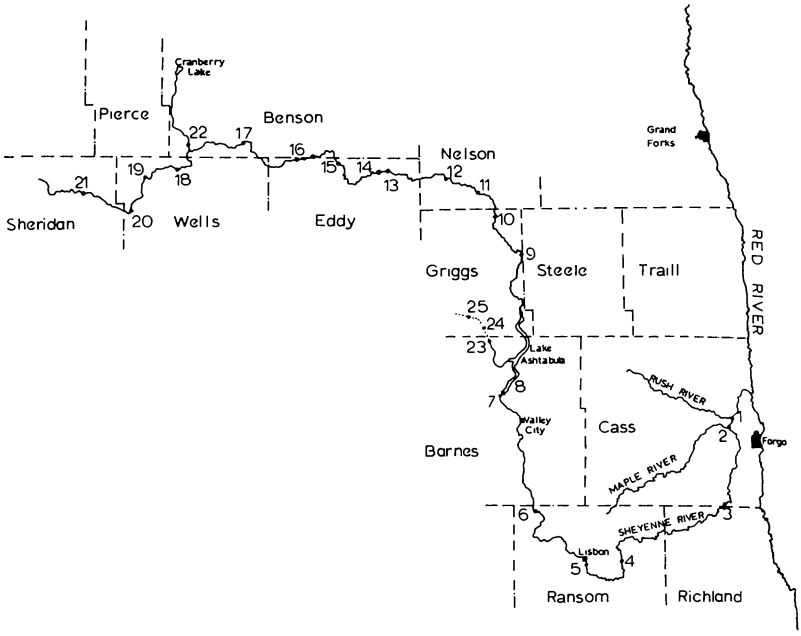

FIGURE 1—Sampling Stations on the Sheyenne River.

COLLECTION SITES

Station 1.—Mouth of the Rush R., T. 141 N, R. 49W, sec. 28, Cass Co., June 25, 1964. Water turbid; unconsolidated bottom mud; Width 30 ft.; depth 8 ft. Species: 2, 3, 7, 9, 10, 12, 15, 16, 22, 24, 38, 39, 42.

Station 2.—Mouth of Maple R., T. 140 N, R. 49W, sec. 18, Cass Co., July 6, 1964. Water turbid; unconsolidated bottom mud; width 25 ft.; depth 8½ ft.; water temperature 74° F; chlorides 480 ppm; bicarbonates 296 ppm; dissolved oxygen 7.6 ppm. Species: 2, 3, 9, 13, 15, 16, 17, 18, 21, 22, 24, 25, 31, 36, 37, 42.

Station 3.—Mill Site Park, T. 136 N, R. 50 W. sec. 5, Richland Co., July 6, 1964. Rising water; depth 7½ ft.; width 30 ft.; water temperature 72° F; chlorides 539 ppm; bicarbonates 240 ppm; dissolved oxygen 7.5 ppm. Species: 3, 9, 12, 13, 15, 16, 17, 21, 27, 42, 43.

Station 4.—T. 134 N, R. 54W, sec. 8 and 17, Ransom Co., July 7, 1964. Riffle area; rising water; depth 3 ft.; width 40 ft.; sand bottom. Species: 2, 3, 7, 8, 9, 10, 11, 12, 13, 15, 16, 20, 21, 26, 27, 35, 39, 42.

Station 5.—Below Lisbon Dam, T. 134 N, R. 56W, sec. 1, Ransom Co., July 7, 1964. Water turbid; rising water; depth 4 to 18 ft., width

70 ft.; rock and gravel bottom. Species: 2, 5, 9, 10, 11, 12, 13, 15, 16, 21, 22, 25, 29, 31, 36, 37, 39.

Station 6.—T. 136 N, R. 58W, sec. 1, Ransom Co., July 7, 1964. Water turbid and rising; gravel and rock bottom; depth 3 to 5 ft.; width 60 ft.; water temperature 73° F; chlorides 68.5 ppm; bicarbonates 451.3 ppm; dissolved oxygen 8.1 ppm. Species: 2, 5, 6, 8, 9, 10, 11, 13, 14, 16, 19, 21, 22, 25, 26, 27, 32, 33, 40, 42.

Station 7.—Below Baldhill Dam, T. 141 N, R. 58W, sec. 18, Barnes Co., July 7, 1964. Water clear; compact clay, sand and rock bottom; heavy concentration of fish visible; depth 3 to 5 ft.; width 100 ft. Species: 2, 11, 16, 21, 22, 25, 36, 37, 39, 40, 41.

Station 8.—Lake Ashtabula, Barnes Co., July 8, 1964. Species list was supplemented by North Dakota Game and Fish collections. Species: 2, 9, 11, 16, 22, 23, 25, 28, 29, 30, 31, 32, 33, 34, 36, 37, 39, 40.

Station 9.—T. 147 N, R. 58W, sec. 36, Grigg Co., July 9, 1964. Water turbid; depth 2 to 4 ft.; width 40 ft.; gravel and mud bottom. Species: 2, 5, 9, 16, 21.

Station 10.—T. 148 N, R. 59W, sec. 12 and 13, Grigg Co., July 9, 1964. Water turbid; depth 2 to 5 ft.; width 50 ft.; mud and gravel bottom. Species: 2, 5, 9, 16, 21, 26, 41.

Station 11.—T. 149 N, R. 59W, sec. 8, Nelson Co., July 10, 1964. Oxbow Lake; turbid water; sand and silt bottom; depth 3 to 5 ft.; width 40 ft.; water temperature 73° F; chlorides 16.3 ppm; bicarbonates 309 ppm; dissolved oxygen 7.1 ppm. Species: 2, 5, 9, 16, 21, 22, 26, 41.

Station 12.—T. 150 N, R. 60W, secs. 29 and 30, Nelson Co., July 10, 1964. Samples were taken above and below a small low water dam. Pool depth 5 ft.; width 40 ft.; mud bottom. Species: 2, 4, 5, 9, 11, 16, 21, 22, 23, 25, 41, 42.

Station 13.—T. 150N, R. 63W, secs. 15 and 22, Eddy Co., July, 1964. Collections were made above and below a small low water dam. Thick emergent vegetation; sand bottom; depth 1 to 4 ft.; width 40 ft.; water temperature 79°F; chlorides 9.1 ppm; bicarbonates 336 ppm; dissolved oxygen 6 ppm. Species: 4, 16, 21, 22, 25.

Station 14.—A small spring near Warwick, T. 150 N, R. 63W, sec. 15, Eddy Co., July 14, 1964. Water clear sand and rock bottom, beaver dams present; water temperature 63°F. Species: 16, 26.

Station 15.—T. 150 N, R. 65W, secs. 11 and 12, Eddy Co., July 14, 1964. Water turbid; sand and mud bottom; depth 1 to 4 ft., width 25 to 30 ft.; water temperature 74°F; chlorides 12.8 ppm; bicarbonates 390 ppm; dissolved oxygen 6.8 ppm. Species: 2, 5, 16, 21, 26, 41.

Station 16.—T. 150 N, R. 66W, secs. 4 and 5; Eddy Co., July 14, 1964. Small check dam 5 feet high; mud bottom; depth 1½ ft to 5 ft., width 60 ft. Species: 1, 2, 4, 16, 22, 26, 30, 40.

Station 17.—T. 151 N, R. 68W, sec. 7, Benson Co., July 14, 1964. Slow moving marsh like area; mud and large rocks on the botttom; depth 1 to 3 ft.; width 40 ft.; water temperature 78°F; chlorides 23.8 ppm; carbonates 92 ppm; bicarbonates 364 ppm; dissolved oxygen 7.4 ppm; Species: 16, 21, 22.

Station 18.—T. 150 N, R. 71W, secs. 20 and 21, Wells Co., July 14, 1964. Sluggish current; mud bottom; depth 1 to 3 ft.; width of pool 30 ft.; water temperature 76°F; chlorides 20.1 ppm; carbonates 270 ppm; bicarbonates 462 ppm; dissolved oxygen 7.0 ppm. Species: 16, 21, 22, 26.

Station 19.—Below Harvey reservoir, T. 150 N, R. 73W, secs. 31 and 32. Wells Co., July 15, 1964. Marsh area; stream channel 3 to 6 ft. wide; depth 1 ft.; some pool areas with depth to 6 ft.; decayed vegetation and mud bottom. Sedges, rushes, and duckweed abundant; water temperature 80°F; chlorides 25.2 ppm; carbonates 252 ppm; bicarbonates 384 ppm; dissolved oxygen 7.0 ppm. Species: 2, 5, 16, 21, 22, 26, 40, 42.

Station 20.—T. 148 N, R. 73W, secs. 8 and 17, Wells Co., July 15, 1964. Marsh area; pools 30 ft. wide; depth 6 ft.; water temperature 82°F; chlorides 5.5 ppm; bicarbonates 297 ppm; dissolved oxygen 6.9 ppm. Species: 16, 22, 26.

Station 21.—T. 149 N, R. 75W, secs. 26 and 27, Sheridan Co., July 15, 1964. Marsh area; channel obscured; bottom composed of decaying plants, depth 6 inches to 4 ft.; sedges, rushes, duckweed abundant. Species: 16, 26.

Section 22.—North branch of Sheyenne River, T. 151 N, R. 71W, secs. 14 and 23, Benson Co., July 14, 1964. Marsh area; sedges, rushes, arrow leaf abundant; no main channel present; depth 1 to 4 ft.; Species 16, 26.

Station 23.—Baldhill creek near Hanaford, N. D., T. 143 N, R. 59W, secs. 2 and 11, Barnes Co., July 9, 1964. A small check dam and the stream below was sampled. Sand and mud bottom; stream depth 2 ft.; stream width 40 ft. Species: 5 9, 16, 21, 22, 40.

Station 24.—Baldhill Creek, T. 144 N, R. 59W, secs. 5 and 8, Griggs Co., July 9, 1964. A small check dam with a surrounding marsh area; depth 3 to 10 ft.; width 125 ft.; sand and mud bottom. Species: 5, 9, 16, 21, 22, 32, 33, 40.

Station 25.—Baldhill Creek T. 145 N, R. 60W, secs. 8 and 9, Griggs Co., July 9, 1964. Marsh area 20 to 40 ft. wide; depth 2 to 5 ft., pH 5.0; brown turbid water. Species: 16, 26.

RESULTS

The distribution of fish is shown in Tables I and II. An attempt was made to sample every type of habitat but the species lists cannot be considered complete. Future sampling should reveal new species.

DISCUSSION

Tables I and II show the distribution of fish in the Sheyenne River. *P. promelas* was the most widespread species. In the intermittent portions of the stream *P. promelas and C. inconstans* (*Eucalia inconstans*) accounted for almost the entire fish population.

Headwater regions of the river supported a small number of species. The harsh environment prevented many species from utilizing the headwaters. High turbid waters and elevated tempratures sharply limited fish populations during the summer months. Although chloride concentrations decreased toward the headwaters, carbonate and bicarbonate concentrations were increased. At Station 19 carbonate concentration was 252 ppm and bicarbonate reached a high of 384 ppm.

During the winter months little or no flow occurred and winter kills of fish were noted. Only 14 species collected above Lake Ashtabula were capable of naturally maintaining a population. Species found in some abundance were: *I. melas, S. atromaculatus, N. cornutus, C. commersoni, E. nigrum, P. maculata, Perca flavescens* and *Notemigonus chrysoleucas.*

Fishes in the lower Sheyenne capable of utilizing the headwater region have been halted by Baldhill Dam. Populations above the dam are the result of planned or indiscriminate stocking. Sports fishermen have apparently introduced *Notemigonus chrysoleucas,* the golden shiner, by way of the illegal bait bucket. Generally, the rough fish removal program has been successful, and *Cyprinus carpio* and *Carpoides cyprinus* have not been introduced above the Baldhill Dam.

Fish fauna below Baldhill Dam is more diversified than that of the headwater region. Many species found in the Red River were missing in the collections.

Species which should occur in the lower part of the river include: *Hiodon alosoides* (Rafinesque), *Hiodon tergisus* Le Seur, *Rhinichthys atratulus* (Hermann), *Chrosomus neogaeus* (Cope), *Chrosomus eos* Cope and *Hybognathus hankinsoni* Hubbs. These species may have been overlooked in collections, or may represent occasional or seasonal migrants into the Sheyenne River.

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TABLE I

SYSTEMATIC LISTS OF FISHES

Family and Species		*Stations*
Salmonidae		
1. *Salmo gairdneri**	Richardson	16
Esocidae		
2. *Esox lucius*	Linnaeus	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 19
Cyprinidae		
3. *Cyprinus carpio*	Linnaeus	1 2, 3, 4
4. *Notemigonus chrysoleucas*	(Mitchell)	12, 13, 16
5. *Semotilus atromaculatus*	(Mitchell)	5, 6, 9, 10, 11, 12, 15, 19, 23, 24
6. *Rhinicthys cataractae*	(Valenciennes)	6
7. *Notropis antherinoides*	Rafinesque	1, 4
8. *Notropis ardens*....	(Cope)	4, 6
9. *Notropis cornutus*	(Mitchell)	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 23, 24
10. *Notropis dorsalis*	(Agassiz)	4, 5, 6
11. *Notropis hudsonius*	(Clinton)	1, 4, 5, 6, 7, 8, 12
12. *Notropis spilopterus*	(Cope)	3, 4, 5
13. *Notropis stramineus*	(Cope)	2, 3, 4, 5, 6, 12
14. *Hybognathus hankinsoni*	Hubbs	6
15. *Pimephales notatus*	(Rafinesque)	1, 2, 3, 4, 5
16. *Pimephales promelas*	Rafinesque	All Stations
Catostomidae		
17. *Ictiobus cyprinellus*	(Valenciennes)	2, 3
18. *Carpoides cyprinus*	(Le Seur)	2
19. *Moxostoma macrolepidotum*	(Le Seur)	6
20. *Moxostoma valenciennesi*	Jordon	4
21. *Catostomus commersoni*	(Lacepede)	2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 17, 18, 19, 21, 23, 24
Ictaluridae		
22. *Ictalurus melas*	(Rafinesque)	1, 2, 5, 6, 7, 8, 11, 12, 13, 16, 17, 18, 19, 20, 23, 24
23. *Ictalurus nebulosus*	(Le Seur)	8, 12
24. *Ictalurus punctatus*	(Rafinesque)	1, 2
25. *Noturus gyrinus*....	(Mitchell)	2, 5, 6, 7, 8, 12, 13

*Species are maintained by a restocking program.

TABLE II

SYSTEMATIC LIST OF FISHES

Family and Species		*Stations*
Gasterosteidae		
26. *Culaea inconstans*	(Kirtland)	4, 6, 10, 11, 14, 16, 18, 19, 20, 21, 22, 25
Percopsidae		
27. *Percopsis omisomaycus*	(Walbaum)	3, 4, 6
Serranidae		
28. *Roccus chrysops**	(Rafinesque)	8
Centrarchidae		
29. *Micropterus dolomieui*	Lacepede	5, 8
30. *Micropterus salmoides**	(Lacepede)	8, 16
31. *Lepomis cyanellus*	Rafinesque	2, 5, 8
32. *Lepomis gibbosus*	(Linnaeus)	6, 8, 24
33. *Lepomis humilis*	(Girard)	6, 8, 24
34. *Lepomis macrochirus*	Rafinesque	8
35. *Ambloplites ruprestris*	(Rafinesque)	4
36. *Pomoxis annularis*	Rafinesque	2, 5, 7, 8
37. *Pomoxis nigromaculatus*	Le Seur	2, 5, 7, 8
Percidae		
38. *Stizostedion canadense*	(Smith)	1
39. *Stizostedion vitreum*	(Mitchell)	1, 4, 5, 7, 8
40. *Perca flavescens*	(Mitchell)	6, 7, 8, 16, 19, 23, 24
41. *Percina maculata*	(Girard)	7, 10, 11, 12
42. *Etheostoma nigrum*	Rafinesque	1, 2, 3, 4, 6, 12, 19
Sciaenidae		
43. *Aplodinotus grunniens*	Rafinesque	3

*Species are maintained by a restocking program.

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DISTRIBUTION AND ECOLOGY OF MUSSELS IN THE TURTLE RIVER, NORTH DAKOTA

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INTRODUCTION

During the late summer of 1964, an investigation was made of the mussel fauna of the Turtle River in Grand Forks County, North Dakota. Twenty stations on the Turtle River and one station at its mouth on the Red River were sampled for mussels. The purpose of this paper is to report on the species of mussels in the Turtle River and discuss certain ecologic factors which may affect their distribution.

Mussels of the Turtle River have not been studied previously. In fact, very little is known of the mussels of the entire state. Coker

and Southall (1915, p. 15) reported six species from the Red River at Fargo, and four species from the Sheyenne River at Lisbon. Winslow (1921, p. 15) listed five species from two localities, Gravel Lake and the Sheyenne River. In 1947, Dawley listed 11 species of mussels for the Red River. Tuthill (1962) compiled a list of North Dakota mollusks and included the mussel species from previous literature.

Field work for this study was accomplished with the aid of National Science Foundation Faculty Research Grant 4263-43 of the University of North Dakota. Mr. George Pike and other members of the Surface Water Branch of the U. S. Geological Survey at Grand Forks aided in water velocity measurements. Professor R. A. Tubb of the biology department at the University of North Dakota critically read the manuscript.

METHODS

Mussels were hand picked with the aid of a Turtox Fishscope, an aluminum alloy cylinder with a glass plate measuring 24 inches long and six inches in diameter. Chemical factors, chloride content, alkalinity and dissolved oxygen, were determined by titration, generally following the manual of the American Public Health Association and others (1960). Chloride content was determined by the Mohr method, titrating with silver nitrate solution and a potassium chromate indicator. Alkalinity was measured by titrating with 0.02N sulfuric acid, using phenolphthalein and methyl purple indicators. The test for dissolved oxygen was made by the Alsterberg (sodium azide) modification of the Winkler method, titrating with 0.025N sodium thiosulfate solution and a starch indicator. All dissolved oxygen measurements were made during daylight hours.

Approximate values of pH were determined by test papers to the nearest 0.5 pH. Turbidity was analyzed by a Hellige Aqua Analyzer, a pre-calibrated photoelectric colorimeter. Light penetration was measured in the field by a homemade Secchi disk (20 cm in diameter) mounted on a rod with 0.1 foot divisions. General sediment type was estimated in the field by visual comparison with a chart of standard particle size. Bottom samples were collected at specific sites of mussel specimens; they were analyzed for particle size by a combination of wet sieving and pipette methods (Folk, 1961, p. 33-37). Water velocity was measured in fps by a Price Pygmy current meter.

GEOLOGIC SETTING

The Turtle River, originating in western Grand Forks County, flows eastward and northeastward and joins the Red River of the North about 17 miles north of Grand Forks (figure 1). At the headward reaches near Niagara, the Turtle River flows through rolling ground moraine of glacial till. The river valley in this area is incised about 50 feet below the upland surface. In places the river has cut through the till into underlying Cretaceous shale.

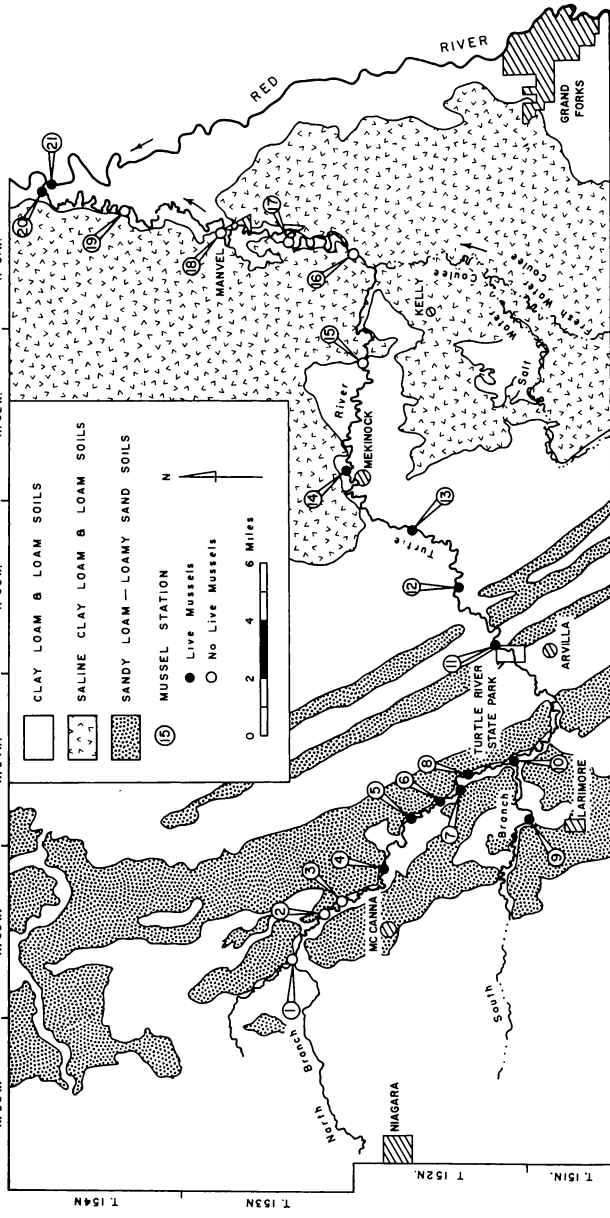

FIGURE 1.—Location map showing mussel stations on the Turtle River and the distribution of general soil types (soils generalized from soils map of North Dakota State University, Department of Soils, 1964).

About six miles east of Niagara the Turtle River enters the bed of former glacial Lake Agassiz, a nearly featureless plain which slopes eastward toward the Red River at about 10 feet per mile. At the western margin of the lake plain the Turtle River cuts through the Elk Valley "Delta," a 5-mile-wide lobe of sandy alluvial sediment. Farther eastward, in the vicinity of Turtle River State Park (figure 1), the river transects several low ridges of sand and gravel. Here, the river has incised a valley about 30 feet into the underlying till. Both the "Delta" and ridges are reflected by sandy loam to loamy sand soils (figure 1).

The surface of the lake bed is composed primarily of wave-washed till, except for the sandy and gravelly sediment of the "Delta" and "beach" ridges. Toward the center of the former lake basin, however, till is overlain by lacustrine silt and laminated clay. This fine-grained sediment predominates on the surface of the lake bed from the general area of Mekinock eastward (figure 1). The valley of the Turtle River is noticeably shallower in this finer sediment, and is only a few to several feet beneath the surface of the lake plain.

STREAM CHARACTERISTICS

The Turtle River drainage basin encompasses an area of approximately 640 square miles, most of which lies on the bed of glacial Lake Agassiz. The gradient of the river, from about station 13 headward (figure 1), is 6.6 feet per mile. Downstream from this point, the gradient is apparently somewhat less, as evidenced by the sluggish appearance of the river and its general lack of riffles.

The mean annual rainfall in the Grand Forks area is 19.8 inches and more than three-fourths of this amount falls between April and September (U. S. Weather Bureau, 1961). An average monthly maximum of 3.85 inches falls during June. Runoff is extremely low, less than one inch per year (Miller, and others, 1962, pl. 10).

Data from a gaging station near Manvel (figure 1) shows the average discharge for the Turtle River for an 18 year period as 45 cfs or 32,580 acre-ft per year. These same records indicate occasional peirods in late summer and midwinter when the river does not flow. Mean velocities (at 0.6 of the total depth) taken at the sites of mussels at several stations in August and September, 1964, ranged from 0.17 to 0.78 fps or about ⅛ to ½ mph. The maximum annual flood typically occurs in April and is usually accompanied by ice jams, which may cause severe high water conditions.

DESCRIPTION OF STATIONS

(University of North Dakota accession numbers follow station numbers; mussel species for each station are given in Table I).

Station 1.—North Branch Turtle River, on section line common to secs. 20 and 21, T. 153 N., R. 55 W., about 3½ miles north-northwest

of McCanna, Grand Forks Co., N. Dak., September 6, 1964. Bottom mainly silty, medium to very coarse-grained sand; along banks sediment finer, of dark gray to black mud. About 210 yds above bridge is a beaver dam; below bridge is presumably another dam as water is ponded, 6 to 7 ft. deep. Water flowing slightly. Bottom examined from about 210 yds above bridge to 120 yds below it for 1½ hours. Banks mainly open, with few scattered trees. Live *Physa* and sphaeriids abundant.

Station 2.—North Branch Turtle River, on section line common to secs. 27 and 34, T. 153 N., R. 55 W., about 2¼ miles north-northeast of McCanna, Grand Forks Co., N. Dak., September 6, 1964. Bottom mainly dark gray to black mud (upper ½ inch light grayish brown), with medium to coarse-grained sand; generally soft to the step with much growing vegetation. Water flowing slightly. Bottom examined from about 94 yds above bridge to 128 yds below it for one hour. Banks open, with only grassy vegetation. Live *Physa* abundant, *Lymnaea* and *Helisoma* common; no other mollusks found alive.

Station 3.—North Branch Turtle River, on section line common to sec. 34 and 35, T. 153 N., R. 55 W., about 2 miles north-northeast of McCanna, Grand Forks Co., N. Dak., September 6, 1964. Bottom mainly silty, medium to coarse-grained sand, but in quiet water and along banks, dark gray to black, sandy, mud; generally soft to the step and with much growing vegetation. Where not ponded, stream 1 to 2 ft wide and few inches deep; where ponded, stream several yards wide and up to about 5 ft deep. Water flowing slightly. Bottom examined from about 132 yds above bridge to 128 yds below it for 1½ hours. Banks mainly open, with grassy vegetation and few bushes. Live *Physa* abundant and sphaerids common.

Station 4 (UND A15).—North Branch Turtle River, on section line common to secs. 2 and 11, T. 152 N., R. 55 W., about 2 miles east of McCanna, Grand Forks Co., N. Dak., September 7, 1964. Bottom mainly medium to coarse-grained sand with particles up to fine gravel. Ninety-three yards above bridge stream is 13½ ft wide; here, maximum depth is 0.9 ft, and mean velocity (at 0.6 depth) and velocity 0.1 ft off bottom is 0.17 fps (depth and velocity measured 7 ft from left bank). *Andodonta grandis* taken in immediate vicinity where depth and velocity measured. Bottom examined from about 112 yds above bridge to 211 yds below it for two hours. Banks largely shaded by trees. Live *Physa* abundant, *Helisoma* and sphaeriids uncommon.

Station 5 (UND A3).—North Branch Turtle River, on section line common to secs. 7 and 12, T. 152 N., R. 55 W., about 3¾ miles east-southeast of McCanna, Grand Forks Co., N. Dak., August 23, 1964. Bottom mainly muddy sand, with sandy mud near banks. About 5½ yds above bridge, stream is 29½ ft wide; here, about 12 ft from left bank, maximum depth is 1.7 ft and velocity at 0.2 ft depth

is 0.25 fps. Bottom examined from 139 yds above bridge to 132 yds below it for 2½ hours. Live mussels found only just upstream (west) side of bridge. Banks open, covered only with grassy vegetation. Live sphaeriids abundant.

Station 6 (UND A4).—North Branch Turtle River, on section line common to secs. 18 and 19, T. 152 N., R. 54 W., about 3¾ miles north-northeast of Larimore, Grand Forks Co., N. Dak., August 24, 1964. Bottom mainly muddy sand with pebbly gravel; few scattered boulders. Thirty-one yards above bridge stream is 14½ ft wide; here, near right bank, maximum depth is 2.4 ft and mean velocity (at 0.6 depth) is 0.14 fps. *Lampsilis siliquoidea* taken where depth and velocity measured. Bottom examined from 217 yds above bridge to 79 yds below it for 3½ hours. Banks partially shaded by trees. Live sphaeriids abundant, small operculids common.

Station 7 (UND A5).—North Branch Turtle River, on section line common to secs. 19 and 20, T. 152 N., R. 54 W., about 3¼ miles north-northeast of Larimore, Grand Forks Co., N. Dak., August 24, 1964. Bottom mainly medium to coarse-grained sand but variable, and particles range up to boulders. Eighty-eight yards above bridge, just below upstream end of "riffle pool," stream is 22 ft wide. Here, maximum depth, at about middle of stream, is 1.5 ft; mean velocity (at 0.6 depth) is 0.47 fps and velocity 0.1 ft off bottom is 0.37 fps. All species of live mussels taken in immediate vicinity where depth and velocity measured. Most mussels in "riffle pool," 66 yards above bridge. Bottom examined from 94 yds above bridge to 138 yds below it for 3 hours. Banks largely shaded by trees. Live sphaeriids common.

Station 8 (UND A6).—North Barnch Turtle River, on section line common to secs. 20 and 29, T. 152 N., R. 54 W., about 3 miles north-northeast of Larimore, Grand Forks Co., N. Dak., August 26, 1964. Bottom mainly medium to very coarse-grained sand (also pebbly gravel common, some particles up to boulder size) where water is flowing; in small backwater areas near banks, sediment is mud to fine-grained sand. Here, *Anodonta grandis* most frequently found. Bottom examined from 104 yds above bridge (washed out) to 156 yds below it for 2 hours. Banks well shaded by trees. Live sphaeriids uncommon.

Station 9 (UND A7).—South Branch Turtle River, NE¼ sec. 1, T. 151 N., R. 55 W., 1 mile north of Larimore, Grand Forks Co., N. Dak., August 26, 1964. Bottom mainly muddy, fine to medium-grained sand, but up to pebble size; generally soft and sinks to the step. Generally, sediment black below depth of a few inches. Seventy-five yards below bridge stream is 5 ft wide and 0.7 ft deep at mid-width. Here, three feet above small riffle, mean velocity (at 0.6 depth) is 0.29 fps and velocity 0.1 foot off bottom is 0.28 fps. *Anodonta grandis* and *Anodontoides ferussacianus* collected 37 yards and 28

yards, respectively, above point where velocity and depth measured. Where *Anodonta grandis* was collected, stream is 13 ft wide with maximum depth of 1.3 feet. Bottom examined from 136 yds above bridge to 165 yds below it for 1½ hours. Banks mainly open with few bushes and small trees. Dead branches and roots of bushes and small trees numerous on bottom. Live sphaeriids uncommon.

Station 10 (UND A8).—Turtle River, just below confluence of North and South Branch, on section line common to secs. 32 and 33, T. 152 N., R. 54 W., about 2¼ miles northeast of Larimore, Grand Forks Co., N. Dak., August 27, 1964. Bottom mainly medium to very coarse-grained sand, pebbles and cobbles (few boulders) also common; some gray, sandy to pebbly mud. About 22 yards below bridge, stream is 18½ ft wide but main channel is 8½ ft wide; maximum depth (3 ft from right bank) is 1.2 ft. Here, mean velocity (at 0.6 depth) is 0.56 fps and velocity 0.1 foot above bottom is 0.44 fps. *Anodontoides ferussacianus* common where velocity and depth measured. Bottom examined from 123 yds above bridge to 183 yds below it for two hours. Banks largely open, with few scattered trees.

Station 11 (UND A1).—Turtle River, Turtle River State Park, NE¼ sec. 36, T. 152 N., R. 54 W., about 1½ miles north of Arvilla, Grand Forks Co., N. Dak., August 2 and 27, 1964. Bottom mainly medium to course-grained pebbly sand; cobbles and boulders common. Left bank of clay till, right bank is edge of river terrace and sandy. Bottom examined over 284 yds above bridge, for 2 hours. Banks well shaded by bushes and trees. Live sphaeriids uncommon.

Station 12 (UND A9).—Turtle River, on section line common to secs. 20 and 21, T. 152 N., R. 53 W., about 2½ miles northeast of Turtle River State Park or 5 miles southwest of Mekinock, Grand Forks Co., N. Dak., August 28, 1964. Besides species listed in Table I, collected empty shells of *Fusconaia flava* and *Strophitus rugosus* along the bottom. Bottom mainly medium to coarse-grained pebbly sand; with mud and gravel up to boulders, surfaced with thin film of silt; water becomes clouded quickly upon walking over bottom. About 100 yds below bridge stream is 23 ft wide with maximum depth of 2.2 ft at 5 ft from right bank; mean velocity (at 0.6 depth) here is 0.14 fps.*Anodonta grandis* and *Lampsilis siliquoidea* collected where depth and velocity measured. Bottom examined from 231 yds above bridge to 165 yds below it for 2½ hours. Banks generally well shaded by trees. Live sphaeriids common; where many specimens collected, about 125 yds below bridge, 6 yds below a boulder crossing and up to 4 feet from left bank, sediment is muddy, fine-grained sand. Four feet from left bank, depth is 0.7 feet and mean velocity (at 0.6 depth) is 0.30 fps.

Station 13 (UND A10).—Turtle River, on section line common to secs. 11 and 14, T. 152 N., R. 53 W., about 2½ miles southwest of Mekinock, Grand Forks Co., N. Dak., August 28, 1964. Mussels more

plentiful than at any other station. Bottom firm, mainly medium to coarse-grained, silty, pebbly sand; examined over 123 yds below bridge for 1½ hours. Banks largely open with scattered trees and bushes. Live sphaeriids common.

Station 14 (UND A11).—Turtle River, on section line common to secs. 31 and 32, T. 153 N., R. 52 W., about half a mile north-northeast of Mekinock, Grand Forks Co., N. Dak., August 28, 1964. Sediment in main channel generally medium to coarse-grained pebbly sand; and along banks, of sandy mud to muddy sand; commonly, surfaced by about one-fourth inch of clayey silt. Sixty-eight yards below bridge stream is 22½ ft wide with maximum depth of 2.3 feet. Here. six feet from left bank, depth is 1.9 ft and mean velocity (at 0.6 depth) is 0.24 fps. Live *Anodonta grandis* common where depth and velocity measured; also many double-valved empty shells in living position, with posterior ends upward. *Lasmigona complanata* taken nine yards upstream. Here, maximum depth is 1.6 feet at 4½ ft from left bank. Mean velocity (at 0.6 depth) is 0.26 fps and velocity 0.1 ft off bottom is 0.19 fps. Bottom examined from 156 yds above bridge to 110 yds below it for 2¼ hours. Banks covered with scattered to numerous trees. Live sphaeriids common.

Station 15.—Turtle River, on section line common to secs. 2 and 3, T. 152 N., R. 52 W., about 3 miles northwest of Kelly (or about 3¾ miles east of Mekinock), Grand Forks Co., N. Dak., September 2, 1964. Bottom mainly soft, black or dark gray sandy mud; and in places, medium to coarse-grained pebbly sand underlain by black to dark gray mud; surfaced with about one-fourth inch of loose clayey silt, making water very turbid when disturbed. Thirty-seven yards above bridge stream is 17 ft wide; about 7½ ft from left bank is maximum depth of 1.2 ft. Here, mean velocity (at 0.6 depth) is 0.46 fps and velocity 0.1 ft off bottom is 0.36 fps. Bottom examined from 112 yds above bridge to 138 yds below it for 1½ hours. Scattered small trees and bushes along banks. Live sphaeriids and *Physa* uncommon.

Station 16 (UND A12).—Turtle River, on section line common to secs. 5 and 33, Tps. 152 and 153 N., R. 51 W., about 3¼ miles northeast of Kelly (or 4¼ miles south-southwest of Manvel), Grand Forks C., N. Dak., September 3, 1964. Bottom mainly firm, dark gray to black mud, upper one-fourth inch light brown; high content of empty snail shells in sediment. Thirty-five yards above bridge stream is 15 ft wide with maximum depth of 1.5 ft, 5 ft from left bank. Here, mean velocity (at 0.6 depth) is 0.78 fps and velocity 0.1 ft off bottom is 0.60 fps (velocities probably high because of moderate downstream wind when measured). Bottom examined from 154 yds above bridge to 119 yds below it for 1½ hours. Banks open except for grassy vegetation. Refuse is dumped at bridge. No sphaeriids noted. Live *Physa* and *Helisoma* uncommon.

Station 17 (UND A13).—Turtle River, on section line common to secs. 21 and 22, T. 153 N., R. 51 W., about 2 miles south-southwest of Manvel, Grand Forks Co., N. Dak., September 3, 1964. Bottom mainly soft (one can sink in up to two feet), dark gray to black mud, surfaced with up to one inch of loose, light brownish gray, clayey silt; black sediment has odor of H_2S gas. Empty shells of aquatic snails very common to abundant in sediment. Bottom examined from 80 yds above bridge to 165 yds below it for 1½ hours. Banks with scattered to many trees. Live, small operculids common, *Physa* uncommon.

Station 18.—Turtle River, NW¼ sec. 10. T. 153 N., R. 51 W., about half a mile north-northwest of Manvel, Grand Forks Co., N. Dak., September 4, 1964. Bottom mainly soft (one can sink in up to two feet), dark gray to black mud, surfaced with up to one inch of loose, light brownish gray clayey silt; black sediment generally with odor of H_2S gas. Twenty-four yards above bridge stream is 20½ ft wide with maximum depth of 1.7 ft, 5½ ft from left bank. Here, mean velocity (at 0.6 depth) is 0.38 fps and velocity 0.1 ft off bottom is 0.29 fps. Bottom examined from 183 yds above bridge to 128 yds below it for 1½ hours. Banks mainly open with few trees. Live *Lymnaea* and *Physa* uncommon; small operculids common. No sphaeriids noted.

Station 19.—Turtle River, on section line common to secs. 23 and 26, T. 154 N., R. 51 W., about 4 miles north-northeast of Manvel, Grand Forks Co., N. Dak., September 4, 1964. Bottom mainly irregular and soft (one can sink in to about 1½ ft), dark gray to black mud, surfaced with up to half an inch of loose, light brownish gray to light grayish brown clayey silt; black sediment generally with odor of H_2S gas. Clay pellets mainly medium to coarse sand size, common. Bottom examined from 92 yds above bridge to 156 yds below it for 1¼ hours. Banks mainly well shaded by trees, lack other vegetation. Much wood debris in stream. Live, small operculids common. Empty shells of other aquatic snails abundant, sphaeriids common in sediment.

Station 20 (UND A20).—Turtle River at confluence with Red River, on section line common to secs. 11 and 12, T. 154 N., R. 51 W., about 7 miles north-northeast of Manvel, Grand Forks Co., N. Dak. (about 1½ miles south-southwest of Oslo, Minnesota), September 5, 1964. Bottom mainly dark gray to black silty clay, surfaced by 1/16 to 1 inch of loose, light grayish brown to brownish gray clayey silt; soft above bridge, one can sink in to two feet. Black sediment with slight odor of H_2S gas. Clay pellets, mainly of coarse sand to very fine pebble size, common. Twenty-six yards above bridge, stream is 18½ ft wide with maximum depth of 1.3 ft at 6½ ft from left bank. Here, mean velocity (at 0.6 depth) is 0.59 fps and velocity at 0.1 ft off bottom is 0.32 fps. Stream velocity faster on down stream side of bridge, because of constriction of channel. Bottom

examined from 145 yds above bridge to mouth of Turtle River (97 yds below bridge) for 1¾ hours. Banks well shaded by trees. Live *Physa* uncommon; empty shells of other aquatic snails common in sediment.

Station 21 (UND A14).—Red River, at mouth of Turtle River, NW¼ sec. 12, T. 154 N., R. 51 W., about 7 miles north-northeast of Manvel (or about 1½ miles south-southwest of Oslo, Minn.), Grand Forks Co., N. Dak., September 5, 1964. Bottom firm, light gray to light tan-gray mud. Eighty-eight yards above mouth of Turtle River, 8½ ft from left bank, depth is 1.9 ft. Here, mean velocity is 0.28 fps and velocity 0.1 ft off bottom is 0.18 fps. *Lampsilis siliquoidea* taken where velocity and depth measured. Bottom examined from 158 yds above mouth of Turtle River to 138 yds below it for 2 hours. Mussels not collected below 2½ ft depth. Banks well-shaded by trees. Station is along undercut bank of Red River. Live limpets (observed attached to mussel shells) uncommon.

ECOLOGIC FACTORS

Selected possible ecologic factors which were analyzed included chloride content, total alkalinity, dissolved oxygen, pH, water turbidity and bottom sediment. Chemical factors, and their variation with station, are shown in figure 2. The chloride content varies from 16 to 2110 ppm throughout the Turtle River. At station 15, about three miles northwest of Kelly, the chloride content (87 ppm) is three times as high as it is elsewhere upstream. At station 16, less than six miles downstream, choride content (2000 ppm) is more than 60 times as high as it is upstream from station 15. The first appreciable increase in chloride content downstream (at station 15) corresponds to an apparent total absence of live mussels (figures 1 and 2), which continues to the mouth of the Turtle River. This eastern limit of live mussels agrees closely with the western margin of saline clay loam and loam soils where it crosses the Turtle River (figure 1). Land is generally poor for crop growing in this belt of saline soils near Kelly. It has been speculated that seepage of saline water from the subsurface, perhaps from Cretaceous rocks of the Dakota Group, may be responsible for these saline soils (Laird, 1944, p. 6).

Total alkalinity varies from 184 to 328 ppm and generally decreases downstream (figure 2). It decreases markedly with a corresponding increase in the chloride content. Negative results with phenolphthalein indicate all alkalinity is present as the bicarbonate ion.

Dissolved oxygen varies from 5.3 to 8.9 ppm. It does not appear to change markedly in any part of the stream (figure 2). The range of values suggests that no serious organic pollution is present.

Approximate values of pH from 5.5 to 6.5 were obtained for the same selected stations as given in figure 2. There appears to be no marked trend of pH values in the river.

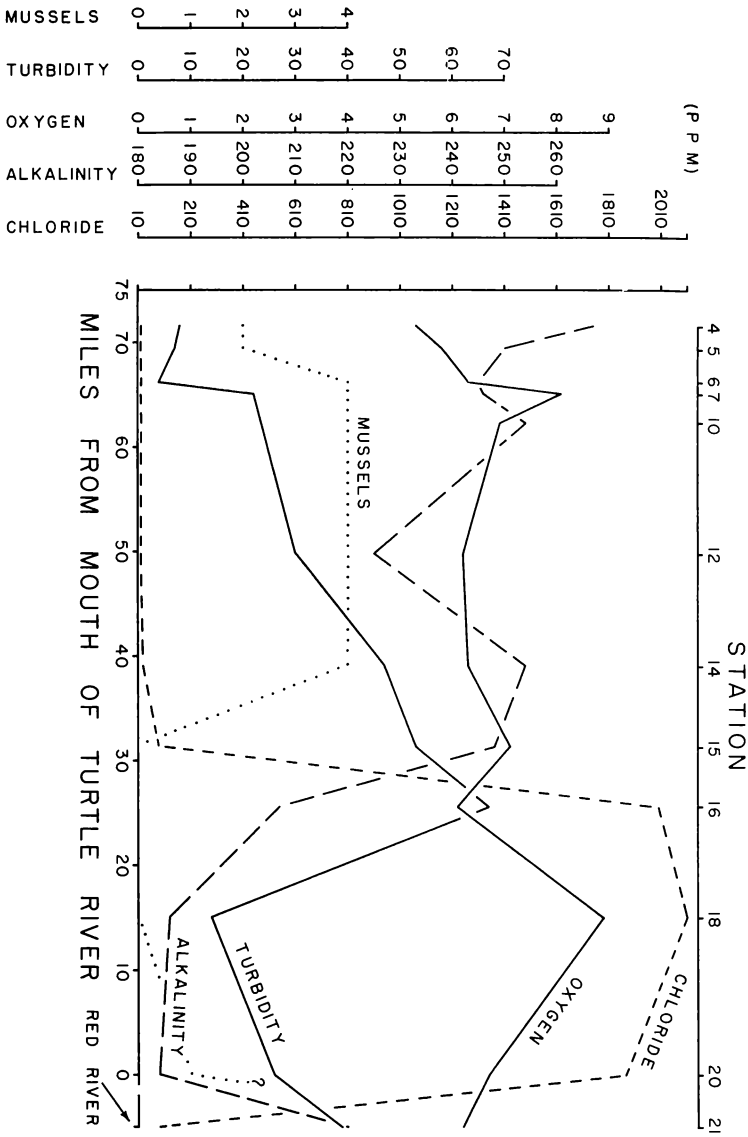

FIGURE 2.—Graph showing the variation of number of mussel species, turbidity, dissolved oxygen, chloride content and total alkalinity with station on the Turtle River.

These pH values are presumably low, however, using a Taylor Model T-O, Hydrogen-Ion Slide Comparator, pH values on August 27, 1965, were 8.1, 8.0, 7.9, 8.3 and 8.7 at stations 10, 11, 13, 15 and 19, respectively.

Water turbidity, expressed as ppm SiO_2, varies from 4 to 67 and shows a general increase to station 16, with lesser values farther downstream (figure 2). It is highest where chloride content increases very markedly (station 16). Secchi disk readings, varying from 4.3 ft to 1.2 ft in the Turtle River, generally correspond inversely with turbidity values and show a similar trend.

Bottom sediment is generally sandy and gravelly in the upper reaches of the river and muddy in its lower reaches. This fact is partially reflected in figure 4, as lower and higher station numbers indicate the upstream and downstream parts of the river respectively.

The writers are well aware that perhaps numerous other factors are more, or as, important ecologically as those briefly described here. A few of these might be aquatic vegetation, fish host, and in particular, food supply (Matteson, 1955, p. 127).

MUSSELS

Species presently in the Turtle River

General.—Four species were found to presently occur in the Turtle River: *Anodonta grandis* Say, *Lasmigona complanata* (Barnes), *Anodontoides ferussacianus* (Lea), and *Lampsilis siliquoidea* (Barnes) (Table I). The relative abundance varies from station to station but, of the four species, *Anodonta grandis* and *Lampsilis siliquoidea* occur most commonly. In the headwaters of the Turtle River, only two species are present, *Anodonta grandis* and *Anodontoides ferussacianus*. At stations 1 to 4 and 9 (figure 1(, these are the only mussels. At the single station sampled for mussels in the Red River, only *Lampsilis siliquoidea* was found present.

Mussels in the Turtle River generally occur most commonly in riffle pools. The highest mussel density observed at one such riffle pool (station 7) was 0.4 mussels per square yard.

Family Unionidae

Subfamily Anodontinae

Anodonta grandis Say "Floater"

Diagnosis.—Shells of this species differ from those of other Turtle River mussels in having the combination of no hinge teeth and double-looped beak sculpture.

Remarks.—The color of the nacre varies from pale blue to bluish white to pale greenish yellow to moderate orange pink (colors after Goddard and others, 1948). Shell measurement ratios of height/length and width/length show no apparent trend downstream.

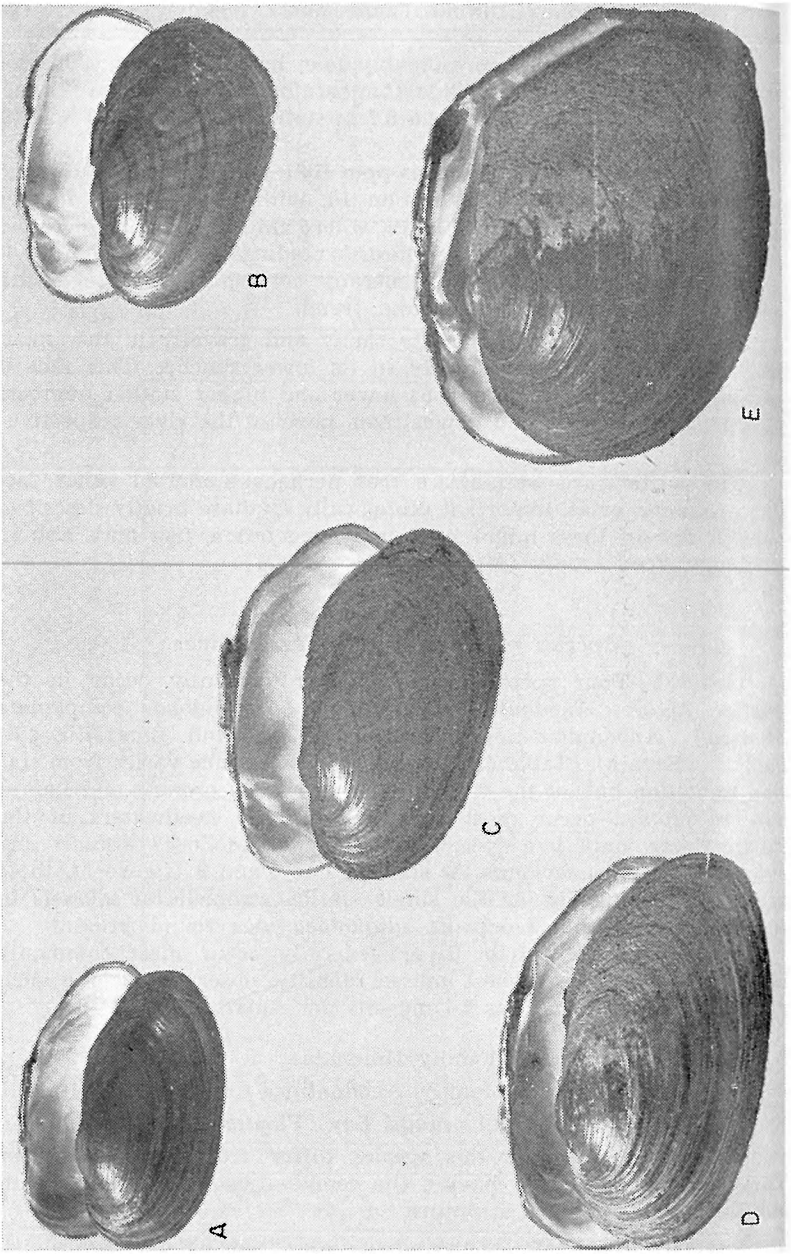

FIGURE 3.—Species of mussels presently inhabiting the Turtle River. All figures are X½. A, *Anodontoides ferussacianus* (Lea), UND Cat. No. 11041, A7. B, C, *Lampsilis siliquoidea* (Barnes), female and male, respectively, UND Cat. Nos. 11048, A10 and 11049, A4. D, *Anodonta grandis* Say, UND Cat. No. 11039, A5. E, *Lasmigona complanata* (Barnes), UND Cat. No. 11044, A8.

TABLE I

Distribution of Live Species of Mussels in the Turtle River*

STATION	MUSSEL SPECIES**			
	a.	b.	c.	d.
1. N. Branch Turtle R., 3½ mi NNW McCanna	U		U	
2. N. Branch Turtle R., 2¼ mi NNE McCanna	C		C	
3. N. Branch Turtle R., 2 mi NNE McCanna	C		C	
4. N. Branch Turtle R., 2 mi E McCanna	C		U	
5. N. Branch Turtle R., 3 3/4 mi ESE McCanna	C	R	U	U
6. N. Branch Turtle R., 3 3/4 mi NNE Larimore	U	R	U	C
7. N. Branch Turtle R., 3¼ mi NNE Larimore	C	R	U	A
8. N. Branch Turtle R., 3 mi NNE Larimore	A	U	R	U
9. S. Branch Turtle R., 1 mi N Larimore	U		R	
10. Turtle R., 2¼ mi NE Larimore	C	R	U	C
11. Turtle R., Turtle R. State Park	U	C	R	A
12. Turtle R., 2½ mi NE Turtle R. State Park	C	R	R	R
13. Turtle R., 2½ mi SW Mekinock	A	U	U	C
14. Turtle R., ½ mi NNE Mekinock	C	U	U	R
15. Turtle R., 3 mi NW Kelly	R			R
16. Turtle R., 3¼ mi NE Kelly			R	R
17. Turtle R., 2 mi SSW Manvel				R
18. Turtle R., ½ mi NNW Manvel				
19. Turtle R., 4 mi NNE Manvel				
20. Turtle R., at mouth (confluence with Red R.)				R
21. Red River, at mouth of Turtle R.				C

* Relative abundance of each species is indicated by: A = abundant, C = common, U = uncommon, and R = rare. Letter symbols underlined indicate a species presence only by empty shells.

** a = Anodonta grandis Say; b = Lasmigona complanata (Barnes); c = Anodontoides ferussacianus (Lea); and d = Lampsilis siliquoidea (Barnes).

Figure 4 shows that *Anodonta grandis* can occur on a variety of bottom type in the Turtle River. However it seems to occur most commonly on a soft bottom of muddy sand in the slow backwater parts of the stream. This species rests relatively high on soft bottom, but on firm substrate its siphons are not uncommonly flush with the bottom.

Lasmigona complanata (Barnes)
"White Heel Splitter"
Figure 3E

Diagnosis.—This is the largest species in the Turtle River. Its shells are heavy, with strong pseudo-cardinal but no lateral teeth, and beak sculpture consists of strong double-looped ridges.

Remarks.—Shell measurement ratios of height/length and width/length show no apparent trend downstream. This species seems to prefer a bottom of muddy, very fine to fine-grained sand (figure 4), and occurs most commonly near banks. It commonly is resting partially on one side in the sediment and usually its siphons are directed upstream.

Anodontoides ferussacianus (Lea)
"Cylindrical Paper Shell"
Figure 3A

Diagnosis.—This species is the smallest of the Turtle River mussels. It has a thin shell which lacks hinge teeth and is characterized by a beak sculpture of low, concentric ridges.

Remarks.—Shell measurement ratios of height/length and width/length show no apparent trend downstream. This species seems to prefer a firm sand or gravelly sand bottom (figure 4) near the upstream end of riffle pools. However, it also occurs in finer sediment and quieter water. *Anodontoides ferussacianus* commonly occurs with its siphons flush with the bottom.

Subfamily Lampsilinae
Lampsilis siliquoidea (Barnes)
"Fat Mucket"
Figures 3B, C

Diagnosis.—Shells of this species are sexually dimorphic, and possess both pseudocardinal and lateral teeth. Beak sculpture consists of low, wavy chevron-like ridges.

Remarks.—Shell measurement ratios of height/length and width/length show no apparent trend downstream. This species occurs on a variety of bottom type (figure 4) and a preference is not apparent in the Turtle River. Not uncommonly, *Lampsilis siliquoidea* occurs with its siphons flush with the bottom. The modified mantle flaps of the female of this species were observed at two localities, stations 11 and 13.

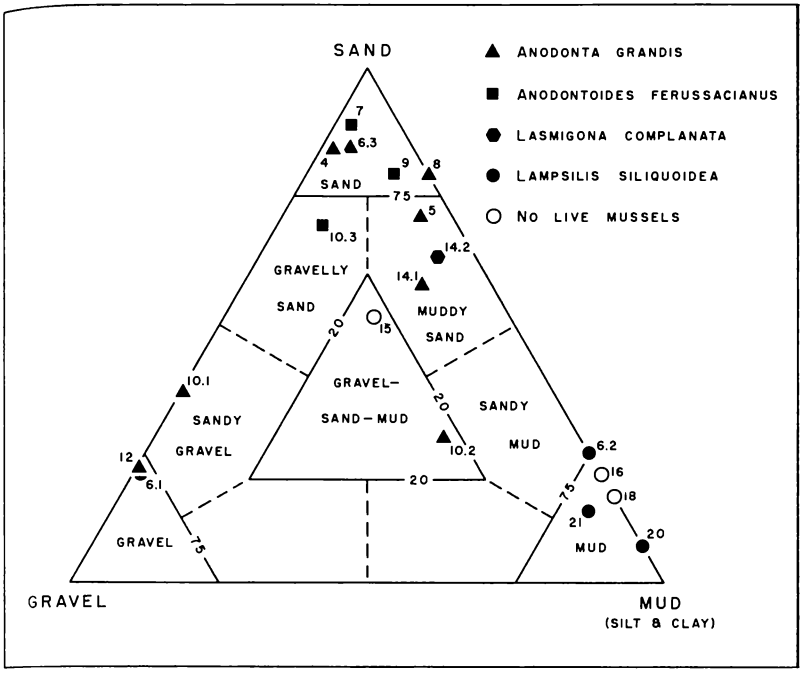

FIGURE 4.—Triangular diagram showing relationship of mussel species to bottom sediment (percentage limits after Shepard, 1954). Numbers at plots correspond to station numbers shown on figure 1. 4 = UND Cat. No. 11031; 5 = UND Cat. 11032; 6.1 = UND Cat. No. 11045; 6.2 = UND Cat. No. 11046; 6.3 = UND Cat. No. 11033; 7 = UND Cat. No. 11040; 8 = UND Cat. No. 11034; 9 = UND Cat. No. 11041; 10.1 = UND Cat. No. 11035; 10.2 = UND Cat. No. 11036; 10.3 = UND Cat. No. 11042; 12 = 11037; 14.1 = UND Cat. No. 11038; 14.2 = UND Cat. No. 11043; 20 = UND Cat. No. 11050; 21 = UND Cat. No. 11047.

Species formerly in the Turtle River

Mussels were also collected at three localities from the terrace-like sediments of the Turtle River. These collections reveal three additional species which presently do not inhabit the river: *Fusconaia flava* (Rafinesque), *Lasmigona compressa* (Lea), and *Strophitus rugosus* (Swainson). All three localities, with mussel species found at each are as follows (numbers in parentheses are accession numbers of the University of North Dakota):

1 (A19). Cutbank (12½ ft. high) in floodplain on west bank of Turtle River, north and south sides of bridge, SW¼ sec. 20, T. 152 N., R. 53 W., about 6 miles southwest of Mekinock, Grand Forks Co., North Dakota.

Anodonta grandis
Anodontoides ferussacianus
Lampsilis siliquoidea

2 (A18). Cutbank (14½ ft. high) on Turtle River, east edge of bridge, west boundary sec. 21, T. 152 N., R. 53 W., about 5 miles southwest of Mekinock, Grand Forks Co., N. Dak. (see Station 12).

Fusconaia flava
Anodonta grandis
Lasmigona compressa
Anodontoides ferussacianus
Lampsilis siliquoidea

3 (A17). Cutbank (11 ft. high) in floodplain of Turtle River, ¼ mile downstream from locality 2 (A18).

Fusconaia flava
Anodontoides ferussacianus
Strophitus rugosus

All species presently inhabiting the Turtle River were also collected from the terrace-like sediments, with the exception of *Lasmigona complanata*. This species will presumably be found upon further collecting.

Analysis of the mussel fauna

The Turtle River mussel fauna, past and present, constitutes a total of seven species. Six of these seven species have been taken from terrace-like sediments previously deposited by the river.

Of the seven species, four are common to both the Turtle and Red rivers: **Fusconaia flava, Anodonta grandis,* **Strophitus rugosus* and *Lampsilis siliquoidea* (those marked with an asterisk do not presently inhabit the Turtle River). In addition, the following species of the Turtle River do not occur in the Red River (Dawley, 1947): *Lasmigona complanata, L. compressa* (does not presently inhabit the Turtle River) and *Anodontoides ferussacianus*. The latter two species are characteristic of creeks or small rivers (Baker, 1928, p. 141 and p. 177, and van der Schalie, 1938, p. 54 and 56). In addition to the four species common to both the Turtle and Red rivers, Dawley (1947) has listed seven other species for the Red River.

The living mussel fauna of the Turtle River is that found in other small rivers or creeks. The three additional species from terrace-like sediments of the present Turtle River also conform with the idea of a small river or creek fauna, and indicate no appreciable difference in the size of the Turtle River when the terrace-like sediments were deposited.

SUMMARY

Four species of mussels presently occur in the Turtle River: *Anodonta grandis* Say, *Lasmigona complanata* (Barnes), *Anodontoides ferussacianus* (Lea), and *Lampsilis siliquoidea* (Barnes). The shells of three other species have been taken from terrace-like sediments of the same river: *Fusconaia flava* (Rafinesque), *Lasmigona compressa* (Lea) and *Stophitus rugosus* (Swainson). This assemblage of seven species is suggestive of a small river or creek fauna, although only *Anodontoides ferussacianus* and *Lasmigona compressa* are particularly characteristic.

Of the four living species, the relative abundance varies from station to station; however, *Anodonta grandis* and *Lampsilis siliquoidea* occur most commonly. In the headwaters of the Turtle River, only two species are present, *Anodonta grandis* and *Anodontoides ferussacianus*.

The Turtle River is apparently barren of mussels from a point about three miles northwest of Kelly (about 12 miles northwest of Grand Forks) to its mouth. Of the ecologic factors analyzed, chloride content, turbidity and total alkalinity seem to be the most important in affecting mussel distribution. The downstream limit of mussels in the Turtle River agrees closely with the western margin of saline clay loam and loam soils where it crosses the river.

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BIOLOGY OF *ENDRIA INIMICA* (SAY), VECTOR OF WHEAT STRIATE MOSAIC

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ABSTRACT

The biology of *Endria inimica* (Say) was studied under greenhouse, environmental chamber, and field conditions. When the environmental chamber had day, interim, and night temperatures of 80°-75°-70°F, respectively, it was found that the females required an average of 12.8 days to oviposit eggs after they mated. Females ovipostited an average of 44 eggs in durum wheat, which required an average of 13.6 days to hatch.

E. inimica has five nymphal instars. The length of the nymphal stage and of each stadium were studied under varying temperature ranges. The nymphs survived best when the day, interim and night temperatures were between 80°-70°-65°F and 90°-80°-70°F. At these temperature ranges the length of the nymphal stage was from 27 to 31 days. Each instar required from 3 to 8 days to complete development. The nymphs could not survive in the environmental chamber when the day, interim and night temperatures were 65°-60°-55°F or below.

In North Dakota *E. inimica* has two generations per year. The leafhoppers hatch from overwintering eggs from late June to early July and can be found until mid-October.

The eggs are oviposted under the epidermis of the leaf sheath, under the epidermis of the leaf blade, and in the soil around the base of the host plant.

In North Dakota smooth brome (*Bromus inermis*) is the most important host plant. Blue grass (*Poa pratensis*), quackgrass (*Agropyron repens*), and wheat are also common hosts.

There is some indication of a migration of *E. inimica* into North Dakota in the spring.

Parasites of *E. inimica* include species from the families Stylopidae, Pipunculidae, and Dryinidae.

HABITAT DISTRIBUTION AND MORPHOLOGICAL VARIATION OF THE DEER MOUSE (PEROMYSCUS) COMPLEX OF NORTHWESTSERN MINNESOTA AND NORTHEASTERN NORTH DAKOTA

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ABSTRACT

Attempts by several authors to distinguish among the three forms of Deer Mouse, *Peromyscus maniculatus bairdii, P. maniculatus gracilis* and *P. leucopus noveboracensis,* on the basis of morphological and ecological variations have been unsuccessful in the ectone region of northeastern North Dakota and northwestern Minnesota. The purposes of the present study were to analyze certain morphological characters and to develop improved criteria for the classification of these three forms by using techniques of numerical taxonomy. Preliminary analyses of variation of 11 morphological characters demonstrated overlap among the three forms so that a single set of measurements could not be reliable for definite identifications. Likewise, habitat preferences were not distinctive for the three forms as they are in other parts of their geographical distributions. One "typical" specimen of each form was chosen on the basis of best fit to "key" characters published by others. All specimens (14 *P. m. gracilis*, 24 *P. m.* bairdii and 51 *P. l. noveboracensis,*) were compared to these "typical" specimens by calculation of the Coefficient of Similarity, a slight modification of the coefficient of community

$$K = \sum_{i=1}^{12} \left(\frac{2w}{a+b} \right)$$

which gave equal weight to each of the 12 characters used in every comparison. The results indicated that misclassifications and subspecific intergrades were present in the original determinations, and that the use of similiarity coefficients in mammalian taxonomy may

be a powerful technique of analysis for problems of the type described here. The lack of sufficient character numbers in the present study prevented the authors from making any final quantitative statement on the classification of members of these three taxa.

DAILY MOTOR ACTIVITY AND CORTICOSTERONE SECRETION IN THE MEADOW VOLE

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ABSTRACT

A study was conducted of daily adrenocortical activity in the meadow vole, *Microtus pennsylvanicus,* with regard to corticosteroid production as a function of daily patterns of motor activity under controlled conditions of temperature and photoperiod, and comparison of corticosteroid levels in males and females, and in wild-caught and laboratory-raised voles. Meadow voles exhibited successive short cycles of motor activity with peaks averaging from one to five hours in length. Longest periods of activity and greatest variability in activity occurred during the hours of darkness. Fluorometric determinations revealed steroid levels several times greater than have been observed in laboratory mice and rats. Voles displayed a daily rhythm of corticosterone secretion, the peak occurring during the lowest ebb of motor activity. Females were generally observed to have higher levels of both serum and adrenal corticosterone than males. Sex differences were more pronounced in wild-caught than in laboratory-raised voles. Wild-caught voles generally had higher corticosterone levels than those born in captivity. These higher levels were particularly pronounced in the wild-caught females.

HIGH TEMPERATURE TOLERANCES OF *BUFO COGNATUS* AND *BUFO HEMIOPHRYS*

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ABSTRACT

Temperature tolerance of *Bufo cognatus* and *B. hemiophrys* were examined by exposing specimens to lethal high temperatures. *Bufo cognatus* was able to tolerate significantly higher temperatures than *B. hemiophrys.* The results yielded an estimated interspecific difference in upper lethal temperature of 2.34 ± 0.41° C. This difference

was correlated with geographic distributions of the two species; *i.e.*, the more southern species, *B. cognatus*, was able to tolerate higher temperatures. This has been taken to indicate that a physiological specialization has taken place which is adaptive for the two species in the microclimates of their respective habitats.

INTRASPECIFIC DIFFERENCES IN DESICCATION TOLERANCE OF THE TIGER SALAMANDER, *AMBYSTOMA TIGRINUM*

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ABSTRACT

Although there is a great deal of overlap, the geographic distributions of three subspecies of tiger salamander, *Ambystoma tigrinum*, are roughly correlated with an east-west climatic gradient across North Dakota.

North Dakota. Specimens of each subspecies were exposed to controlled desiccation to determine tolerance to water loss. The westernmost form, *A. t. melanostictum*, lost 63.1 percent of its total water at death, which was significantly different from the losses of 54.2 and 52.6 percent for *A. t. diaboli* and *A. t. tigrinum*, respectively. These differences have been interpreted as subspecific specializations to a component of the environment which may be limiting to the geographic distribution of these members of the salamander complex in North Dakota.

ACKNOWLEDGEMENTS

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WATER PERMEABILITY AND LIPID CONTENT OF AMPHIBIAN SKIN

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Significant differences in permeability to water and lipid content of skin were observed among three species of anuran amphibians which were correlated with differences in habitat preference. The skin of the aquatic mink frog, *Rana septentrionalis*, was less permeable

[1]N.S.F. Undergraduate Research Participant, Grant No. G.E.-2836.

to water and had a higher lipid content than that of *Bufo hemiophrus* a terristrial toad. The skin of the semiaquatic tree frog, *Hyla versicolor*, was intermediate to these. The adaptive significance of these observations was discussed in regard to the physiological specialization of amphibians to environmental conditions. The inverse correlation between permeability to water and lipid content of skin was interpreted as being of regulative importance.

SOME SEASONAL CHANGES IN THE MOURNING DOVE, *ZENAIDURA MACROURA*, IN RELATION TO AUTUMNAL MIGRATION

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ABSTRACT

Total body weight, gonadal weight, daily caloric intake and lipid content of liver tissue were studied in Mourning Doves of northeastern North Dakota during the spring, summer and fall of 1964. The observed increase in body weight (lipids?) prior to migration may not be a simple function of increased feeding activity. The decrease in gonadal weights during August, which lagged behind the onset of a gradual increase in body weights, indicated a complex sequence of endocrine-physiological changes prior to autumal migration.

STUDIES ON THE SIGNIFICANCE OF BIRDS IN THE EPIDEMIOLOGY OF LISTERIC INFECTION

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INTRODUCTION

There is at present very little conclusive information regarding the epidemiology of infections caused by *Listeria monocytogenes*. This organism, once considered as solely an animal pathogen, is now recognized as the etiological agent of a number of disorders in man. In addition, it is felt that because of the difficulty encountered in

culturing the organism numerous cases of human listeriosis have not been bacteriologically confirmed.

A number of investigators indicate that ingestion is the portal of entry for Listeria (1, 2, 3). Experimental work performed on both domestic and laboratory animals has produced evidence that *L monocytogenes* in the alimentary tract is capable of overcoming normal barriers of the host, particularly during conditions of stress (4, 5, 6, 7, 8, 9). The organism's ability to attack mucous membranes is easily demonstrated by its ability to produce a conjunctivitis experimentally. It may be, therefore, that the epithelial barriers of the nasal mucosa, digestive tract, or genital tract might also serve as portals of entry. Andrews (10) produced listeric infections in mice by using aerosols containing the organism. Rappaport *et al.* (11) repeatedly isolated Listeria from the vagina of 25 of 34 women with a history of repeated abortions. The belief that Listeria is part of the normal flora of the animal gastro-intestinal tract has been expressed by a number of invesigators (12, 13).

Regardless of the varied opinions as to the portal of entry, the source of the organism in nature is of importance. Soil is a natural suspect, as listeriosis is world-wide in nature. Welshimer (14) and Lehner (15) have found that the organism remains viable for months particularly if agents which inhibit competitive organisms are used. Listeria has also been isolated from silage with considerable regularity by a number of investigators (16, 17).

As listeriosis most commonly comes into focus as a disease of domestic ruminants, it is natural that they be considered the dominant factor in its epizootiology. However, these animals generally display a fairly high order of natural resistance. Then, too, we would think that the numbers of escaping listeria would be far greater from species of birds, rodents, and other small mammals where the disease is characterized by massive involvement of liver, spleen and other organs. When *L. monocytogenes* causes encephalitis in ruminants, it would seem that the organism would have a limited opporunity to escape. This is, however, not true in the abortion syndrome of listeriosis, although the period of excretion would be relatively brief.

There has been and is presently a considerable amount of research with *L. monocytogenes* carried on at the Department of Veterinary Science, North Dakota State University. The possibility of contamination of control animals with Listeria has been a source of concern. Preventive measures to insure against such contamination included the procedures generally used to inhibit the transmission of infectious agents. This brought to focus the possibility of contamination via the birds in the area. Numerous pigeons and English sparrows live in the vicinity of the Departmental barns, and have on occasion had access to areas of these structures which harbor diagnostic animals or feed for such animals. A number of birds were trapped and the tissues examined bacteriologically.

MATERIALS AND METHODS

A total of 38 sparrows and 8 pigeons was used in the study. Following euthanization, the liver, lung, and intestinal contents were cultured. Blood agar plates (BAP) were inoculated from the liver tissues and incubated aerobically and under a 10% CO_2 atmosphere. The lung tissues were cultured on pleuropneumonia-like-organisms (PPLO) plates. BAP, Mac Conkeys, Salmonella-Shigella, eosin methylene blue, and brilliant green plates were used for the intestinal contents.

TABLE I

THE INCIDENCE OF LISTERIA AND CERTAIN OTHER MICROBES IN THE TISSUES OF THIRTY-EIGHT SPARROWS

Microorganism	Liver	Intestine	Lung
Listeria monocytogenes	5	2	N/A*
Staphlococci H + C + S + M+	13	3	N/A
Streptococci, alpha	4	1	N/A
Proteus vulgaris	6	N/A	N/A
Pseudomonas aeruquinosa	1	1	N/A
Corynebacterium sp.	2	—	N/A
Salmonella enteriditis	—	1	N/A
Paracolobactrum intermedium	—	1	N/A
Paracolobactrum arizonea	—	1	N/A
PPLO**	N/A	N/A	16
Aspergillus sp.	5	5	2
Candida sp.	2	4	1

*N/A—Isolation not attempted.

**Pleuropneumonia-like-organisms

TABLE II

THE INCIDENCE OF LISTERIA AND CERTAIN OTHER MICROBES IN THE TISSUES OF EIGHT PIGEONS

Microorganism	Liver	Intestine	Lung
Listeria moncytogenes	3	1	N/A*
Staphlococci H + C + S + M+	2	2	N/A
Streptococci, beta	1	—	N/A
Proteus vulgaris	1	N/A	N/A
Pseudomonas aeruquinosa	—	1	N/A
Paracolobactrum intermedium	—	1	N/A
Diplococcus pneumoniae	1	—	N/A
PPLO**	N/A	N/A	5
Asperigillus sp.	1	—	1
Candida sp.	1	—	1

*N/A—Isolation not attempted.

**PPLO—Pleuropneumonia-like-organisms.

RESULTS AND DISCUSSION

The incidence of *L. monocytogenes* in the tissues is shown in Tables I and II. Five sparrows of 38 and 3 pigions of 8 yielded *L. monocytogenes* on examination. Of interest was the isolation of this organism from the intestinal tract in 3 of the birds indicating a definite source of transmission of the disease. The birds were probably in the carrier state, as no gross pathological lesions were observed on any visceral organs. The incidence of hemolytic staphlococci, PPLO, and other organisms generally considered pathogenic were also of interest. The PPLO isolates differed serologically from Mycoplasma gallarum, which is found in the upper respiratory tract of fowl, and has been the most studied member of this group of organisms.

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PIGMENT PRODUCTION BY A SOIL BACTERIUM

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ABSTRACT

A microorganism was isolated from the soil of the grounds of the University of North Dakota. Biochemical tests and morphological characteristics indicate that this organism belongs to the family *Achromobacteraceae*, species unknown. The organism produced a red pigment when grown in a medium containing tryptophan and yeast extract. The pH optimum for pigment production was about 8.0 and the temperature optimum 23°C. During a study of the nutritional requirements for growth and pigment production, it was found that the organism grew and produced pigment in a medium containing tryptophan and nucleosides, although at a reduced rate. The organism grew well in the presence of acid hydrolyzed casein without producing any pigment thus indicating that the pigment is not necessary for growth. The exact amino acid requirements were not determined. Resting cell experiments definitely established that the sole exogenous requirement for pigment production was tryptophan. The pigment was extracted from yeast extract-trypotphan media with chloroform. The solubility of the pigment in water was reduced by adjusting the pH to approximately 3.0, facilitating the chloroform extraction. The pigment was further purified using paper chromotography. Whatman 3mm paper and an ethanol-water-acetic acid (65:34:1) solvent system were employed. Thin-layer chromotography was employed using various solvent systems to check purity. The pigment served as an electron acceptor when coupled with formic

dehydrogenase indicating its possible function as an oxidation-reduction pigment. The pigment had an absorption peak at 500 mμ which disappeared when reduced with sodium sulfite. Shaking the reduced pigment in air proved to be an unsatisfactory method for returning the reduced pigment to its oxidized, colored state.

THE APPLICATION OF THE ELECTRONIC PARTICLE COUNTER IN DETERMINING BACTERIAL CELL COUNTS

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INTRODUCTION

The determinations of bacterial cell numbers have, until recently, depended on the tedious and somewhat unprecise microscopic or viable count methods. By the introduction of the electronic particle counter (EPC) of Coulter (1953) new possibilities of determining bacterial cell numbers were introduced. Kubitschek (2) showed that the EPC, originally designed for counting blood cells, could be adapted to the much smaller size range of bacteria by replacing the original aperature, about 100 μ in diameter, with a smaller one, about 10 μ in diameter, through a glass sheet about 50 μ thick. The circuitry of the EPC was changed as to prevent multiple recordings of a given cell, and a pulse-height analyser was added to give a more direct measurement of the distribution of cell volumes.

The objective of this study was to analyze the application of the EPC in determining bacterial cell counts utilizing an EPC equipped with an aperture tube 30 μ in diameter and calibrated to count the particles in a volume of 0.05 ml.

MATERIALS AND METHODS

The electronic particle counter.—The mechanism of the EPC includes an aspirator which produces a controlled external vacuum initiating the flow of the suspension from the beaker through an aperture having an immersed electrode on either side. As a particle passes through the aperture a change in the resistance between the two electrodes is produced. The resulting voltage pulse, which is proportional to the size of the particle, is amplified and fed to a threshold circuit having an adjustable threshhold level. If this pulse is comparable to or surpasses the level, the pulse is counted. The threshhold level is indicated on an oscilloscope screen by a brightening of the pulses above the threshold level.

In this study all the various combinations of settings of the aperture current control, threshold, and the gain trim and gain switch were utilized in the counting procedure.

Preparation of organism.—*Staphylococcus aureus* was selected for counting on the basis of present research being done with the organism. Initial inocula into Brain Heart Infusion Broth were taken from Brain Heart Infusion Agar slants. The cultures were incubated at 37°C for 4 hours and 18 hours of the growth period. To prepare the organisms for counting the broth cultures were prepared in numerous ways as to find the preparation which would be the simplest and fastest means of preparation and yet yield accuracy on the part of the EPC which was compared with two other means of counting, the hemocytometer and the standard plate method.

Serial dilutions of the organisms from 10^2 to 10^8 were made for all the various preparations and means of counting, the hemocytometer, the standard plate method, and the EPC. The dilution which would yield the easiest and most accurate count by the standard plate method was selected. For the 18 hour period of growth the 10^6 dilution was selected; the 10^4 dilution was selected for the 4-hour growth period.

Preparation of organisms for counting.—The first method utilized in the preparation of the organisms was that method utilized by Swanton, Curby, and Lind (1962). To prepare the organisms for counting, the broth cultures were centrifuged at 900 x g for 25 minutes, and the supernatant fluid discarded. The procedure was repeated for two 0.85 percent saline washes and then the organisms were suspended in 10 ml of saline. Serial dilution from 10^2 to10^6 were made for the standard plate counts. After plating the organisms on Brain Heart Infusion Agar the remaining portions of the dilutions were placed in a 56° C water bath up to two hours to kill the bacteria. After killing, the bacteria growth was checked by plating in Brain Heart Infusion Agar. The remaining portions of the dilutions were shaken mechanically with glass beads to aid in the breaking up of bacterial clumps for 15 minutes and then were counted with the EPC utilizing all the various settings of the aperture current control, threshold, and gain trim and gain switch. The remaining portions of the dilutions were shaken mechanically for two hours with glass beads and then were counted with the EPC utilizing the same settings as in the previous counts.

The second method utilized in preparation of the organisms for counting was the same as that of the first method except instead of killing the organisms in a water bath, the organisms were killed in an autoclave and then were counted using the same settings of the EPC as previously described. This method of killing the organisms was utilized to find a more rapid means of preparation for counting.

The third method of preparation included the taking of the organisms from the broth cultures, not killing them, and making

serial dilutions from 10^2 to 10^7 in saline. The organisms were then refrigerated for 30 minutes in order to restrain further growth. After refrigeration the organisms were shaken mechanically for 15 minutes using glass beads.

This same procedure of preparation was used in another method. Here the live organisms were centrifuged at 900 x g for 25 minutes and the supernatant discarded. The procedure was repeated for two .85 percent saline washes and then resuspended in 10 ml of saline. Serial dilutions were made from 10^2 to 10^8 in saline and were plated in Heart Brain Infusion Agar and counted by the EPC.

Obtaining a background count.—Before organisms were counted with the EPC, the saline which was used as a dilutent for the organisms was passed through the EPC in order to obtain a background count. This count which was also obtained at the various combinations of settings of the aperture, current control, threshold, gain switch and gain trim to be substracted from the bacterial counts for the identical settings in order to get the true bacterial count for a particular setting.

Analysis of counting procedures.—The counts obtained from the EPC were compared with those counts obtained by two other counting methods, the hemocytometer and the standard plate method. Because of the lack of reliability and difficulty of counting with the hemocytometer, it was excluded as a means of comparison to the EPC. This same lack of reliability of the hemocytometer counts concurs with that reported by Mattern, Brackett, and Olson (1957), and Swanton, Curby, and Lind (1962).

After the exclusion of the hemocytometer the EPC counts were to be compared entirely to the standard plate method of determining bacterial cell counts.

The number of organisms were obtained per milliliter for each method of the counting, the EPC and the standard plate method. The combination or combinations of the settings of the EPC which compared the most closely to the standard plate was determined. This indicated that the most efficacious adjustments for the EPC were an aperture current setting of 2, and a threshold setting of 30 to 40. The gain trim and gain switch setting of 4 was the most satisfactory of all investigated. After these settings were determined successive runs were made of the different methods of preparations to determine which one stayed constant with the EPC as compared to the standard plate method, and also if the settings which were obtained were constant.

RESULTS

The difference in gain trim and gain switch settings is demonstrated on figure 1. The aperture current control setting was maintained at a setting of 2 for the repeated runs of the same sample. As indicated in the analysis of counting procedures in this paper the gain

trim and the gain switch setting of 4 was most satisfactory as it coincides with the threshold setting of 30 to 40 in which the standard plate counts agreed most closely. The gain trim and gain switch settings of 5 and 6 were too high and the gain trim and gain switch setting of 3 which is not indicated on figure 1 was irregular in its pattern with repeated runs.

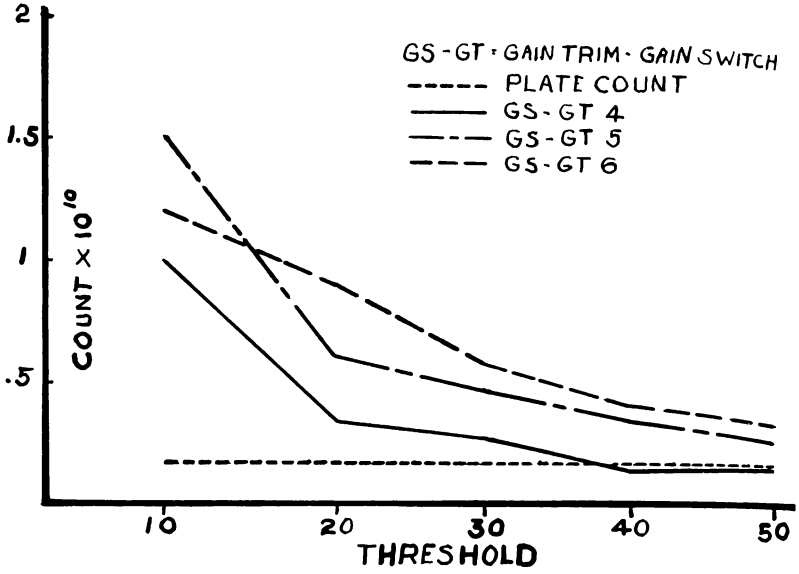

FIGURE 1—The comparison of various gain trim and gain switch settings to the standard plate count maintaining the aperature current control setting of 2 for all counts.

The aperture current control setting of 2 was determined as being the most efficacious adjustment for the EPC. Figure 2 indicated the relationship of the aperture current control setting of 2 to the aperture current control settings of 3 and 4. The aperture current control setting of 5, which was not placed on the graph, produced a count which was very high and irregular. The results indicated on Figure 2 were taken from the same preparation.

Figure 3 shows four different preparations that were counted with the EPC setting the aperture current control at 2, the gain trim and the gain switch at 4. The standard plate counts coincided with the threshold setting of 30 to 40 in all preparations.

Of the different preparations of organisms investigated the following results were indicated. In the preparation in which the organisms were autoclaved a very high EPC count was obtained as compared to the standard plate counts made before the cells were

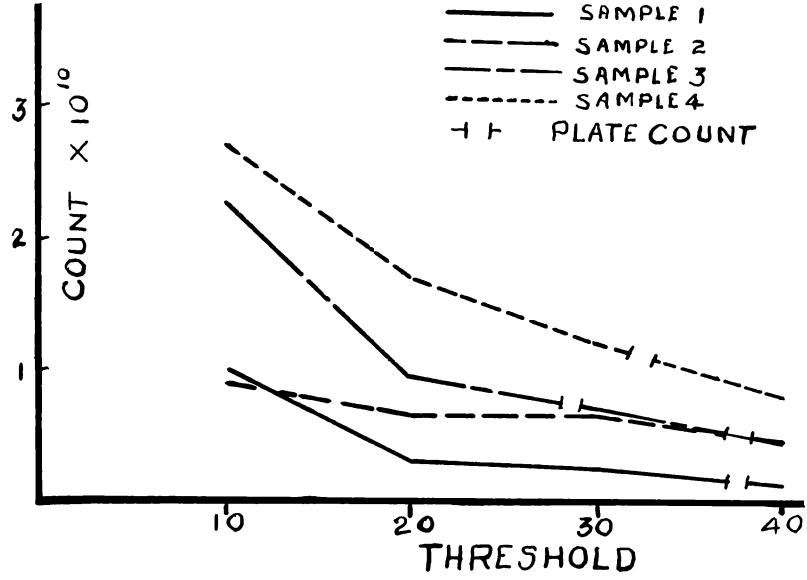

FIGURE 2—The comparison of various aperature current control settings to that of the standard plate counts maintaining a constant gain trim and gain switch setting of 4.

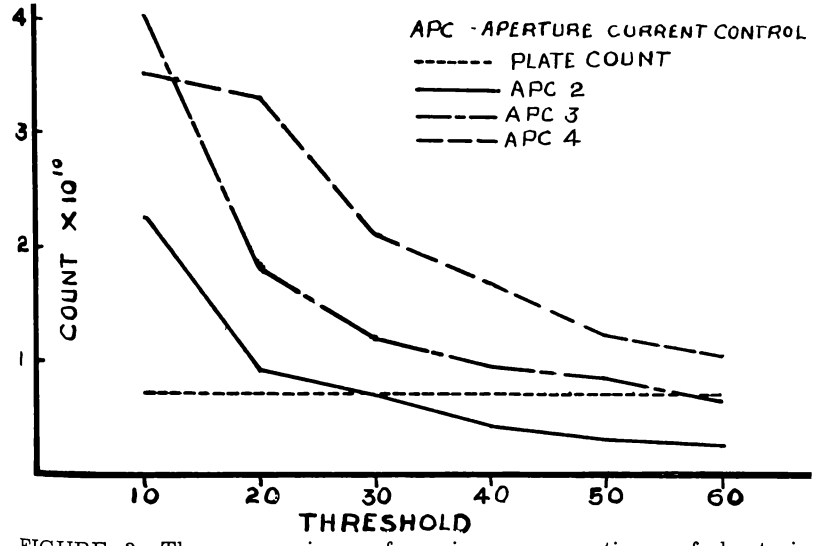

FIGURE 3—The comparison of various preparations of bacteria maintaining a constant aperature current control setting of 2 and a constant gain trim and gain switch setting of 4.

autoclaved. Cell lysis probably interrupted these results thus increasing the number of particles indicated by the EPC. The same results were also indicated when cells were shaken mechanically for two hours with the aid of glass beads. Here it is presumed that the cells were ruptured by mechanical means thus increasing the particle count. The other procedures used were satisfactory in that the results obtained from the EPC and the standard plate counts were within range of each other.

CONCLUSION

On the basis of this investigation utilizing the standard plate count as a recognized means of determining bacterial numbers, comparisons were made indicating the most efficacious settings of the EPC, an aperture current control setting of 2, a threshold setting of 30 to 40, and a gain trim and gain switch setting of 4. One must recognize that these settings are estimates and that there still is a need for more investigation.

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STUDIES OF THE CHANGES IN COLONIAL AND CELLULAR MORPHOLOGY OF *VIBRIO FETUS*[1]

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INTRODUCTION

Frequently the aborted fetal bovine and ovine specimens presented to the diagnostic laboratory, Department of Veterinary Science, are received in a state of advanced post mortem decomposition. The bacterial contaminants interfere with the isolation of the pathogenic, etiological agent. In order to suppress the contaminants, but not the suspected pathogen, various agents were added to the standard Difco Blood Agar Base.

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[1]Published with the approval of the Director, Agricultural Experiment Station. North Dakota State University, Fargo, North Dakota.

Numerous investigators (1, 2, 3, 4, 5, 6) have studied and identified *V. fetus* as the etiological agent of one contagious type of abortion. The eight *V. fetus* cultures in this study were isolated from the stomach contents of the bovine fetuses. The 7th Edition of *Bergey's Manual of Determinative Bacteriology* simply states that *V. fetus* grows on blood agar plate in 10 percent CO_2. Merchant and Packer, *Veterinary Bacteriology and Virology,* 6th Edition, describes growth on blood agar plate as ". . . fine pin-point, bluish areas from 1 to 3 mm. in diameter visibly raised above the surface of the medium". No mention was made of hemolysis of sheep erythrocytes. Hagan and Bruner, *The Infectious Diseases of Domestic Animals,* 3rd Edition, describe the cellular morphology as ". . . comma-shaped or S-shaped bodies; occasionally as longer spirals. In young cultures the organisms are short, but in older cultures very long spirals are seen. . ."

MATERIALS AND METHODS

Original inoculation was made directly from the stomach contents of the aborted fetuses onto the following media:

Blood Agar Plate (BAP)
- 40 gm. Difco Blood Agar Base
- 50 ml. sterile sheep blood (defibrinated)
- 950 ml. distilled H_2O
- pH 7.4

Brucella Plates (Bruc.)
- 40 gm. Difco Blood Agar Base
- 50 ml. sterile sheep blood (defribrinated)
- 950 ml. distilled H_2O
- 25,000 I. U. Polymixin B. Sulfate
- 10 ml. Dimethyl Sulfoxide
- pH 7.3

Alkaline Tellurite Sodium Lauryl Sulfate Salt Medium (SL)
- 40 gms. Difco Blood Agar Base
- 05 ml. sterile sheep blood (defibrinated)
- 15 gms. NaCl
- 800 ml. Distilled H_2O
- 50 gms. $NaCO_3$
- 50 ml. of a 20% Millipore-filtered solution of sucrose
- 100 ml. of a 0.1% autoclaved solution of sodium lauryl sulfate
- 1 ml. of a 1% Millipore-Filtered solution of K_2TeO_3 pH 7.8

No Proteus Plates (NP)
- 40 gms. Difco Blood Agar Base
- 50 ml. sterile sheep blood (defibrinated)
- 950 ml. distiled H_2O
- 2 gms. Difco Bile Salts
- 25 gms. Difco Agar*

(*Total agar concentration of 4.0%)

The inoculated plates were incubated at 37 degrees C for twenty-four hours under 10 percent CO_2 atmosphere. The individual colonies were then selected and subcultured to the same media and also crossed over to the other mdia. The effect of subculturing on this media was then studied.

RESULTS AND DISCUSSION

The control of *Proteus* sp., because of their prevalence as a contaminant and their capacity to spread, was our major objective. In order to achieve initial isolation of *V. fetus,* it was found that blood as a media nutrient and 10 percent CO_2 atmosphere were essential. With this as our starting point, we then invesigated various ingredients and concentrations of each to determine their effect.

The variations in cellular and colonial morphology are noted below as compared to the translucent, low profile, circular, convex colonies with an entire margin as noted on the BAP and the normal comma-shaped cell.

A more coccoid form and a translucent colony very closely resembling growth on BAP were produced on the Bruc. media. Subculturing intensified the prominence of the coccoid form.

On NP media there was a thin, hair-like rod produced which exhibited a long filamentous form after repeated subculturing. The colonies were translucent, but smaller and more discreet with the highest profile.

On SL media the colonial morphology resembled the growth on NP; small convex, translucent and discreet. The celluar structure became rod-shaped and thicker in diameter.

Long filamentous forms increased in the above cultures in proportion to the age of the cultures.

Both cellular and colonial morphology reverted to that described above on each media during the linear and cross serial subculturing in this study. Apparently the media is the determining factor in the cellular and colonial morphology. Hemolysin production increased with subculturing as measured by the ability to hemoyze the RBC's on the BAP or Bruc. media within 24 hours.

Subculturing caused no apparent loss in virulence. I. P. injection of a saline suspended inoculum prepared from the fourth subculture produced abortion and caused death in a pregnant Guinea pig within 24 hours. *V. fetus* was isolated from the uterus of the Guinea pig and the aborted fetus.

SUMMARY AND CONCLUSIONS

To facilitate the isolation of *Vibrio fetus* from the contents of the fetal stomachs of bovine and ovine specimens submitted to this laboratory for bacteriological examination, various ingredients were added to Difco Blood Agar Base and 5 percent sterile defibrinated

sheep blood to enhance its growth and suppress contaminants. Concurrently, a study of the effect of these additives on the cellular and colonial morphological characteristics of *V. fetus* was made.

We feel, from our observations, that adequate control of contaminants and growth of *V. fetus* was achieved, improving the opportunity of primary isolation. Recognition of the organism microscopically would be facilitated if the respective changes in cellular morphology were kept in mind.

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ADRENERGIC BLOCKADE IN REPRESENTATIVE BOVINE VASCULAR SEGMENTS

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ABSTRACT

Although the smooth muscle cells which invest the walls of arteries, arterioles, vascular sphincters, and veins appear histologically identical, they respond in widely different ways to the various vasomotor activators. Moreover, the functional disparity between the vessels of different organs is so great that a single substance will produce decreased blood flow in one organ and increased flow in another. The most popular explanation for this phenomenon is the difference in affinity of these drugs for postulated receptor sites in the smooth muscle.

Strips of arteries of approximately the same size taken from the heart, lungs, and peripheral areas of freshly slaughtered cattle were placed individually in the tissue bath of a Casella Automatic Bioassay Apparatus. The reactions of the strips to norepinephrine, an alpha (motor) receptor stimulating drug, isoproterenol, a beta (inhibitory)

receptor stimulating drug, epinephrine, a drug stimulating both types of receptors, and carbachol, a parasympathomimetic drug stimulating neither type of receptor, were recorded by an E & M Physiograph. The reactions to the same drugs were observed on artery strips exposed to Inderal, a beta receptor blocking agent, or Dibenzyline, an alpha receptor blocking agent.

Norepinephrine caused the pulmonary arteries to constrict and the dorsal metatarsal (peripheral) arteries to constrict strongly. Blockade with Inderal did not alter these reactions. Isoproterenol produced relaxation of dorsal metatarsal arteries and slight relaxation of pulmonaries. Inderal caused a slight reversal of these reactions. Epinephrine produced strong constriction in dorsal metatarsal and pumonary arteries, which was reduced slightly in pulmonary arteries following Inderal Blockade. Carbachol caused relaxation in dorsal metatarsal arteries and a slight constriction in pulmonary arteries which was slightly diminished following Inderal blockade. Reactions of coronary arteries were equivocal. In general, norepinephrine and epinephrine cause a slight relaxation, isoproternol caused relaxation, and carbachol caused constriction. Inderal blockade produced a slight diminution of the relaxation caused by isoproterenol. Data will be presented on the effects of Dibenzyline blockade on these tissues.

PENTOBARBITAL HYPNOSIS IN MICE

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ABSTRACT

Thirty female mice were divided into two equal groups and the average sleeping times of each group was determined. Group A slept 91.6 minutes and group B slept 100.2 minutes. To study pentobarbital's hypnotic action, both groups of animals were given weekly injections of 75mg/kg for ten weeks. In addition, group B mice were given subhypnotic injections of 30 mg/kg pentobarbital four days prior to each trial of the hypnotic dose. Shorened sleeping times appeared during the second to the fourth weeks and significant tolerance was developed by both groups to the pentobarbital during this time. In addition, group B developed a significant tolerance during the sixth and eighth weeks. However, at the tenth week group A acquired a significant increase in sensitivity to pentobarital. Group B did not appear to have a significant tolerance at this time. At the end of the ten weeks, the mice were decapitated and their livers removed. These livers were frozen and saved for further investigation.

EFFECTS OF *SOLIDAGO ALTISSIMA* EXTRACTS UPON ISOLATED TISSUES OF THE RAT[1]

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ABSTRACT

Solidago altissima, commonly called goldenrod, has been used as a medicinal agent by the Indians in the Standing Rock Reservation. Preliminary studies of the leaves and flowering tops of a sample obtained from the reservation indicated that a water extract of the plant was toxic when administered intraperitoneally to mice. Consequently an attempt was made to study the effects of a water extract of this plant upon the smooth muscles of the rat. It was observed that jejunal strips of the rat were depressed when the extract was added to the bath. The degree of depression varied with the quantity of extract added; ranging from 2.0 mg o 20.0 mg. It was observed that the depression was readily reversed with acetylcholine. Employing the experimental procedure used in studying the effects of the extract upon jejunal strip, uterine horn strips of adult females were also depressed and readily reversed with exytocin. The results obtained suggest that the water extract of *Solidago altissima* was depressant o isolated jejunal and uterine strips and readily reversed by acetylcholine and oxytocin respectively.

[1]Supported in part by U.N.D. grant 4214-16 and AMA Grant 4537.

FUNCTIONAL DEVELOPMENT OF THE CAROTID SINUS PRESSOR REFLEX IN DOGS

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ABSTRACT

The carotid sinus pressoreceptor mechanism for the homeostatic regulation of arterial blood pressure has been described as being present in the newborn puppy, but the functional development of this reflex has not been studied using dogs of known ages between birth and adult.

In this study, 28 dogs, divided into 7 age groups, were used. The carotid arteries were occluded, and systolic and diastolic blood pressures recorded from the aorta during a 15 minute period of time.

Results are reported as the percent increase of mean arithmetic blood pressure and heart rate after 15 minutes occlusion of both carotid arteries. Blood pressure and heart rate changes with age were respectively: 1-6 days, 16% and 1%; 7-10 days, 4% and 2%; 11-18 days, 7% and 15%; 19-58 days, 42% and 30%; 59-75 days, 36% and 24%; 76-120 days, 44% and 14%; adult, 53% and —1%.

The results indicate that although there is an increase of blood pressure and heart rate in response to a decrease in blood pressure in the carotid sinus area, the period of significant development of the maximal sensitivity of this reflex is in the age bracket of 19-58 days. Further discussion related the results with the functional development of the circulatory system.

[1]Supported by the North Dakota Heart Association.

CHANGES IN THE HEART WEIGHT-BODY WEIGHT RATIOS IN THE DOG WITH AGE

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ABSTRACT

Previous studies have described the heart weight-body weight ratio in dogs of various age groups but the exact ages were unknown. In this study, the heart weight-body weight ratio was determined for 211 dogs of various known ages. The body weight was measured to the nearest 10 gm and the heart was weighed to the nearest 0.1 gm. The heart weight-body weight was expressed as gm/Kg. The fat and fat-free water content of the heart and the whole body were also determined.

Results suggest a general rise in the heart weight-body weight ratio with age, but were not as clear cut as other investigators have shown. The average heart weight-body weight ratio postnatally was in gm/Kg as follows: 1-3 days of age, 7.17; 10-12 days, 7.67; 18-23 days, 7.09; 57-120 days, 8.41; and in adults, 8.87. The ratio was 6.52 in 4 prenatal, later-term puppies. However, there were large variations within each age group and it was found that there was a statistically significant difference only between the ratios determined for the puppies less than 4 days of age and that of adults.

The fat content of the whole heart increased from 0% at the prenatal, lateterm age to 0.44% at 1-3 days of age and to 5.62% in the adult. The fat-free water content of the whole heart decreased from 86.05% prenatally to 84.6% at 1-3 days of age and dropped further

[1]North Dakota Heart Association Fellow.

to 80.24% in adults. The fat-free water content of the whole body was found to be 84.6% in puppies 1-3 of age as compared to 60-70% in adults.

The reasons which might explain the large variation in the heart weightbody weight ratios found within one age group and the disagreement with the data of other investigators was discussed. The changes in the heart weight-body weight ratios, fat-free water content of the heart and whole body, and the fat content of the heart were considered in relation to changes in the functions of the cardiovascular system.

THE RELATIONSHIP BETWEEN PLASMA AND SPINAL FLUID SODIUM LEVELS AND OSMOTIC PRESSURE IN ACUTELY INDUCED HYPO- AND HYPERNATREMIC STATES

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ABSTRACT

Dogs in the post-absorbative state were made hyponatremic by (1) intravenous infusion of 0.028% NaCl or (2) by intraperitoneal dialysis with 5% glucose in water. Hypernatremia was induced by intravenous 5% NaCl. Initial and hourly cisternal spinal fluid and blood samples were obtained for 3 hours. Sodium, water, and osmotic pressure (measured by freezing point depression) were obtained on all samples. Initial relationship CFS Na/plasma Na = 0.966, indicating that the spinal fluid is not a simple dialysate of the plasma when the Donnan factor is taken into consideration. In acute water intoxication the plasma Na level and the CSP Na level fall at the same rate. In acute NaCl depletion the NSF Na level falls only slowly when compared with the plasma Na level. In acute NaCl loading in dogs the plasma Na level exceeds the CSF Na level for as long as 6 hours after loading. It was concluded that while water is freely permeable between the extracellular and spinal fluid spaces a restriction is placed on the outward and inward movement of sodium from and to the CSF. Osmotic pressure parallels sodium level and is predictable from a simple linear equation of osmotic pressure as a function of sodium concentration.

PHOTOSYNTHESIS IN LEAVES AND STEMS DURING EARLY ONTOGENY OF *PINUS RADIATA*

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Distinct juvenile and adult stages are characteristic of the early ontogenetic development of many woody species. In pine, transition from the juvenile to adult stages is marked by more or less pronounced morphological changes in foliar shape and anatomy in addition to marked differences in physiological behavior of foliage. Bormann (1956 and 1958) has pointed out that the morphological and physiological differences between the juvenile and adult foliar forms may have great ecological significance in nature in the establishment of *Pinus taeda* L. seedlings under relatively low light intensities, for young seedlings with needle-like primary leaves are photosynthetically more efficient at low light intensities than older seedlings with mature secondary needles.

Several questions arose from this study. To investigate these questions, $C0_2$ exchange rates were measured for seedlings of *Pinus radiata* Don. ranging in age from two weeks to two years. In this species, cotyledons are the principal photosynthetic organs immediately following germination, with needle-like primary leaves developing after two to four weeks and secondary needles in fascicles appearing in the axils of primary leaves midway through the first growing season. Older primaries begin to brown and die at the end of the first growing season and are dropped usually by the middle of the second growing season.

In addition to these morphological shifts in predominant foliar types, the *Pinus radiata* seedlings showed significant shifts in absolute rates of net photosynthesis and in relative photosynthetic efficiencies at low light intensity with age.

Two to sixteen-week-old seedlings, possessing either primary, or secondary leaves, or a mixture of both, attained maximum rates of net photosynthesis between 2,000 and 3,500 ft-c. Older seedlings, possessing predominantly secondary needles, reached maximum net photosynthesis at the highest intensity utilized (5,000 ft-c).

Absolute rates of net photosynthesis were significantly higher in the younger seedings than in the 1 to 2-year-old seedings; net photosynthesis being almost double at 5,000 ft-c in the 2-16-week-old seedlings.

The shifts in light response and absolute rates of net photsynthesis are attributable to several factors, including: effects of mutual

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shading of needles and decreased response due to changes in leaf anatomy or physiological capacity.

These data could help to explain the early establishment, but later failure, of pine seedlings under unchanging weak light intensities.

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PRELIMINARY STUDIES ON BUD DORMANCY IN SEEDLINGS OF *EUPHORBIA ESULA*

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ABSTRACT

Euphorbia esula L. is difficult to eradicate due to the presence of a massive root system and of propagative buds on the hypocotyl and root. The first bud appears when the hypocotyl is about 4 cm long (7 days old). In most cases 3-5 buds develop on the lower half of the young hypocotyl. The buds on each hypocotyl are usually at different developmental stages. The lowermost one is commonly the most developed, but the uppermost may or may not be the youngest.

These buds remain dormant and do not attain further growth in the seedlings under normal conditions. However, if the seedling is decapitated, there is an immediate effect on the buds. As early as 24 hours after decaptation a few new buds arise and the uppermost one shows a distinct increase in growth. Within a period of 6-8 days the bud develops into a new shoot. The decapitated hypocotyl does not show any further growth and eventually withers.

The newly developed shoot takes over the photosynthetic activity, while in most cases the remaining buds stay dormant. However, in some cases more than one bud grows into a new shoot after decapitation. The immediate response of a dormant bud to the removal of the cotyledons suggests the presence of a factor in the cotyledons which exerts an inhibiting effect on the buds. The removal of one of the two cotyledons lessens the inhibitory effect to the extent that one of the buds shows growth activity but does not

continue to grow into a new shoot. Thus, the buds stay dormant as long as this inhibitory factor is being produced by the cotyledons.

If the seedling is decapitated above the cotyledons two new shoots arise one from the axil of each cotyledon. All the buds continue to remain dormant. This is an additional indirect evidence of the present of an inhibiting factor for bud developing in the cotyledons. The biochemical studies necessary to extract this presumed inhibitor from the cotyledons are in progress in our laboratories.

NET CARBON DIOXIDE EXCHANGE OF THE SUCCULENTS: TWO *KALANCHOË* SPECIES AND THEIR INTERSPECIFIC HYBRID

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INTRODUCTION

Interspecific hybrid derivatives frequently seem intermediate between their parental species in morphological characteristics, suggesting that the hybrid might also be intermediate in physiological factors. Previous studies have indicated, however, conflicting findings on the effects of hybridization and/or increased ploidy level on the rates of photosynthesis and respiration (CO_2 exchange rates) of plant species. Hybridization and polyploidy (auto and allo-) frequently have resulted in reduced rates of photosynthesis or respiration, or both. Other investigators have reported increased or similar rates of CO_2 exchange in certain cases (Andersson 1943, Bordeau and Mergen 1959, Johnsson 1945, Larsen 1943, and others). These conflicting results have indicated the necessity of more research on the interrelationships of hybridization, polyploidy, and CO_2 exchange rates.

A succulent species was chosen for this study because the succulents have the characteristic capacity of fixing large quantities of CO_2 in the dark ("crassulacean acid metabolism") with resultant diurnal fluctuations in organic and amino acid contents (Bonner and Bonner 1948, Gregory, *et al.* 1954, Thomas 1951, Zabka, *et al.* 1959, and others). Kunitake and Saltman (1959) have observed that some of this CO_2 fixed in darkness was preferentially re-utilized directly in photosynthesis in subsequent light periods. Thus the capacity for dark fixation of CO_2 may be of tremendous ecological significance to succulents in arid environments by minimizing the requirement of stomatal opening to allow gas exchange. This is in

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accord with the observations of Loftfield (1921) that, unlike non-succulent species, the stomata of succulents opened at night but remained closed during the day.

This study sought to investigate the capacity for dark CO_2 fixation in the diploid *Kalanchoe daigremontiana* Hamet and Perrier ($2n = 34$), the tetraploid *K. verticillata* Scott-Elliot ($2n = 68$), and their triploid hybrid derivative ($2n = 51$), and the effects of hybridization and changes in ploidy level both on that capacity and on rates of CO_2 exchange. Vegetatively-reproducing stocks of the three taxa of *Kalanchoë* were supplied to the author by the Biology Greenhouse, Brookhaven National Laboratory, Upton, New York.

MATERIALS AND METHODS

Rates of CO_2 exchange for populations of *K. diagremontiana* (2N), *K. verticillata* (4N), and the hybrid derivative (3N), were measured under constant temperature conditions (22±°C) under both 12- and 24-hour light (5,000 ft-c) and dark periods utilizing infra-red gas analysis methods and an open system (Bourdeau and Woodwell 1965, Hadley and Woodwell 1965).

Net CO_2 exchange rates for shoots of intact plants were measured by placing them in photosynthetic-respiratory chambers consisting of polyethylene film bags (3 mils thickness) fitted over and supported by two rings made of copper tubing. Air was supplied through the top ring at a constant rate (3 liters/min in darkness, 11 liters/min in light). This air was allowed to exhaust through the bottom of the bag, which was gathered tightly around the stem of the plant. The exhaust was sampled through the bottom copper ring, and then passed through a drierite column for water removal and the analyzer for CO_2 measurement. A manifold and a series of timed selenoid valves allowed the automatic sequential sampling of each assimilation chamber once every 35 minutes.

Rates of CO_2 exchange were determined for 8-month-old, actively growing plants of the three taxa of *Kalanchoë* grown in the greenhouse under a 10 to 12 hour photoperiod for at least one month prior to CO_2 measurements. All plants were placed in the assimilation chambers (located within a controlled environment growth room) at least 24 hours prior to measurement, and were watered during measurements by continuous subirrigation. Rates of CO_2 exchange were then measured during continuous 24-hour light and dark and 12-hour light and dark periods.

Shoots were harvested following CO_2 measurements for determinations of ovendry weight (80°C for 48 hours), and net CO_2 exchange rates were expressed as mg CO_2 per gram ovendry weight of shoot per hour. All rates are based on four replications, and differences significant at the 5% level will be discussed.

RESULTS

Rates of CO_2 exchange for intact shoots of the three taxa of *Kalanchoë* are presented in Tables I and II and figure 1.

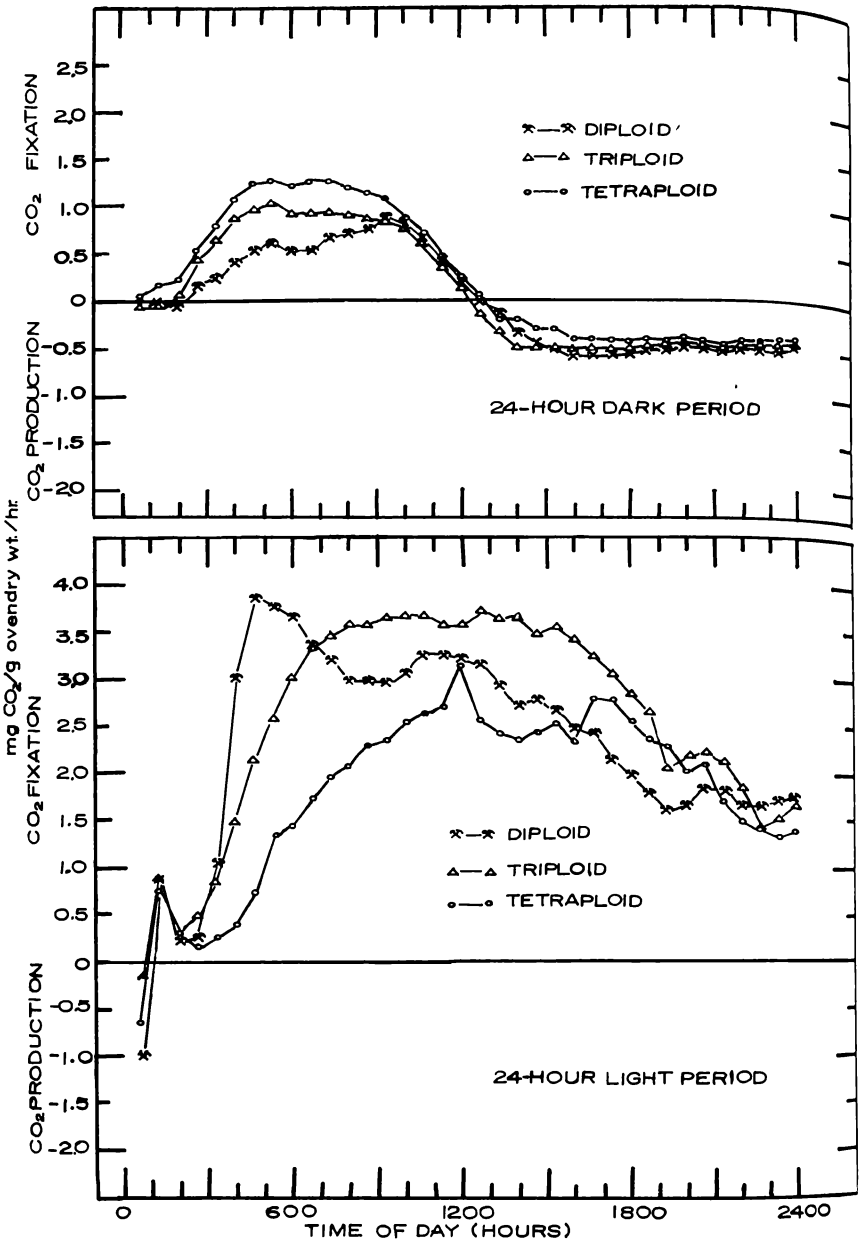

Figure 1—Rates of net CO_2 exchange of three taxa of *Kalanchoë* under constant temperature (22±2°C) and continuous 24-hour dark (top) and light (bottom) periods.

TABLE I

NET CO_2 EXCHANGE OF A DIPLOID AND TETRAPLOID SPECIES OF KALANCHOË AND THEIR TRIPLOID HYBRID DERIVATIVE UNDER 12-HOUR (12 HOUR LIGHT AND DARK PERIODS) AND 24-HOUR (24 HOUR LIGHT AND DARK PERIODS) PHOTOPERIODS AND CONSTANT TEMPERATURE (22±2°C). RATES ARE EXPRESSED AS MG CO_2/G OVENDRY WT. PER HR.

	TAXA					
	2N		3N		4N	
	12 Hr	24 Hr	12 Hr	24 Hr	12 Hr	24 Hr
Hourly average net CO_2 fixation in light*	2.32	——	2.41	——	1.52	——
Maximum net CO_2 fixation in light	4.56	3.93	3.79	3.70	3.20	3.17
Hourly average net CO_2 fixation in darkness	0.46	——	0.62	——	0.82	——
Maximum net CO_2 fixation in darkness	1.00	0.93	1.28	1.03	1.32	1.29
Daily net CO_2 fixation (12-hour photoperiod)	33.4	——	36.4	——	28.1	——

*5,000 ft-c

TABLE II

ANALYSIS VARIANCE "F" VALUES AND DUCAN'S MULTIPLE RANGE TESTS FOR RATES OF CO_2 EXCHANGE OF THE THREE TAXA POPULATIONS UNDER A 12-HOUR PHOTOPERIOD (DATA PRESENTED IN TABLE I)

Source	"f" Value	Table F Value (5% level)	Duncan's Multiple Range[1]		
Maximum rates of net photosynthesis					
Ploidy level	4.9	5.1	2N	3N	4N
2N vs. 3N and 4N	5.8	6.0			
3N vs. 4N	1.4	6.0			
Average hourly net photosynthesis					
Ploidy level	6.8*	5.1	2N	3N	4N
2N vs. 3N and 4N	5.1	6.0			
3N vs. 4N	8.6*	6.0			
Maximum rates of dark fixation					
Ploidy level	8.5*	5.1	2N	3N	4N
2N vs. 3N and 4N	8.1*	6.0			
3N vs. 4N	9.4*	6.0			
Average hourly dark fixation					
Ploidy level	6.3*	5.1	2N	3N	4N
2N vs. 3N and 4N	6.5*	6.0			
3N vs. 4N	6.0*	6.0			

*Significant at 5% level

[1]Underlined ploidy populations are not significantly diffferent from each other.

Light CO_2 Fixation.—Maximum rates of net CO_2 fixation in light (5,000 ft-c) ranged from 3.17 to 4.56 mg CO_2/g/hr in the three taxa and did not differ significantly either between taxa or with length of light period. Although, diploid plants (*K. daigremontiana*) did consistently average the highest (3.93 - 4.56 mg CO_2) and tetraploid plants (*K. verticillata*) the lowest (3.17 - 3.20). These maxima occurred within the first five hours in the diploid but not until 8-10 and 11-12 hours of exposure to light in the triploid and tetraploid plants, respectively.

Average hourly net CO_2 fixation rates were calculated for the plants exposed to 12-hour photoperiod (Table I). These rates did not differ significantly in the diploid and triploid plants, but were significantly lower in the tetraploids.

Net Dark CO_2 Fixation.—The pattern of maximum rates of dark CO_2 fixation within the three taxa was the reverse of that of light fixation, being consistently highest in the tetraploid (1.29 - 1.32 mg CO_2 per hour) and lowest in the diploid (0.93 - 1.00 mg) plants. These differences in rate were minor, but statistically significant. Time interval required to achieve optimal dark CO_2 fixation ranged from 5-7 hours in both the triploids and tetraploids, but 8-10 hours in the diploid plants.

Average hourly net dark CO_2 fixation in those plants subjected to a 12 hour dark period ranged from 0.46 to 0.82 mg CO_2 in the diploids and tetraploids, respectively; the former being significantly lower than the latter.

Net Dark CO_2 Production.—Net dark CO_2 fixation by the plants exposed to a 24-hour dark period essentially ceased in all three taxa after a total of 12-14 hours in darkness (figure 1). Relatively stable rates of net dark CO_2 production (respiration) were noted after 13-15 hours. These did not differ significantly between the three taxa. Although, rates averaged highest in the diploid and lowest in the tetraploid plants.

DISCUSSION

Hybridization and changes in ploidy level appear to affect the important capacity for dark CO_2 fixation, but not the rates of net photosynthesis and dark respiration.

Dark CO_2 fixation increased with increasing ploidy level. Maximum rates of dark CO_2 fixation were significantly higher in the tetraploids than in the diploid species, with the triploid hybrid plants being intermediate. In addition, the time interval in hours required to reach the maximum rates was longest in the diploid, and shortest in the tetraploid. Both of these factors resulted in total net CO_2 fixed in darkness being significantly higher in the tetraploid than in the diploid (9.8 vs. 5.5 mg CO_2/12 hours).

Plants of all three taxa were able to fix large net quantities of CO_2 during the dark period (Table I). Under a 12-hour protoperiod, net CO_2 exchange in darkness in diploid *K. daigremontiana* plants

amounted to a net fixation of 5.5 mg CO_2 per gram (12 hours x 0.46 mg CO_2). An additional 27.8 mg CO were fixed during the subsequent 12-hour light period, for a total net daily gain of 33.4 mg CO_2 per gram (corresponding to approximately 23.4 mg organic matter). Comparable figures for the tetraploid *K. verticillata* averaged 9.8 and 18.2 mg CO_2 fixed in the dark and light periods, for a net daily gain of 28.0 mg CO_2 (19.6 mg organic matter).

Thus 16-35 percent of the total daily CO_2 fixation occurred in the dark period. The potential importance of the capacity for dark fixation to the total metabolic economy of the *Kalanchoë* plants is apparent from these figures.

Under the conditions of this experiment, these plants were never subjected to drought. Under arid conditions, normal CO_2 fixation in light would be curtailed in non-succulents due to closure of the stomata and resultant lack of gas exchange. The large quantities of CO_2 fixed in darkness by the succulents, and preferentially re-utilized in subsequent light periods (Kunitake and Saltman 1959) would facilitate photosynthesis, yet minimize the necessity of stomatal opening and resultant water loss.

Rates of net photosynthesis and dark respiration were not significantly different in the three taxa. However, rates of both of these processes showed a similar pattern being highest in the diploid and lowest in the tetraploid species. These differences were minor, and thus were not significant. However, the pattern of reduced photosynthetic rates with higher ploidy level is in accord with the findings of Larsen (1943) for autotetraploids of *Solanum,* Andersson (1943) for tetraploids of *Hordeum,* and Bordeau and Mergen (1959) for colchicine-induced polyploids of *Pinus elliottii.* On the other hand, Johnsson (1945) reported no significant difference in the photosynthesis of half-sibling diploid and triploid plants of *Populus tremula.*

The lower respiration with higher ploidy levels agrees with that reported by Larsen (1943) and Schwanitz (1950). Schwanitz found that autotetraploids of *Digitalis* and *Brassica* respired at only 78 to 87 percent of the rates for the diploid plants. On the other hand, Bordeau and Mergen (1959) noted no significant difference in the respiratory rates of diploid and colchicine-induced *Pinus elliottii.*

Any changes in photosynthetic and respiratory capacities with hybridization and increased ploidy level have been variously attributed to several causes, including changes in: water content of leaf cells, chlorophyll content of cells, thickness of leaves, or metabolic activity due to effects on the enzymes of the CO_2 exchange processes. Whatever the causes, the resultant effects on metabolic rates are not consistent among the plant species studied to date. Much more research on this question will be necessary before generalizations will be possible.

SUMMARY

Rates of net photosynthesis and dark respiration (following cessation of dark fixation) were measured in diploid, triploid, and tetraploid taxa of *Kalanchoë*, a succulent. Maximum rates of both processes were not affected significantly by interspecific hybridization and changes in ploidy level, however, total net photosynthesis during the entire light period (12-hour photoperiod) was significantly reduced in the tetraploid species. In addition, increases in ploidy level resulted in increased capacity for dark CO_2 fixation ("crassulacean acid metabolism").

Total net carbon dioxide fixation in the diploid species under a 12-hour photoperiod, amounted to 27.8 and 5.5 mg CO_2 per gram for the light and dark periods, respectively, for a total net daily gain in fixed CO_2 of 33.4 mg. Comparable averages for the tetraploid species were 18.2 and 9.8 mg CO_2, respectively, for a net daily gain of 28.0 mg CO_2.

Thus net dark fixation accounted for 16-35 percent of the total net gain in fixed CO_2 per day in the diploid and tetraploid populations, respectively. The ability of these plants to fix large amounts of CO_2 in darkness and re-utilize this fixed CO_2 for photosynthesis during subsequent light periods could be of tremendous importance under arid conditions, for such a mechanism would facilitate photosynthesis and yet minimize the need for day-time gas exchange through the stomata.

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A CHROMATOGRAPHIC STUDY OF THE JUNIPERS OF WESTERN NORTH DAKOTA[1]

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INTRODUCTION

Recent studies have indicated the significance of modern chemotaxonomic methods in complementing morphological, cytological and anatomical data in elucidation of systematic relationships of dubious plant taxa (Alston *et al.* 1962, Alston and Turner 1959, 1962, 1963, Lester *et al.* 1965. Smith and Levin 1963, Stebbins *et al.* 1963, Torres and Levin 1964, Turner and Alston 1959, among others). Such chemotaxonomic techniques as paper chromatography have been of particular use in studies of hybridization, polyploidy and speciation (Smith and Levin 1963, Stebbins *et al.* 1963).

Chemical criteria can be used as reliable taxonomic characters because of the biochemical complementation of species-specific chemical substances in the interspecific hybrid derivatives. The biochemical constituents of these hybrids are therefore a summation of the substances found in the parental species; that is, the species-specific constituents of the parental species must be combined in their hybrid derivatives (Alston and Turner 1962, 1963).

[1]We wish to acknowledge the helpful suggestions and comments of Dr. O. A. Stevens and Mr. D. L. Green during the early phase of this work. Thanks are also due Dr. W. D. Schmid for help with photography.

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The present investigation is a preliminary phase of a larger study of the ecology of the "columnar junipers" of western North Dakota. These columnar junipers have been variously thought to be: merely a variety of *Juniperus scopulorum* Sarg.; an ecological variant of true *J. scopulorum;* a hybrid, between *J. scopulorum* and one of several other juniper species that occur in the area; or a distinct species. Fassett (1945) considered the columnar to be a variety (v. *columnaris* Fassett) which differed from *J. scopulorum* in the ratio of height to width of trees. The height of *J. scopulorum* trees varied from 1-2 times their width, while that of *columnaris* ranged from 2.5-3.0 times width. A paper chromatographic study of the 4 most likely parental species, (*J. scopulorum, J. communis* v. *depressa* Pursh., *J. horizontalis* Moench., and *J. virginiana* v. *crebra* Fern. & Grisc.) and the columnar juniper was initiated to obtain additional evidence bearing on the ancestry of the columnar junipers.

The data discussed in this paper now essentially eliminate the possibility of the columnar junipers having raisen by hybridization between *J. scopulorum* and any of the 3 other juniper species.

MATERIAL AND METHODS

During March and April 1965, paper chromatograms were prepared from leaf tissue of individual plants of the 5 taxa of *Juniperus.* All plants were collected in November, 1964, and March, 1965, from western North Dakota (Slope County, Section 12, T136 N, R 102 W), except for 1 specimen of *J. virginiana* v. *crebra* collected for comparison from western Minnesota (April, 1964).

For each chromatogram, needles were utilized from several positions within the crown of the tree to minimize any differences due to age of needles, crown position or exposure. A total of 2, 3, 4, 1, and 6 plants of *J. communis* v. *depressa, J. horizontalis, J. scopulorum, J. virginiana* v. *crebra,* and the columnar juniper, respectively, were analyzed to assay intraspecific chromatographic variability. Little intraspecific variability was noted, however, in these specimens.

Chromatograms were prepared by crushing the dried leaves (dried at 55-60° C. for 24 hours), and extracting them for 24 hours in the dark in approximately 3 ml of absolute methanol. A total of 7 drops of the extract was applied with a pipette on Whatman No. 2 filter paper (30 x 30 cm), and each drop was allowed to dry before the next application was made. The chromatograms were then run in 2 dimensions by the ascending method. The first dimension solvent system was utilized for 12 hours, and consisted of n-butanol: glacial acetic acid: distilled water (8:2:3, v/v). Chromatograms were run for 9-10 hours in the second dimension utilizing n-butanol: glacial acetic acid: distilled water (3:4:3, v/v). Both solvent systems resulted in excellent separation of the individual biochemical constituents.

The chromatograms were analyzed after viewing in ultraviolet light (long wave) and visible light. Spots were grouped both by color and by R_f values in each dimension. Only those spots which occurred for all plants of each taxon were utilized in these studies, and detected substances occurring in a lower frequency were ignored. No attempt was made to identify the detected chemical substances. Previous studies by Smith and Levin (1963) and Torres and Levin (1964) using diazotized sulfanilic acid have established that the majority of the observed spots would be phenolic or polyphenolic compounds. The repeatability of chromatographic patterns and the presence or absence of species-specific biochemical substances, not the identity of these compounds, were important for this study.

RESULTS

Analyses of the chromatograms of the 5 taxa of *Juniperus* are tabulated in Table I. The representative chromatographic pattern of each taxon is diagrammed in figure 1.

The data show that the chromatographic patterns of *Juniperus scopulorum* and the columnar junipers were identical, and did not differ even in the presence or absence of a single detectable constituent (Table 1). Chromatograms of each of the other 3 species studied deviated from this "*J. scopulorum*-columnar" pattern, with each of the 3 containing 2 or 3 substances absent in both of the former taxa.

The ranges of R_f values for the 15 detected biochemical constituents on the 6 replicate specimens of columnar juniper are presented in figure 1E. These R_f values show little intraspecific chromatographic variability. Although only a limited number of chromatograms were run for each of the other 4 taxa, a similar low extent of variability was apparent for each taxon, with the highest variability in the chromatograms of J. communis v. depressa.

DISCUSSION

A large body of accumulated scientific evidence from a wide range of plant taxa indicates that the chemical constituents of interspecific hybrid derivatives are a summation of those found in the parental species. This investigation of the 5 taxa of *Juniperus* seems to eliminate the possibility of the columnar juniper having resulted from the hybridization of *J. communis* v. *depressa, J. horizontalis,* or *J. virginiana* v. *crebra* with *J. scopulorum,* for each of the former 3 taxa possesses species-specific biochemical substances not found in the proposed interspecific hybrid derivative. The isolation of the geographical range of the columnar junipers from those of any *Juniperus* species, other than the above three, would greatly diminish the possibility of another species being involved in a hybridization with *J. scopulorum.*

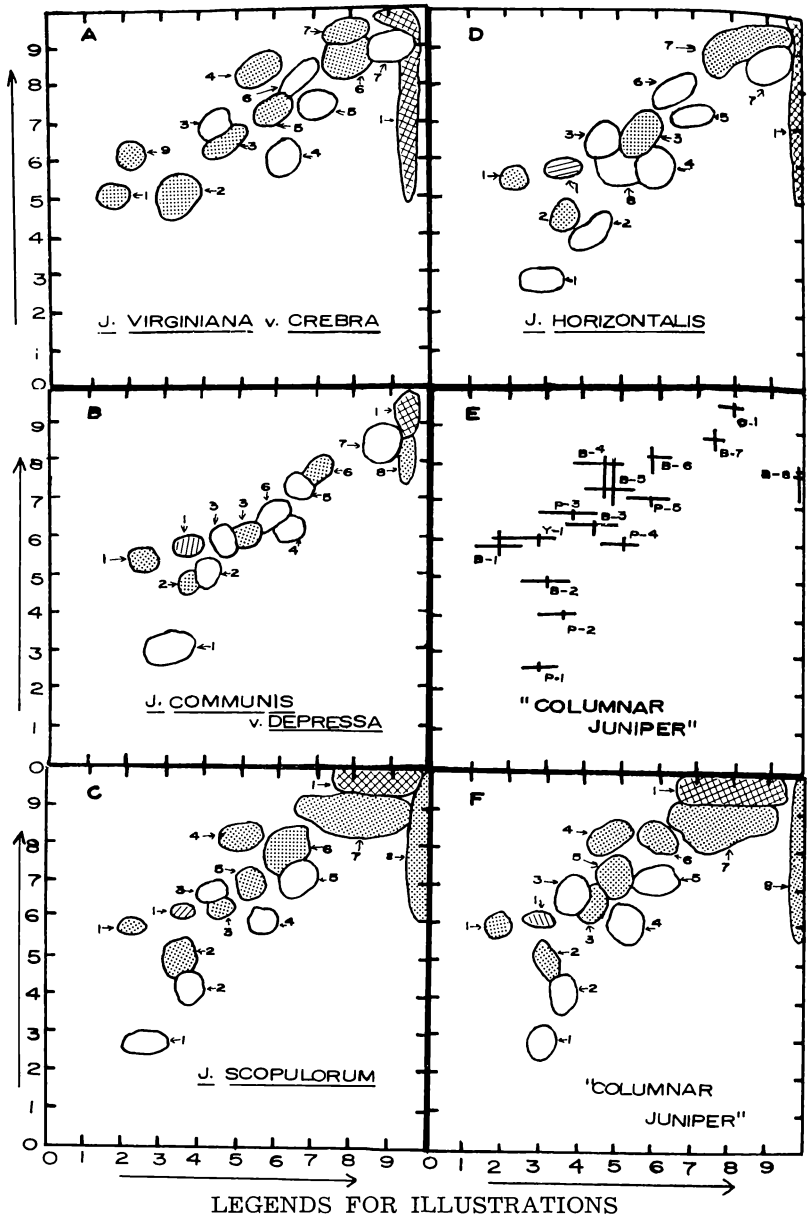
Chromatographic determinations on specimens along a transect from a grove of "typical" *J. scopulorum* to one of "typical" colum-

TABLE I

DETECTED BIOCHEMICAL CONSTITUENTS ON THE CHROMATOGRAMS OF FOUR TAXA OF JUNIPERS FROM WESTERN NORTH DAKOTA

Taxon	Detected chromatographic spots*																	
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	Y_1	O_1
J. communis v. depressa	+	+	+	+	+	+	+	—	+	+	+	—	—	+	—	+	—	+	+
J. horizontalis	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—	+	+
J. scopulorum	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	+	—	+	+
"Columnar juniper"	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	+	—	+	+
J. virginiana v. crebra	—	—	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—	+

*Spots agree with those in Fig. 1.
The symbols "P", "B", "O", and "Y" are used for purple, blue, orange, and yellow; the colors of these spots under U-V light.



LEGENDS FOR ILLUSTRATIONS

FIGURE 1. Diagrammatic representation of the characteristic chromatographic patterns for the 5 taxa of *Juniperus*. The degree of intraspecific chromatographic variability in the range of R_f values of the columnars is shown in Figure E. Colors under ultra-violet are as follows stippled = blue, outlined = purple, cross-hatched = orange, lined = yellow.

nars involving "intermediate" forms showed no differences between any of these specimens (a portion of a similar transect is shown in figure 2). The similarity of the chromatographic patterns of *J. scopulorum* and the other columnar juniper could result either from the columnar being a varietal or an ecological-induced polymorphoric type of the former. On the basis of this chromatographic data, we are unable to distinguish between these two possibilities.

O. A. Stevens (personal communication) has informed us that only 1 of approximately 40 columnar trees transplanted to the campus at Fargo has come true to type. This would seem to support the hypothesis of ecological polymorphism.

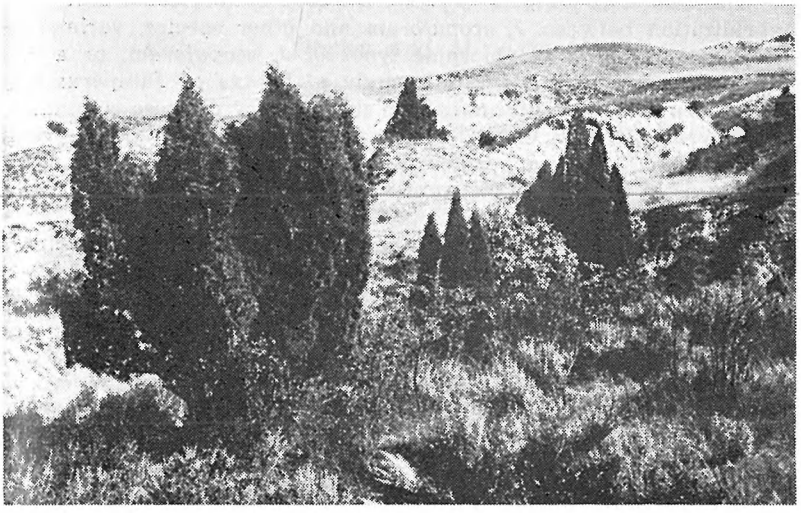


FIGURE 2. A view along a potion of a transect from "typical" *J. scopulorum* to "typical" columnar trees. (Slope County, Section 12, T 136N, R 102 W). —Photo by R. W. Seabloom.

Three groves of the columnar juniper are known to occur in North Dakota. These are in Slope County (Sections 12 & 13, T 136 N, R 102 W and Section 18, T 136 N, R 101 W) and Billings County (Section 21 or 22, T 140 N, R 101 W) (D. L. Green, Deputy State Forester, Bottineau). All three stands appear to be located in close proximity to burning coal veins, suggesting that possibly these burning coal seams either are a cause of the ecological polymorphism or influence the distribution of the columnar junipers.

A few junipers of unknown species and having a columnar appearance have been noted near Sargent, Nebraska (D. L. Green, personal communication), near Williston, North Dakota (E. Nielsen, cited in Fassett), and in central Montana (O. A. Stevens, personal

communication). These would not be associated with burning coal seams. These sites and specimens from them are currently being examined.

Further chromatographic studies as well as detailed cytological and ecological studies of *J. scopulorum* and the columnar juniper will be required to establish the exact nature of the columnar. Such studies are currently in progress. The present chromatographic data for the five taxa of *Juniperus* do appear, however, to severely diminish the possibilities of the columnar juniper having resulted from hybridization.

SUMMARY

The columnar junipers of western North Dakota could represent: hybridization between *J. scopulorum* and other species, varietal or ecologically-induced polymorphic types of *J. scopulorum*, or a distinct species. A chromatographic study of 5 taxa of *Juniperus* was undertaken to obtain information on the ancestry of these columnars. This revealed that 3 of the 4 species possess species-specific substances which are absent in the columnar juniper, while the chromatograms of *J. scopulorum* were identical with those of the columnar.

These data severely diminish the possibility of columnar junipers having resulted from hybridization. Further studies are in progress to further clarify the nature of and causes for these columnar junipers.

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INFLUENCE OF THE INTERACTION OF INDOLE-3-ACETIC ACID AND P-CHLOROPHENOXYISOBUTYRIC ACID ON THE ABSCISSION OF DEBLADED PETIOLES OF *PHASEOLUS VULGARIS* L.

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ABSTRACT

The influence of indole-3-acetic acid (IAA) and p-chlorophenoxyisobutyric acid (PCIB), alone and in combination, on the rate of abscission and obscission layer formation of debladed petioles of *Phaseolus vulgaris* L. was investigated. When one of a pair of opposite debladed petioles was treated at its distal tip with 1% IAA, it did not abscise during a 10-day test period. All control petioles abscised within this time. Application of PCIB with IAA failed to counteract the inhibitory effect of IAA on abscission. In fact, application of PCIB alone retarded abscission, but to a lesser extent than IAA. Anatomical observations were made in order to detect the first signs of abscission layer formation. On the seventh day, a definite abscission layer could be observed in petioles treated with PCIB or lanolin paste. Abscission layer formation was not observed when 1% IAA, or combinations of 1% IAA and PCIB were used as treatments.

The abscission of *untreated* petioles opposite petioles treated with 1% IAA was accelerated. This acceleration was counteracted by applying PCIB with IAA to the opposite petiole. Application of PCIB alone, however, to the distal tip of a debladed petiole had little effect on the rate of abscission of its opposite *untreated* petiole. Although PCIB counteracted the influence of IAA on the abscission of *untreated* petioles, it did not counteract the acceleration of abscission layer formation by IAA in these petioles. Abscission layers were observed in *untreated* petioles three days after treatment of the opposite petiole with 1% IAA or with combinations of 1% IAA and PCIB. This contrasted with the seven days required for abscission layer formation in the lanolin paste control and when PCIB

was used alone. It appears that PCIB, whether used alone or in combination with IAA, has no effect on the rate of abscission layer formation. This is most strikingly illustrated in the lack of correlation between abscission and abscission layer formation in *untreated* petioles opposite petioles treated with combinations of 1% IAA and PCIB. Although PCIB, under the above circumstances, counteracted the acceleration of abscission by 1% IAA, it had no influence on the accelerating effect of 1% IAA on abscission layer formation.

Additional evidence supporting the observation that PCIB has no effect on abscission layer formation was obtained from a variation of the above methods. Instead of both leaves being debladed, one was left in tact. The debladed petiole was treated with IAA and PCIB. The effect of PCIB on abscission layer formation was identical to that of the control.

It is suggested by the authors that IAA may exert its influence on abscission through several different sites of activity. At one site, PCIB may counteract the effects of IAA, while at another site have no effect. In this study, PCIB appears to counteract the effect of IAA in the actual abscission of petioles but is completely neutral as far as abscission layer formation is concerned.

ABSCISSION OF *PSORALEA ARGOPHYLLA* PURSH

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ABSTRACT

Abscission in *Psoralea argophylla* is similar to the abscission of leaves, flowers, fruits, and stems of other angiosperms. In late abscission stages foliage changes of the plant are accompanied by changes in the pectic materials. These pectic changes involve a conversion of the hard pectic material in the compound middle lamella into pectic acid and highly esterfied pectin which is water soluble. Cell division occurs immediately preceding abscission throughout much of the stem. This production of thin, soft cross walls together with the previously existing reduction of supporting tissue in the vascular region produces a mechanically weak area as well. However, mechanical breakage occurs in only a negligible amount of tissue, and separation of the cells across most of the abscission zone proceeds through the chemical dissolution of the middle lamella.

In several respects abscission in *P. argophylla* is interesting. First, the entire plant body is separated which enables *P. argophylla* to act as a tumble-weed. Secondly, the separation often proceeds through more than one chemically active abscission layer. Thirdly, extreme

morphological modifications of tissue in the abscission zone are present to reduce the amount of mechanical breakage and thereby indirectly promote separation through the middle lamella. Finally, the protective layer is only indirectly associated with abcission since it does not form a part of the abscission zone as is typical in angiosperms, but rather is formed some distance underground at the base of the shoot.

APICAL ORGANIZATION IN THE ROOTS OF *EUPHORBIA ESULA*

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ABSTRACT

Changes in the pattern of organization of the root apex are common in primary roots of different developmental stages in *Euphorbia esula* L. The boundary separating various histogens at the root apex exists in the same root at different ages and fluctuates in position with time during normal growth.

The primary roots of a certain developmental stage show a common organizational pattern in which the uppermost single tier of initials give rise to the central cylinder. A distinctly separate single layer of initials located below the central cylinder gives rise to the cortex. The cap and epidermis have their own common initials directly adjacent to the cortical initials. A central columella and a lateral region can be recognized in the root cap. Absence of longitudinal divisions is conspicuous in the columella and the cells are arranged in longitudinal files. Histological differentiation of the epidermis begins with an oblique division of the cell outside the columella initials and below the immature cortical cells. A file of immature epidermal cells continue to the outer limit of the columella. This same oblique division also gives rise to a cell which further undergoes oblique and periclinal divisions and adds cells to the inner portion of the lateral cap. In the developmental stage of the root at which the histogens show clear boundaries no mitotic figures are observed in the initials, and the divisions are very infrequent in the immediate derivatives of these initials.

ANOMALOUS CYTOLOGICAL BEHAVIORS DURING MICROSPOROGENESIS IN SELF-STERILE PLANTS OF *BROMUS INERMIS* LEYSS

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INTRODUCTION

Bromus inermis is an octoploid forage grass with a genomic number of seven. It has been recognized that a high degree of structural irregularities of chromosomes exist in bromegrass.

Besides these conveniently recognized structural irregularities, which include lack of homology and the presence of inversion and translocation, anomalous cytological behaviors occur, particularly in plants of low fertility levels. Nielsen in a study of small plants of brome and first generation *Bromus* hybrids reported the occurrence of pycnosis, stickiness, coenocyte formation and the appearance of globular structures, etc., during microsporogenesis (5, 6). Nath (4) observed these anomalous behaviors in the self-sterile plants of *Phleum pratense*. L. Walters (7, 8) has reported extreme fragmentation of chromosomes in the products of inter-specific hybridization of *Bromus*.

Th purpose of his investigation was to determine the nature and frequencies of anomalous cytological behaviors during microsporogenesis of relatively self-sterile smooth bromegrass plants. These plants were obtained from several generations of inbred and open-pollinated progenies.

An extensive report on the 'self-sterile' and 'relatively self-fertile' plants is under preparation, which will be submitted to the journal of Crop Science.

MATERIALS AND METHODS

Fifty plants from various inbred and open-pollinated progenies were included in the study. The family lineage are shown in Table I. These plants were characterized by low self-seed set (1.5% or

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This work was done under the supervision of Dr. E. L. Nielsen, Professor of Agronomy, University of Wisconsin and geneticist ARS, U.S.D.A. grass breeding project.

An extensive report on the 'self-sterile' and 'relatively self-fertile' plants is under preparation, which will be submitted to the journal of Crop Science.

lower) and a variable, but usually low, cross seed set (Table II).

The cytological data are based on panicles fixed at three developmental stages. Carnoy's solution was employed for fixation. This was followed by storage in 70% alcohol at 42° F. Meiotic studies of microsporocytes were made on anthers smeared in acetocarmine. The use of ferric-acetate and heating over a steambath helped the staining reactions appreciably.

The data compiled at different meiotic stages were based usually upon a minimum of 50 sporocytes, sporad pairs, second-division cell pairs or quartets. Estimates of pollen stainability were obtained either by the reaction to 2% IKI in lactophenol or to acetocarmine.

The estimate of cross-seed set was obtained from three panicles selected randomly from each clone. Spikelets from top, middle and base areas of each panicle, were used to determine the number of florets and caryopses. Self-pollination was enforced by enclosing 5 panicles of approximately equal size in parchment selfing sleeves. Three such bags were applied to each clone, wherever possible. The selfed seed set was based on a total of 10 panicles from 2 bags. It was assumed that the average number of florets per panicle was the same in selfed- and open-pollinated panicles.

RESULTS

A total of 50 plants were analyzed for structural disturbances and for anomalous behaviors during microsporogenesis. Only the anomalous disturbances at various stages of meiosis are enumerated in some detail in this report.

Phophase 2. In general it is difficult to analyze the chromosomal associations, and chiasma frequency in bromegrass because of the tendency of the chromosomes to form a tangled mass at late prophase. The plants analyzed for chromosomal associations exhibited a preponderance of bivalent formations with occasional uni- or quadrivalents.

Several forms of anomalous behaviors were observed at this stage.

Prophase pycnosis: This is regarded as the occurrence or accumulation of pycnotic material in the developing nuclei. The sporocytes were classified as either pycnotic or non-pycnotic. A range of 8 to over 60 percent pycnotic nuclei was encountered. The sporocytes shown represent moderate to heavy pycnosis, with a greatly increased accumulation area and the obliteration of visible chromatic strands. The result is a chromosomal mass that is completely clumped (figures 1 and 2).

Coenocyte formation: Coenocytes that were bi- and multinucleate occurred with low frequency. The nuclei within the coenocytes varied in shape and size. They appeared to arise due to premeiotic failure of cytokinesis or to complement fractionation. (figures 3, 4, 5). A maximum of eight nuclei were observed within a cell.

Frequently, the coenocytes had a heavy-wall precocious exine or exine-like deposition.

Extreme nuclear fragmentation: Extreme nuclear fragmentation was observed in the sporocytes of plants 824-18 and 829-8. Figures 6 and 7 reveal progressive fragmentation of the chromosomes.

Uncoiled chromosomes: Occasionally the chromosome groups within the same cell exhibited non-synchronization in degrees of chromosome coiling (figures 3, 4). In these some chromosomes are well spiralized whereas others remain attenuated.

Metaphase - 1. Only a few plants rendered themselves suitable for analysis for chromosome association and chiasma frequency. This difficulty was due to the occurrence of dense-configurations and interchromosomal adnations. Unoriented univalents could be identified rather easily in most nuclei.

Stickiness: The sticky nuclei were characterized by dense configurations and inter-chromosomal adnations (figure 8). The range of stickiness in an array of sporocytes varied from 13 to over 60 percent.

Bi-nucleate cells: A number of binucleate cells exhibited extreme non-synchronization of development. Often the chromosomes of one nucleus were oriented at metaphase while those of the other were still at early prophase (figures 10, 11). Occasionally, chromosomes that appeared to fragment or to lag as stretched bivalents were associated with the binucleate cells.

Anaphase-telophase 1: The commonly encountered abnormalities at these stages were lagging univalents, dicentric bridges and acentric fragments. Chromosome stickiness was observed with a more reduced frequency than in earlier stages.

Multi-polarity: Tripolar and quadripolar spindles formed with rarity. These might arise due to the occurrence of binucleate cells in addition to a misdivision of the centriole.

Dyad: The most common dyad irregularity was the formation of micronuclei from the lagging chromosomes. Occasionally, however, aberrant dyad cells were observed that had sticky interchromosomal adnations, stretched chromosomes, extreme-fragmentation and globular bodies (figures 14, 15).

Anaphase-telophase 2. These stages were encountered less frequently than the first division sporocytes. Common irregularities included lagging chromosomes, bridges, bridge frements, and nonsynchrony of the developmental stages of the sporad pairs. At times bridges occurred in both sporad pairs which was assumed to have arisen from a crossover in the interstitial region and two crossovers within the loop of the inversion heterozygote. Stickiness appeared to be much reduced at these stages.

Small sporocytes: Very small sporocytes were observed in a few plants. Usually these were associated with normal sporads or cells that appeared to contain more than a normal number of chro-

mosomes (figures 16, 17). They might arise due to abnormal chromosomal disjunction or complement fractionation of chromosomes into groups at, or approximately at, the genomic number.

Quartets: Micronuclei occurred with frequencies that varied from 1.116 to 2.28 per quartet per plant. Sometimes large micronuclei were seen which presumably arose from lagging univalents or due to the fusion of half-univalents.

Sometimes, very heavily stained rather large non-descript pycnotic bodies were observed (figure 21). Occasionally, aberrant quartets exhibited extreme-chromosomal adnation and irregular chromosome distribution (figures 22, 23).

Pollen stainability: Stainability of pollen-grains of all the plants was based on the reaction to IKI. Occasionally acetocarmine was used in addition to IKI. The percentages of pollen stainability ranged from 1.8% to 96.0%.

Three rather distinct pollen grain sizes occurred in small plants. The tiny sporocytes gave rise to very small pollen grains that were always non-stainable. The middle class often had a chromosome number reduced to half ($n = 14$) the larger grains had $n = 28$ chromosomes. The pollen grains represented in figures 24, 25, 26 are all at the same magnification.

DISCUSSION

It has been suggested that sterility in high polyploid *Bromus* and *Phleum* species could be attributed to direct genic causes, to structural disturbances of chromosomes, and to anomalous aberrations (3, 4, 5, 6). The present investigation emphasizes the occurrence of anomalies during microsporogenesis in the highly self-sterile plants.

Pycnosis occurred regularly in the selfed progeny of *Phleum pratense* (4). Nielsen (5, 6) observed a high percentage of pycnosis and stickiness at earlier stages of meiosis. This expression was highly correlated with low pollen stainability. He suggested that a high proportion of these pycnotic nuclei appeared to be eliminated at earlier stages, which is in accord with the present finding. Nielsen, attributed this behavior to an interaction between nucleus and cytoplasm.

Coenocytes have been reported to occur in *Phleum* and *Bromus* among other plants. Usually they have been assumed to result from failure of pre-meiotic cytokinesis. In the present material, however, coenocytes appeared to arise, at least in part, from complement fractionation of the prophase nucleus. A thick transparent precocious exine or exine-like deposition was often observed surrounding the coenocyte.

Extreme nuclear fragmentation has been reported to occur in the interspecific hybrids of *Bromus* (Walters, 7). Walters suggested that this behavior was caused by an excess, shortage, or both of certain elements during cell metabolism. In the material examined

TABLE 1
LINEAGE OF FAMILIES EXAMINED CYTOLOGICALLY*

OP-1	OP-2	OP-3	S-1 OP-1	S-2
585	1180-2	822-5	913-11	910-5
615	1188-10	-7	-13	-14
348	1296-26	-12	917-1	912-1
352	1297-7	-15	-8	-3
346		824-4	919-1	-12
366		-7	-2	-16
		-12	-15	-29
		-14		916-1
		829-5		-3
		-8		-11
		-15		-17
		830-2		-39
		-7		918-2
		-9		-4
		-20		-12
				-28
				-33
				-34

*Abbreviations Used:
OP—Open pollinated generation
S—Self pollinated generation

fragmentation was observed in plants very low in fertility and was restricted primarily to earlier stages of meiosis. No definite opinion was arrived at concerning the cause of this behavior.

Multiple spindles in the form of tri- and tetra-polar structures were observed. In view of the bi- and multi-nucleate cells in the present material, it is assumed that at least some of the tetra-polar cells result from binucleate cells.

Complement fractionation, irregular chromosomal disjunction and multi-spindle operation resulted in a few very small sporocytes. Such sporocytes subsequently gave rise to very small non-stainable pollen grains (figure 4).

The basic cause for and the function of such anomalous structures remains obscure. It is conjectured however, that these behaviors are related to some form of physiological disturbance in cell metabolism.

SUMMARY

Fifty plants of *Bromus inermis* from inbred- and open-pollinated progenies were analyzed for cytological behaviors during microsporogenesis. These plants were characterized by a very low self- and variable but usually low cross-seed set. Anomalous behaviors observed at different stages of meiosis includes pycnosis, coenocyte

TABLE II

AVERAGE FREQUENCIES OF MEIOTIC IRREGULARITIES, PERCENTAGE OF STAINABLE POLLEN, PERCENTAGE OF SELF- AND CROSS-SEED SET, IN THE FAMILIES OF RELATIVELY SELF-STERILE *BROMUS INERMIS* PLANTS

Families	Prophase pycnosis	Metaphase stickiness	Pollen stainability	Seed Set Self	Seed Set Cross
585	29	32	78.6	0.38	19.6
615	21	19	84.3	0.89	33.0
348	14	17	90.1	1.00	74.0
346	6	10	92.4	1.36	70.0
366	4	7	90.3	0.37	65.0
1180	20	18	82.2	0.16	30.0
1188	34	38	83.5	0.10	48.0
1296	31	35	91.3	0.23	47.8
1297	19	18	79.4	0.16	50.0
822	41.7	42.2	71.8	0.01	28.2
824	46.5	43.8	52.3	0.24	24.8
829	31.0	30.0	56.4	0.00	16.2
830	37.2	7.2	70.6	0.35	57.8
913	12.0	14.5	93.9	0.34	56.2
917	34.0	30.0	34.5	0.09	51.4
919	40.0	41.0	82.6	0.48	27.6
910	37.5	24.0	45.2	0.00	7.3
912	31.3	32.0	53.5	0.14	35.6
916	30.2	38.6	75.8	0.46	37.0
918	27.5	31.7	74.3	0.27	47.2

formation, chromosomal-fragmentation, multi-spindle formation, occurrence of uncoiled and non-condensed chromosomes, precocious exine or exine-like deposition, complement fractionation and the production of very small sporocytes. Only prophase pycnosis and metaphase stickiness were encountered in measurable frequencies, which were related to lack of pollen stainability.

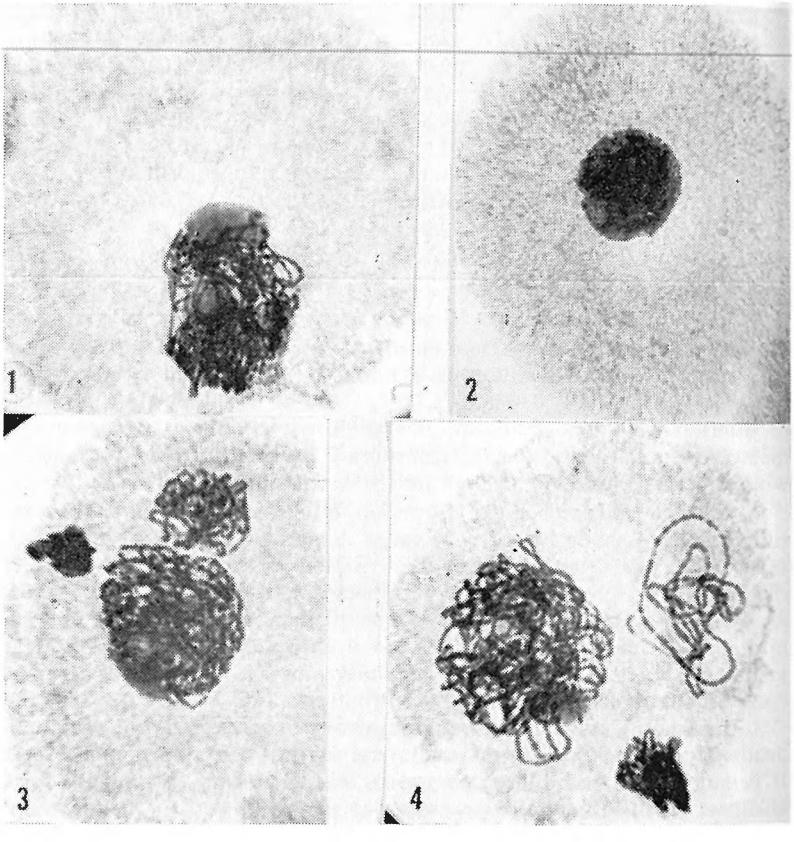
Irregular chromosome disjunction due to several anomalies resulted in the production of very large dispersion of pollen grain sizes (120μ to 82.6μ). Furthermore, these pollen grains appeared to form roughly three groups. Those with diameters of about 12.0μ were always non-stainable. The second group, varying from 28.0μ to 35.0μ in diameter, had several instances of a chromosome number reduced to half (n = 14). The normal pollen grains (n = 28) had a modal class of about 52.0.

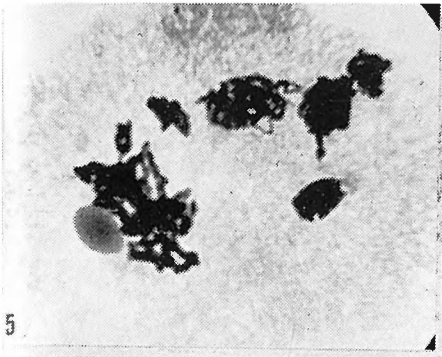
The anomalous irregularities appear to arise and behave independently of the recognized structural errors in chromosomes (3, 6). It is suggested that these anomalous behaviors are associated with an upset in the metabolic processes of the cell.

FIGURES 1 - 26

CYTOLOGICAL ANOMALIES DURING MICROSPOROGENESIS OF SELF-STERILE PLANTS OF *BROMUS INERMIS*

Figures 1, 2—Moderate to intense pycnosis with several accumulation areas. Figures 3, 4, 5—Coenocytes with dense fractionated nuclei that exhibit differential condensation and spiralization. Figures 6, 7—Moderate to extreme fragmentation of the nucleus. Figure 8—Intensely sticky M-1 with 2 unoriented bivalents. Figure 9—Double M-1 plates. Figures 10, 11—Binucleate nonsynchronous cells with disorganized chromosomes. Figure 12—Anaphase I with sticky adnations. Figure 13—A binucleate cell with a group of unorganized chromosomes. Figures 14, 15—Aberrant dyads with pycnotic nuclei. Figure 16—Anaphase II with a tiny cell. Figures 17, 22, 23—Irregular late second division configuration with intense pycnosis. Figures—18, 19, 20—Quartets with hexad and pentad formation. Figures—24, 25, 26—Pollen grains of different sizes.

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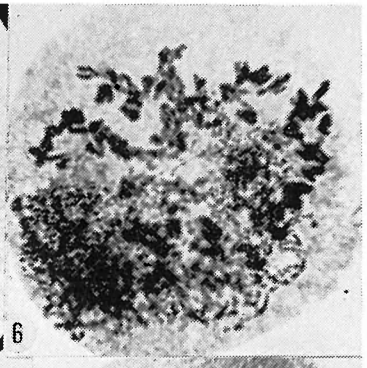
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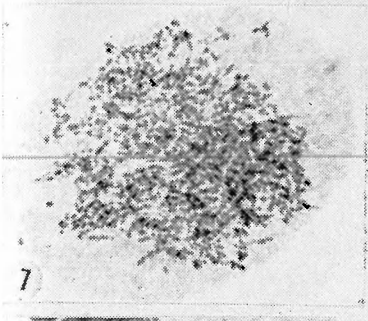
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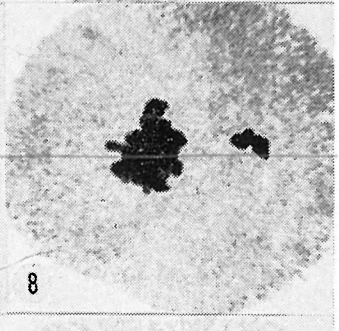
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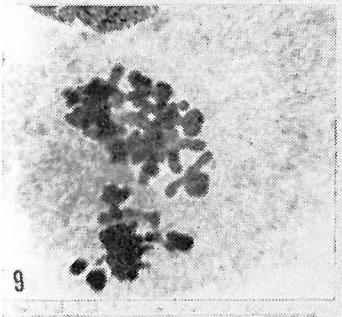
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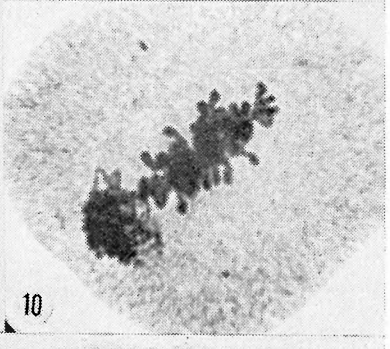
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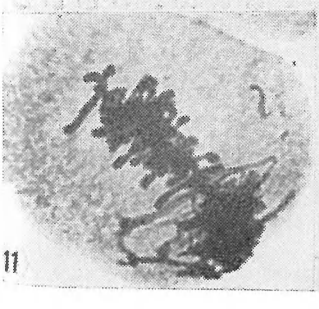
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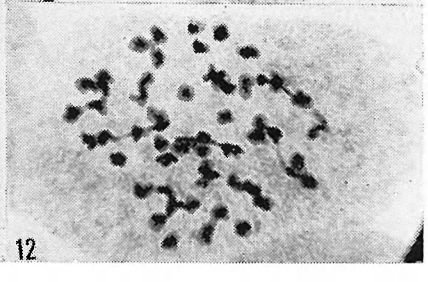
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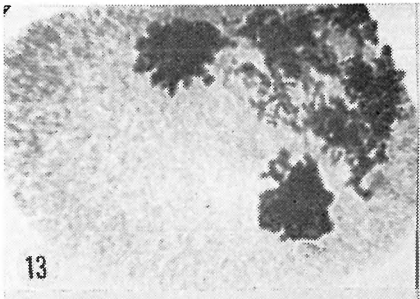

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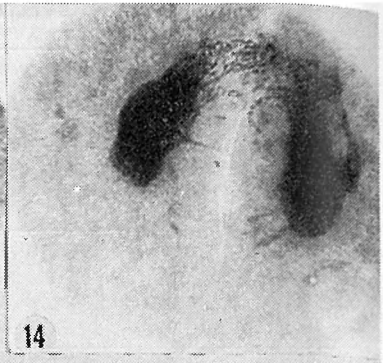

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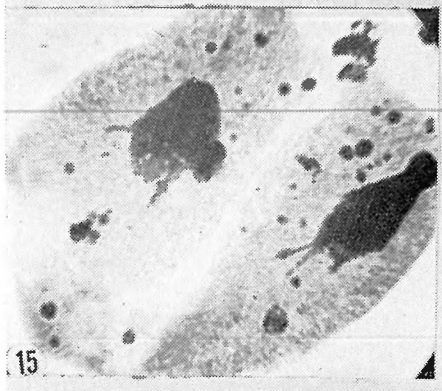

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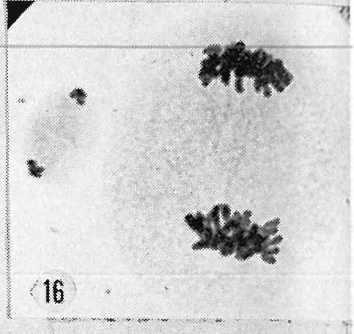

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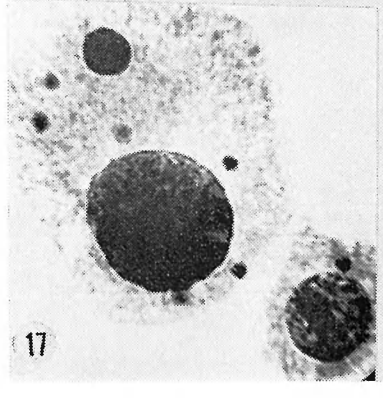

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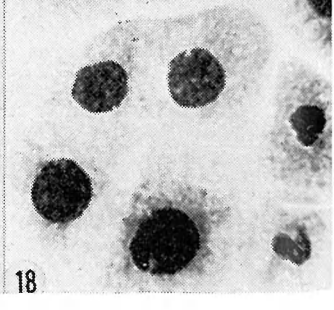

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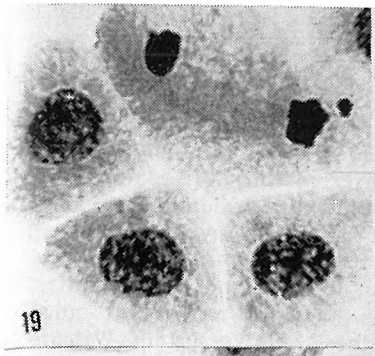
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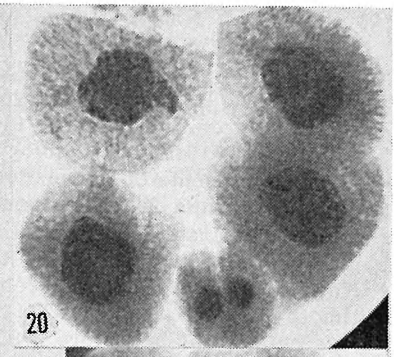
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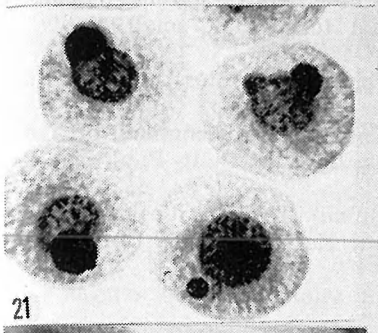
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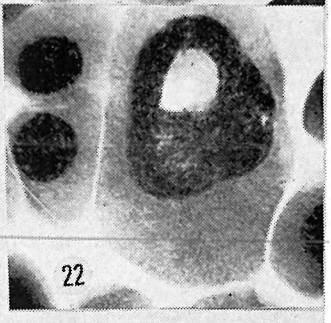
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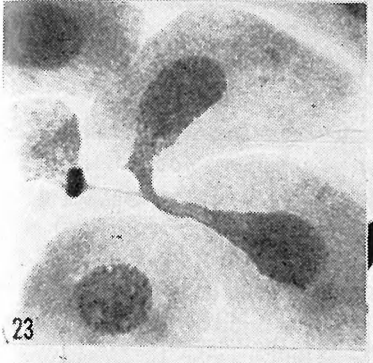
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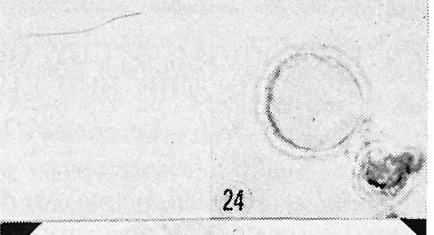
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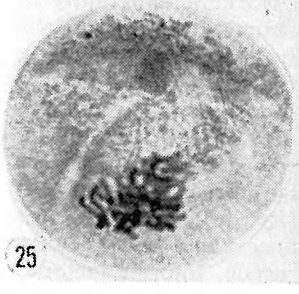
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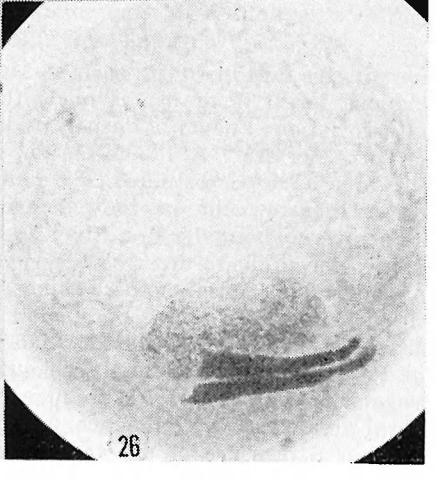
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AN ECOLOGICAL STUDY OF ASPEN COMMUNITIES IN THE RED RIVER VALLEY

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ABSTRACT

In the northwestern part of Minnesota the transition from deciduous forest to prairie is made through an intermediate complex of associations known as "aspen parkland", the "aspen grove region" or "brush prairie". Although the *Populous tremuloides* communities of this area have been studied by a number of workers, apparently they have failed to recognize the existence of a number of isolated aspen stands throughout the Red River Valley. The purposes of the present study were: (1) to describe the vegetation, distribution, growth rate of aspen, and habitat of these stands, and (2) to attempt to interrelate these factors by the use of biometric methods. The study area consisted of Grand Forks County, North Dakota and the western half of Polk County, Minnesota. The quantitative characters used in the vegetation analyses were frequency, density, relaive density, total basal area, relative dominance, and importance value. Site index was used as a measure of the growth rate of aspen. Habitat factors in-

cluded in the study were soil moisture, water retaining capacity, soil texture, and soil salinity. The results of this study indicate that the aspen stands are limited to areas which have a high water table. Soil texture and water retaining capacity varied greatly from stand to stand and therefore were not considered to be important limiting factors.

UPTAKE OF FUMIGANT GASES BY CEREALS AND CEREAL PRODUCTS

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ABSTRACT

Analyses of gas samples taken from elevator annex bins of wheat fumigated with ethylene dibromide, ethylene dichloride and carbon tetrachloride (EDB, EDC and CT) showed kinetic differences in the rates and amounts of fumigant migration. Temperature and moisture content of the wheat were major influencing factors. Field and laboratory experiments supported the hypothesis (Berck, 1956) that wheat could behave as a chromatographic column towards fumigant gases. The location of the peak (analogous to retention time in gas chromatography) varied for each of 5 fumigants (EDB, EDC, CT, acrylonitrile, and chloropicrin) applied in the vapor phase. Experiments with binary mixtures were further explored with EDB-EDC-CT mixtures applied in the vapor phase in micro fumigation chambers to 51 cereals and cereal products. The sorption equilibria patterns showed that each substrate had a different affinity for the gases. Even moderate decrease in particle size by coarse grinding markedly increased the uptake of the 3 gases. The resultant exposure of reactive endosperm appeared to have a greater influence on sorption than multiplication of surface area as such. Wheat gluten sorbed considerably more of the 3 gases than did wheat starch.

NEW LIVER AND KIDNEY MICROSOMAL NUCLEOTIDE-GLUCOSE PHOSPHOTRANSFERASE ACTIVITIES[1]

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ABSTRACT

Deoxycholate-treated rat liver and kidney microsomes contain previously undescribed enzymic activities catalyzing Reactions 1 and 2.

Nucleoside 5'-triphosphate + glucose → glucose-6-P + nucleoside 5'-diphosphate (1)

Nucleoside 5'-diphosphate + glucose → glucose-6-P + nucleoside 5'-monophosphate (2)

CTP, GTP, ATP, ADP, and ITP, but not CMP, function as phosphoryl group donors. These activities differ from previously characterized hexokinases in their *a*) sub-cellar location, *b*) marked activation by deoxycholate, *c*) acidic pH optima, *d*) presence in kidney, *e*) inhibition (and not stimulation) by Mg^{++}, f) extremely broad range of specificity both for phosphoryl donor and acceptor, *g*) elevation in diabetes and depression by insulin administration, and *h*) close association with potent phosphohydrolase activities. Results of differential thermal inactivation, combined substrate studies, and kinetic analyses suggest the identity of these activities with microsomal inorganic pyrophosphate (PP_i)-glucose phosphotransferase (Reaction 3), which we (1) recently demonstrated to be catalyzed by classical liver glucose 6-phosphatase.

$$PP_i + \text{glucose} \rightarrow \text{glucose-6-P} + P_i \quad (3)$$

These phosphotransferases are, potentially, the most active glucose phosphorylating enzymes yet reported in rat liver and kidney. At their pH optima, phosphotransferases catalyze the formation of as much as 20 μmoles of glucose-6-P per minute per gram wet liver. Stimulation of enzyme formation by *in vivo* treatments producing hyperglycemia and acidosis, as well as the further favoring of activity by elevated blood sugar levels, suggest that these enzymic activities may constitute a physiologically significant compensatory mechanism for glucose phosphorylation in, for example, the diabetic state.

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[1]Supported in part by a grant from the Hill Family Foundation and by Research Grant AM 07141 from the National Institutes of Health, United States Public Health Service.

[2]National Defense Graduate Fellow.

AN INVESTIGATION OF SOME FACTORS CONTROLLING THE TIME OF ONSET OF RAT LIVER MITOCHONDRIAL SWELLING

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ABSTRACT

Mitochondrial swelling is used extensively as a tool for the study of respiratory and phosphorylative functions. The swelling process, as observed by following optical density at 515 millimicrons with time, can be described by three criteria: time of onset (TO), rate and extent.

The "time of onset" of swelling is defined as the point in time at which the active swelling process begins. The "rate" of swelling is defined as the slope of the optical density-time plot between the time of onset and the lower plateau. The "extent" of swelling is defined as the difference between the initial and final optical densities.

An invesigation of several factors known to affect mitochondrial swelling was conducted to observe their effects, specifically on the time of onset. The factors selected for investigation included pH and varying concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), respiratory substrate and swelling agent. Inorganic phosphate (Pi) was used as swelling agent in all experiments.

In the pH studies the time of onset was observed to vary directly with pH at all Pi concentrations used, *i.e.*, the time of onset increased with increasing pH.

Swelling agent (Pi) concentration studies revealed that the time of onset varies inversely with the agent concentration.

The time of onset was found to vary directly with ATP concentrations from 0 to 2 x 10^{-3}M. Oligomycin reverses this effect of ATP. In contrast, while the time of onset increases with ADP concentrations from 0 to 1.67 x 10^{-4}M, additional ADP does not change the time of onset. Furthermore, oligomycin does not reverse the effect of ADP on the time of onset. Succinate also increases the time of onset in a manner similar to that of ADP, i.e., a level is reached which is not affected by increasing concentration. The time of onset observed with succinate in the presence of oligomycin is markedly increased over that seen with succinate alone. Succinate was found to increase the times of onset seen with either ATP or ADP, although the effect was much greater with ADP. Oligomycin reduced the time of onset seen with the ATP-succinate combination to that seen with succinate plus oligomycin. On the other hand, oligomycin enhanced the effect of the ADP-succinate combination to a level

much greater than that seen with succinate plus oligomycin. The concentration of ADP required in the presence of succinate plus oligomycin is relatively small. This is indicated by the observation that in the presence of succinate and oligomycin, concentrations of ADP greater than 2.5×10^{-5}M have little or no effect on the time of onst, whereas, at constant ADP concentration (2.5×10^{-5}M) increasing succinate concentration produces a linear increase in the time of onset of swelling.

A scheme is proposed which accounts for all of the observations in terms of the following five reactions:

1) the utilization of substrate, *2*) the phosphorylation recation, *3*) the formation of a "maintenance" intermediate, *4*) the degradation of the "maintenance" intermediate and *5*) an adenylate kinase reaction.

EVIDENCE OF RNA-RICH PARTICLES FROM BEEF LIVER MITOCHONDRIA[1]

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ABSTRACT

Purified beef liver mitochondria after being subjected to sonication and deoxycholate treatment have been separated into three fractions by differential centrifugation. The 20,000 X g sedimentable fraction was found to be rich in protein, neutral fat, and phospholipids but low in RNA; whereas the (105,000 X g) sedimentable fraction was low in lipids and rich in protein and RNA (25-30%). Ultracentrifugation analysis of the RNA-rich fraction revealed the presence of three kinds of particles with sedimentation coefficients of 57S, 85S, 110S, with 85S particles in predominance. Antisera to the mitochondrial soluble protein fraction was prepared in rabbits. This antisera was cross-absorbed with beef liver soluble protein, yielding antibodies specific to mitochondrial proteins. Antibodies specific for soluble proteins were prepared in smilar manner. Using antibodies to mitochondrial protein it was possible to precipitate 25% of the RNA-rich particles from mitochondria while only 5% of these particles were precipitated with antibodies to soluble proteins. These results indicate that the particles are of mitochondrial origin and not the result of contamination.

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[1]Supported by NSF Grants #GB-1748 and #GU-923.

COMPOSITION AND RATE OF SYNTHESIS OF INDIVIDUAL PHOSPHOLIPIDS IN SUB-CELLULAR PARTICLES OF TISSUES DURING EMBRYOLOGICAL DEVELOPMENT

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ABSTRACT

A number of enzymes have been shown to have biochemical differences during embryological development. Some of these are found in microsomes and mitochondria and are lypoenzymes. Fetal rabbits of known ages (—12, —9, —3, and +2, +9, +14) were available. Pregnant mothers or young rabbits were injected with P_i^{32} and sacrificed at 2,4 and 6 hours after administration of the isotope. Liver, kidney, and heart were removed from fetuses, pooled, weighed, and homogenized in ice-cold 0.25 M sucrose. An aliquot was taken for inorganic phosphate and radioactivity. Mitochondria and microsomes were separated by differential centrifugation; an aliquot of each fraction was taken for protein nitrogen determination. The lipids were extracted from the sub-cellar fraction and chromatographed on silicic acid impregnated glass paper. The individual phospholipids were identified, the chromatogram spots hydrolyzed, and phosphorus and radioactivity determined. From the data, the concentration of the various individual phospholipids of mitochondria and microsomes (micrograms of phospholipid per mg of protein nitrogen) was calculated from the radioactivity and phospholipid P data. There is a progressive increase of liver mitochondrial protein concentration per whole organ with embryological development. The total phospholipid phosphorus concentration per whole organ of liver, heart, and kidney mitochondria increases during development. There is very little difference in the concentration of phosphatidyl inositol, sphingomyelin, phosphatidyl ethanolamine, and phosphatidyl serine (micrograms of phospholipid P/mg of protein) from liver mitochondria during pre- or post-natal development. However, the concentration phosphatidyl choline increases at birth, subsequently returning to the previous level. There is an increase in total phospholipid P/mg protein in liver microsomes during embryological development. This increase primarily is reflected in the concentration of phosphatidyl choline. It would appear from the concentration data of the phospholipids in liver mitochondria that the structural pattern mitochondria is developed at an early age before birth.

EFFECTS OF ETHIONINE UPON THE INTESTINAL ABSORPTION OF METHIONINE[1]

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ABSTRACT

Studies have been made using L-ethionine in an attempt to antagonize the absorption of methionine in the small intestine of the intact rat. Radiolabelled L-methionine (methyl ^{14}C) was perfused at various concentrations (0.28 mM to 5.7 mM) in a mixture of sixteen amino acids at twice the concentration found in blood plasma. Test perfusates contained 10.5 mM ethionine. The amino acids were analyzed chemically with the Beckman-Spinco Amino Acid Analyzer; radioactivity was measured by liquid scintillation counting. It was found that ethionine had no apparent effect upon the absorption of methionine by recirculated perfusion. However, singlepass perfusion experiments demonstrated an inhibition of absolute absorption of methionine induced by ethionine.

[1]Supported in part by a NIH training grant and by NIH research grant AM-02023 NTN.

ZIRCON VARIATION IN THE TUNK LAKE GRANITE, SOUTHEASTERN MAINE

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ABSTRACT

Study of the variation and distribution of the accessory mineral zircon in the Tunk Lake granite body shows that abundance, size, and shape vary throughout this concentrically zoned intrusion. Zircon populations of the granite body appear to be systematically related; zircon in the outer rocks is more abundant, larger, more elongated, and crystallographically less complex than in the central rocks while zircon of intermediate rocks has intermediate characteristics. The prevalent concept that zircon generally does not vary in nature and abundance within a single intrusion is questioned and qualified by this study. Possible causes for the observed variation are multiple intrusion, regular change of the environment of crystalization of zircon, and separation of an originally homogeneous population by gravity settling.

INTRODUCTION

Zircon studies have been used frequently to establish genetic relationships between adjacent igneous rock bodies. It is generally emphasized, c. f. Poldervaart (1956), Larsen and Poldervaart (1957), Taubeneck (1957), Larsen and Poldervaart (1961), that zircon populations from a single intrusion are identical. Even where mild differentiation has produced several somewhat similar rock types, zircons, because of early crystallization, are thought to be uniform throughout the differentiated sequence. Different zircon populations in spatially related rocks are usually assumed to indicate different magma sources. In a series of genetically related, chemically dissimilar, rock types zircons are believed to vary systematically throughout the series (e.g., Poldervaart, 1956, Forbes and Eckelmann, 1962). Initial thin-section study by the junior author[1] of zircon from the zoned Tunk Lake granite body in southeastern Maine suggested that zircon distribution and character varied systematically in this granite body which is best interpreted as having developed from a homogeneous magma. It was observed (Karner, 1963A, 1963B) that vircons in the marginal rocks of the body were larger, more abundant, and crystallographically less complex than in the core. Preliminary results of a statistical study of the zircon population are presented here, and show that the concept of a relatively uniform zircon population in a zoned magmatic body must be seriously qualified even if the zones are chemically similar and have developed from an originally homogeneous magma.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the National Science Foundation through the Undergraduate Research Participation Program (Grant GE4035) in which the senior author was a participant during the summer of 1964 and the 1964-65 academic year. The Geology Department of the University of North Dakota provided laboratory space and equipment for this study and the university provided the use of an IBM 1620 computer. The authors wish to thank Mr. Robert Freiburghouse and Mr. Richard Everson for aid in computer programming.

GENERAL GEOLOGY OF THE TUNK LAKE GRANITE

The Tunk Lake granite body is one of the "younger granites" (Chapman, 1962) intruded into the Bays-of-Maine igneous complex and older metamorphic rocks in southeastern Maine. The area of outcrop of the Tunk Lake body is 70 square miles and nearly circular (figure 1). The outermost rocks are aegirine augite and magnetite granites. These grade inward to hornblende and biotite granites and biotite quartz monzonite. Six concentric zones are recognized. Numbered from the margin inward they are:

Type I Aegirine augite and magnetite granite chill zone.
Type II Hornblende-aegirine augite granite.

[1]F.R.K.

Type III Horblende granite.
Type IV Hornblende-biotite granite.
Type V Biotite granite.
Type VI Biotite quartz monzonite.

The types are graduational and form a continuous sequence from margin to core. Their distribution is shown on figure 1.

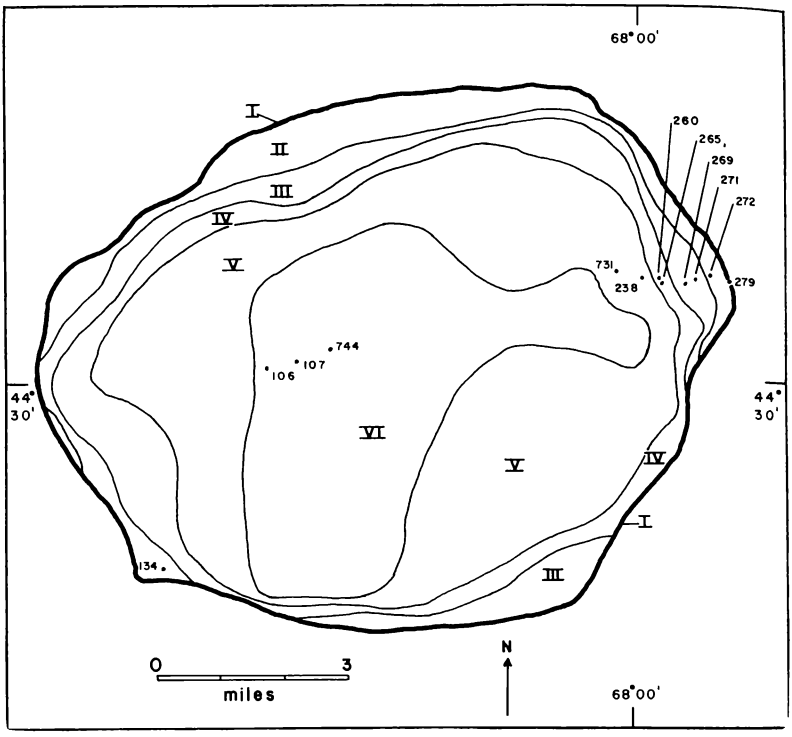

FIGURE 1—Map of the Tunk Lake granite body and zircon sample locations. Roman numerals refer to petrographic zones described in text.

It has been shown (Karner, 1963A, and in preparation) that the Tunk Lake body is best interpreted as a single intrusion in which the different zones have developed by differentiation of a magma of composition similar to the calculated average of the body. Crystallization began along the margins where a sample of the original magma was quickly frozen against the enclosing rocks forming the chill zone (I). The mineralogy of the marginal rocks indicates that they crystallized at relatively high temperature compared to the core which contains minerals which apparently crystallized at lower

temperatures in the presence of abundant water. This water is believed to have streamed from the partially crystalline margin to the still molten core as a hot silica-rich fluid. This accounts for the enrichment of SiO_2 in the core, the most striking, and perhaps only, chemical distinction between the marginal rocks and the core. The chill zone is chemically similar to, but mineralogically different than the core rocks (Karner, 1963A).

METHODS OF ZIRCON SEPARATION AND MEASUREMENT

The procedure used in obtaining zircon crystals from the rock involved crushing, sieving, and magnetic and heavy mineral separations. Preliminary study showed that a 50 gram sample of each rock specimen would yield adequate zircons for measurement. This amount was obtained from several rock chips available from each location shown in figure 1. The sample was pulverized with mortar and pestle and was then sieved to obtain four different size classes (U. S. sieve numbers 20,40,100, and the pan).

Each of the four was separated by tetrabromoethane (S. G. 2.96 at 20°C) into two fractions, the lighter containing quartz and feldspar, and the heavier containing zircon in addition to hornblende, biotite, aegirine augite, magnetite, sphene, apatite, and allanite-epidote. The light minerals of the two largest size classes were crushed and separated again by heavy liquid to free additional zircon. Next, an electromagnet was used to separate magnetic minerals from the non-magnetic heavy minerals zircon, sphene, apatite, and allanite-epidote. The magnetic fraction was set aside for further study of the constituent minerals. Some zircon was included in this fraction but was not significantly different than that recovered. A final separation with Clerici solution (S. G. 4.33 at 20°C) resulted in a heavy fraction of essentially pure zircon.

The zircons were mounted on cardboard slides for microscopic investigation. It was concluded, using the method of Dennison (1962), that examination of the characteristics of 100 crystals would give results within 0.95 confidence limits for means of zircon length and width. Numbered grids printed on the slides were utilized to assure that 100 crystals were selected randomly from the total number on the slide. Size and crystal form were noted for each of 92-100 complete crystals for each of the sample sites of figure 1 with the following exceptions: 744-22 crystals, 107-60 crystals, 279-186 crystals. Length and width were measured using an ocular micrometer and a magnification of 72x. Crystallography was determined with a magnification of 160x. Crystals consist of tetragonal prisms and pyramids and were classified according to the following scheme:

Class 1 Crystals with either first order prisms and pyramids or second order prisms and pyramids.

Class 2 Crystals belinging to classes 4 or 5 (see below).

Class 3 Crystals with combination of first, second, and third order prisms and pyramids.

Class 4 Crystals with combinations of first and second order prisms and pyramids.

Class 5 Crystals with combinations of either first and third order prisms and pyramids or second and third order prisms and pyramids.

Zircon abundance was determined by a method utilizing rock thin sections. The number of zircons occurring in a measured area of a thin section was multiplied by the average area of zircon crystals as determined in that section. This area when compared with the measured area of the thin section gives the volume percent of zircon in the rock. An area of about 600mm^2 was utilized for at least one thin section from each zircon location. Results from measurements on different thin sections from the same location were variable, probably because of loss of zircon during the making of some thin sections. More precise and accurate methods for determining zircon abundance are being developed but it is felt that results of these preliminary measurements are sufficiently accurate to show trends and the approximate magnitude of zircon abundance (Table I).

TABLE I

CHARACTERISTICS OF ZIRCON POPULATIONS OF THE TUNK LAKE GRANITE

	Zone I	Zone II	Zone III	Zone IV	Zone V	Zone VI	Average
Mean zircon length (mm)	0.243	0.212	0.162	0.177	0.169	0.138	0.165
Standard deviation of length (mm)	0.101	0.100	0.074	0.081	0.057	0.053	——
Mean zircon width (mm)	0.104	0.079	0.066	0.072	0.072	0.066	0.070
Standard deviation of width (mm)	0.039	0.032	0.025	0.033	0.024	0.025	——
Mean elongation (L/W)	2.352	2.685	2.481	2.533	2.403	2.125	2.379
Standard deviation of elongation	0.500	0.660	0.585	0.583	0.547	0.430	——
Zircon abundance (volume percent)	0.13	0.14	0.16	0.07	0.07	0.04	0.08
Crystal complexity	1.80	1.67	1.86	1.98	2.12	2.11	2.03

ZIRCON VARIATION

Microscopic examination showed that zircons occur as euhedral to subhedral crystals, although many appeared broken, probably as a result of crushing during separation. Most are translucent and pale-brown to reddish brown. A few are dark red, possibly because of an iron oxide coating.

The University of North Dakota IBM 1620 computer was used to analyze the size and crystal form data. The calculation of elongation (ratio of length to width) and the classification of all zircon measurements were both accomplished using Fortran programs written by the junior author and R. Everson. A standard statistical program, (Correlation co-efficients with means, sigmas, covariance, and the regression line bX, Y or bY, X) written by Robert Freiburghouse and on file at the UND computer center, was used to calculate means, standard deviations, and the correlation coefficients for zircon parameters. These calculations were made for zircon populations of the six petrographic zones of the Tunk Lake granite and for the total population by weighting the values for the individual populations according to areal extent of the zones. The results are summarized in Table I, in addition to abundance and relative crystallographic complexity of zircon. Crystal complexity is expressed as a numerical value equal to the proportion of crystals having prisms and pyramids of one order (crystal class 1) plus two times the proportion having two orders (crystal class 2) plus three times the proportion having three orders (crystal class 3). Variation of zircon length, width, and elongation are also shown in figure 2 as frequency distributions for zircon populations of zones I-VI and of the average weighted as described above.

The characteristics of zircon are seen to vary markedly and fairly regularly from zone I at the margin of the body to zone VI at the center. Mean lengths and widths of zircon crystals show a general decrease from zone I (.243mm x .104mm) inward to zone VI (.138mm x .066mm). Standard deviations of length and width also decrease from zone I to VI. Mean elongation is slightly lower in the central zones. The frequency distribution curves readily show these variations as well as a conspicuous difference in skewness. The curves for the total zircon population indicate a nearly normal distribution, whereas the populations of the outer zones tend toward a more positively skewed distribution. Frequency distribution for the zone V zircon population most nearly resembles that for the total population in all cases. Zircon abundance is much greater in the marginal zones (about 0.14 percent) than in the central zones (about 0.04 percent). The crystal complexity of zircons changes uniformly throughout the body from relatively simple at the margin to progressively more complex in zone VI. Simple prisms and pyramids are most common in the outer portions of the Tunk Lake body, while

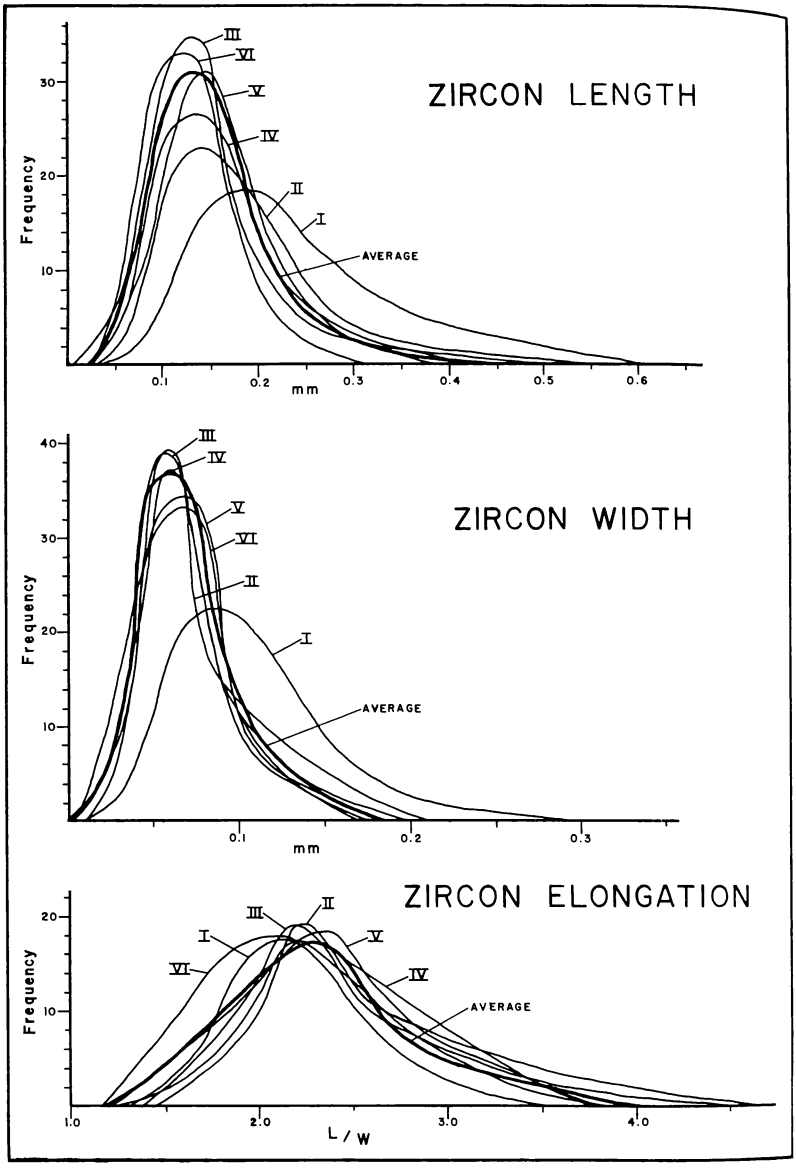

FIGURE 2—Frequency distribution curves for length, width, and elongation of zircons in the Tunk Lake granite.

crystals composed of combinations of prisms and pyramid faces are concentrated in the central zones.

DISCUSSION

This study has shown that zircon size and shape may vary extensively throughout a granite body derived from a homogeneous magma and exhibiting little chemical variation. Further, the variation shows systematic trends which may yield significant data as to the processes operating during crystallization of the body. There are several possible explanations for the observed zircon variation:

1. *Multiple Intrusion.*—A major feature of the results of this study is that the central zone, VI, has fewer and smaller zircons than the outer zones of the body. If this zone represents an intrusion of different age than the rest of the body a part of the zircon variation could be explained. However, this interpretation would still not explain the continuous zircon variation in the outer zones.

2. *Environment of Crystallization.*—Zircon size and shape, like that of most minerals, may be affected by temperature, pressure, and chemical environment during crystallization. The fact that zircon populations of different rocks vary is the strongest evidence for the importance of this factor (see, for example, Poldervaart, 1956, and Forbes and Eckelmann, 1962). An explanation, based largely on the concept of variable environment of crystallization, has been used by Larsen and Poldervaart (1961) for part of the zircon variation in the Bald Rock batholith, California. Recently Silver and Deutsch (1963) have found variations in zircons within single blocks of the Precambrian Johnny Lyon granodiorite and evidence that although zircon began to crystallize early it continued over a relatively long part of the crystallization history.

3. *Gravity Settling.*—Since zircon has a very high specific gravity (4.68) and is thought to crystallize early it could be depleted from some portions of a magma chamber and concentrated in others. Crystal settling in granitic magmas has recently become a mechanism of considerable interest to petrologists (see for example Shaw, 1965) and has been used to explain zircon variation in a granodiorite dike at Bradford, Rhode Island by Quinn (1943). However investigation of the same (?) dike by Hall and Eckelmann (1959) showed no evidence of gravity settling.

Each of these processes may have been significant to some extent, or one may have produced most of the zircon variation. At present, gravity settling with depletion of zircon from the central part of the body seems to be the most likely explanation. Further investigation by the authors is being conducted to test these three possible explanations for zircon variation in the Tunk Lake granite.

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REPORT OF THE OLIGOCENE RHINOCEROS, *SUBHYRACODON*, IN NORTH DAKOTA

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ABSTRACT

During 1964 bones assigned to the rhinoceros genus, *Subhyracodon,* were found in the White River Formation in a channel sandstone known as the "Fitterer Channel" (Skinner, 1951) on the Leo Fitterer ranch, NW¼, Sec. 17, T. 137 N., R. 97 W., about 15 miles south of Dickinson, Stark County North Dakota. A skull, a humerus, an atlas, and several lower jawbones and ribs of several individuals have been collected and prepared.

The gently sloping skull, long thin nasals, and the internal and external cingulum indicate the middle Oligocene *Subhyracodon.* The specimens have, however, certain affinities with the genus *Diceratherium* which is characteristic of later Oligocene deposits. The prominent antecrochet and incipient crochet on the upper molars and premolars suggests *Diceratherium.* Also the skull, over 500 mm in length, is longer than reported for *Subhyracodon*; and the humerus is more than one-third longer than previously reported for this genus.

It is hoped that further collecting, study, and comparison of fossils from this badland area will aid in correlation of the Oligocene deposits of North Dakota with the Chadron and Brule formations of South Dakota and Nebraska.

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A POSSIBLE *BISON (SUPERBISON) CRASSICORNIS* OF MID-HYPSITHERMAL AGE FROM MERCER COUNTY, NORTH DAKOTA

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INTRODUCTION

In 1963 the operator of a drag-line shovel recovered a partial bison skull (N.D.S.U. Geology Department Specimen 165) from a depth of 20 feet in a high-water diversion cut being made in the valley of Spring Creek near Zap, Mercer County, North Dakota.

Dr. J. Knox Jones of the Museum of Natural History, University of Kansas made a tentative identification of the skull as *Bison (Superbison) crassicornis.*

In 1964 I visited the site and while clearing the cut face discovered a second, more complete skull (N.D.S.U. Geology Department Specimen 166) at about the same depth.

GEOLOGY

Spring Creek, a tributary of Knife River, is cut in Tertiary bedrock and thin glacial drift. Various segments of its valley carried diversion waters of Little Missouri River via Hans Creek and Goodman Creek which lie to the northwest prior to the retreat of Wisconsin glacial ice from the Missouri Valley. Meltwater drainage from the ice front at the Krem moraine about 5 miles north also moved through parts of Spring Creek (Colton, et al., 1963).

The measured section in the north wall of the diversion cut at the discovery site of Specimen 166 (Table I) shows fine-grained alluvium over coarser stream deposits. The very coarse material at the base of the cut (Unit d)) is probably a lag of glacial boulders, but high water prevented penetration which might have determined its thickness and nature. A more complete areal study remains to be made.

SKULL IDENTIFICATION

Skinner and Kaisen (1947) have prepared the most recent general review of fossil bison, and their procedure for cranial measurements, calculation of horn-core indices, and skull photography were followed.

The general appearance of the skulls (Fig. 1), particularly the length of the horn-cores, aroused suspicion that they might not belong to the modern species *Bison bison.*

Detailed cranial measurements were then made for both skulls

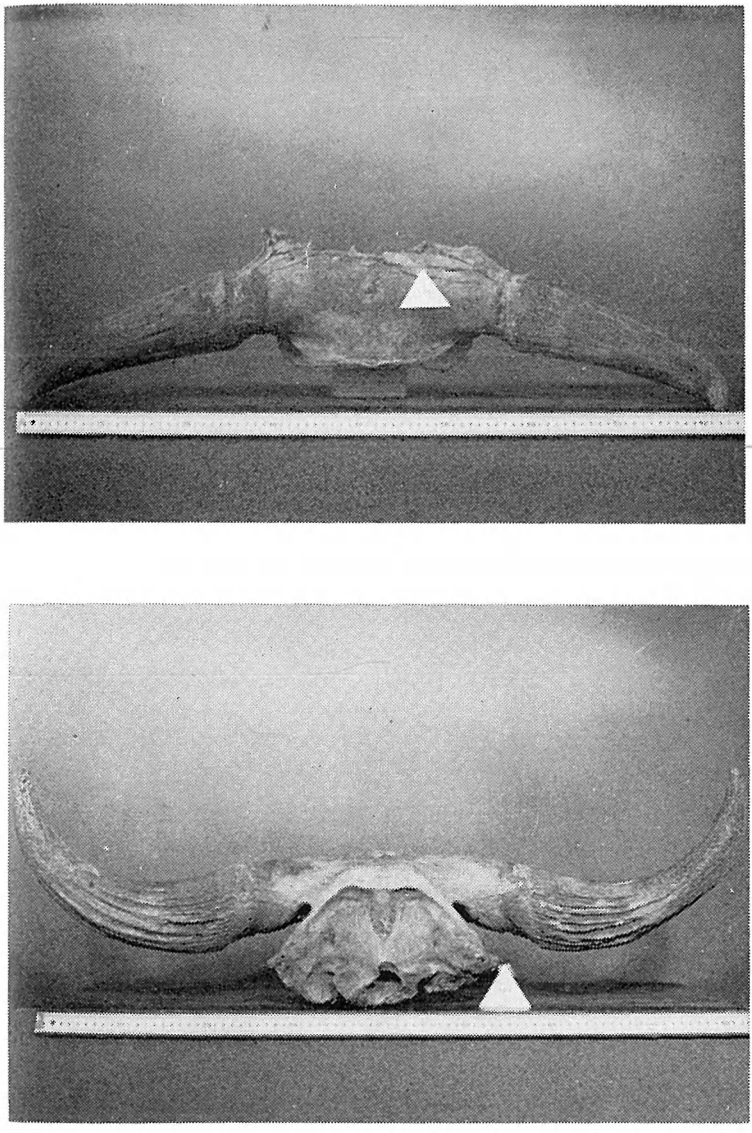
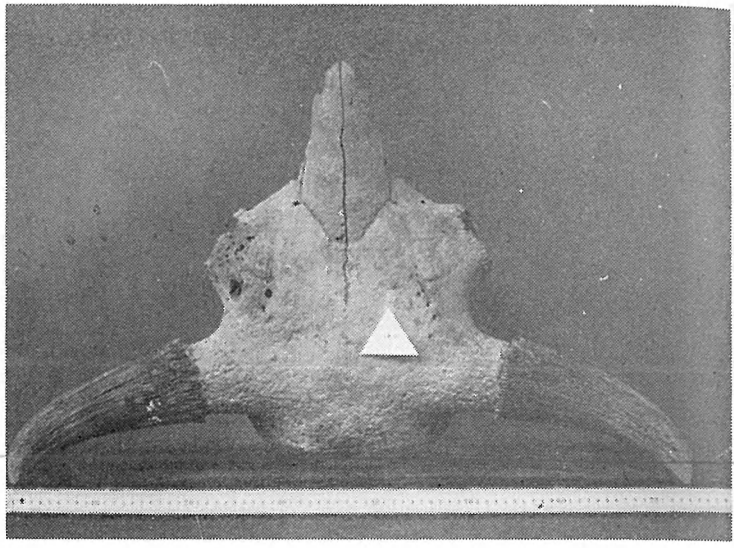


FIGURE 1—Dorsal and posterior views of specimen 165. (Also see page 216).



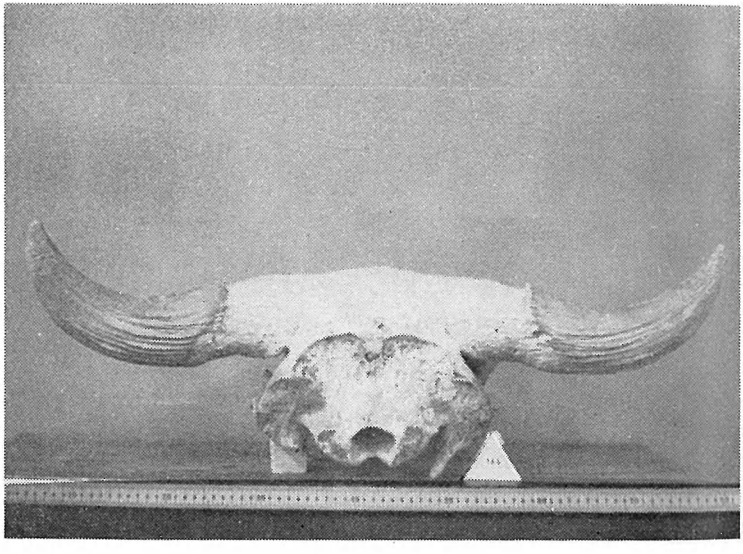


FIGURE 1—(Continued)—Dorsal and posterior views of specimen 166.

(Table II), and from these, horn-core indices were derived. According to Skinner and Kaisen (1947) these indices, which eliminate deceiving impressions of size and shape, constitute one of the best criteria for species differentiation. Figure 2 shows the position of key measurements used in horn-core index calculation. Calculations are as follows:

$$\text{Index of curvature} = \frac{Ll}{D} \times 100$$

$$\text{Index of compression} = \frac{dv}{dt} \times 100$$

$$\text{Index of proportion} = \frac{Lu}{C} \times 100$$

$$\text{Index of length} = \frac{Lu}{W} \times 100$$

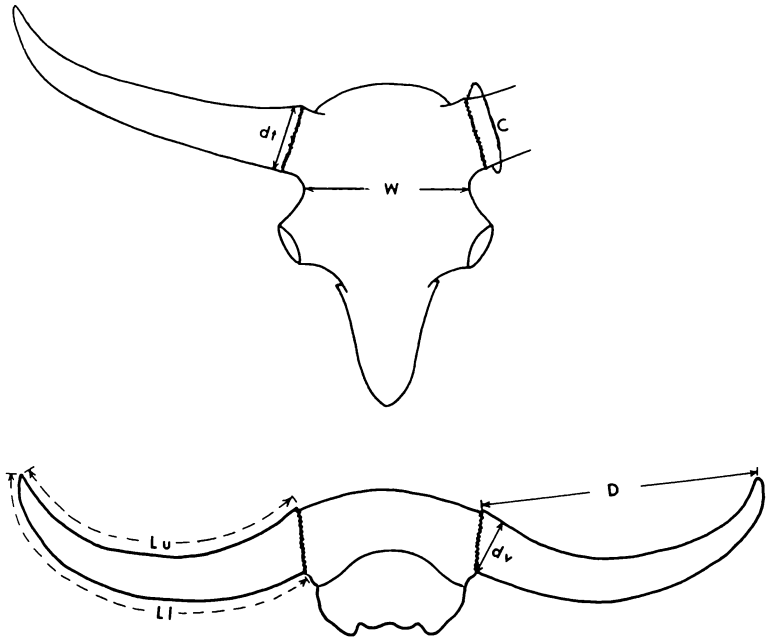


FIGURE 2—Idealized diagram of bison skull showing measurement points from which horn-core indices are derived. *Ll* is horn-core length on lower curve, *Lu* is horn-core length on upper curve, *dv* is vertical diameter of core at base, *dt* is transverse diameter of core at base, *C* is circumference of core at base, *D* is distance from core tip to upper base at burr, and *W* is cranial width between horn-cores and orbits. (Modified from Skinner and Kaisen, 1947).

In figures 3 through 6 these horn-core indices for specimens 165 and 166 are compared with ranges of indices of eight species of bison, as cited by Skinner and Kaisen.

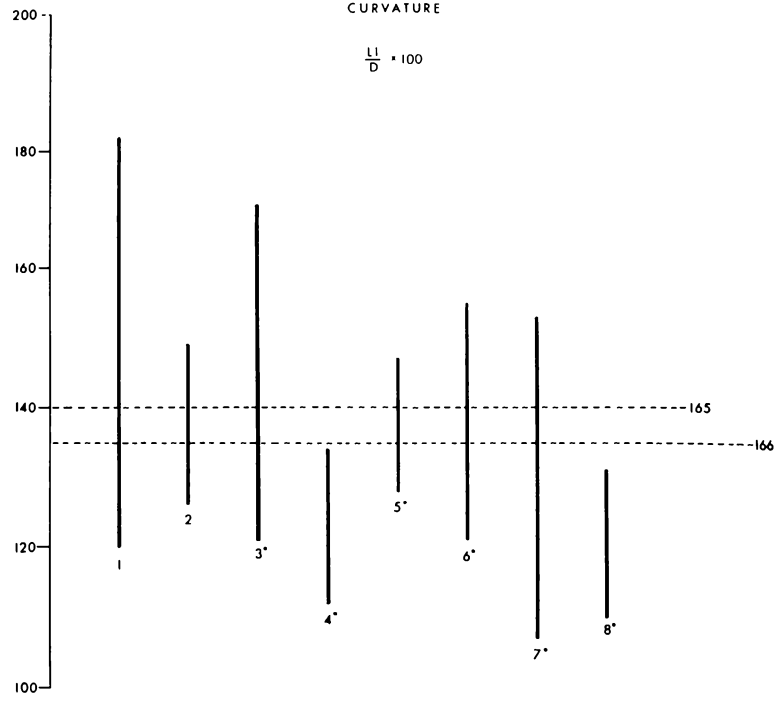


FIGURE 3—Horn-core curvature indices of Mercer County skulls (165, 166) compared with range of horn-core curvature indices of eight species of North American bison: (1) *Bison* (*Bison*) *bison bison*, (2) *B.* (*B.*) *bison athabascae*, (3) *B.* (*B.*) *occidentalis*, (4) *B.* (*B.*) *preoccidentalis*, (5) *B.* (*Simobison*) *antiquus antiquus*, (6) *B.* (*S.*) *alleni*, (7) *B.* (*Superbison*) *crassicornis*, (8) *B.* (*Gigantobison*) *latifrons*. Asterisk indicates extinct species.

Analysis of these charts shows that all four indices of Specimen 165 fall within the known range of indices of *Bison* (*Superbison*) *crassicornis*. The fit of this specimen with all other species is less good.

Specimen 166 is more equivocal, with its indices failing to fall within the known range of any single species. It fails to fall within the range of *B.* (*S.*) *crassicornis* in only one index, that of core proportion, which is 94 compared with the *B.* (*S.*) *crassicornis* minimum of 100 (Fig. 5). It comes equally close, however, to matching *B.* (*B.*)

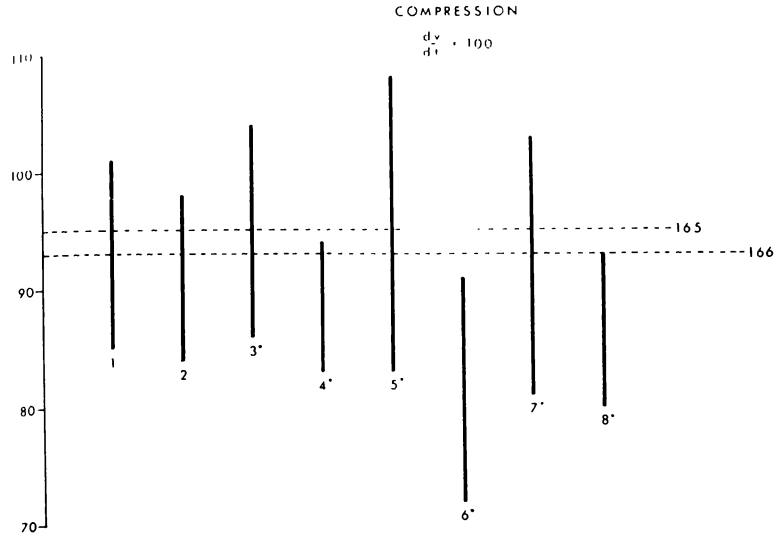


FIGURE 4—Horn-core compression indices of Mercer County skulls (165, 166) compared with range of horn-core compression indices of eight species of North American bison. (Species are numbered the same as in figure 3).

occidentalis (failing only in index of core length) (Fig. 6) and *B.* (*S.*) *antiquus antiquus* (failing only in index of core length) (Fig. 6). In *B.* (*B.*) *antiquus* however, the horn-cores tend to project at right angles to the longitudinal axis of the skull, while in Specimen 166, they are posteriorly directed at about 18°. The strong deviation of the core length index of Specimen 166 from the known range of *B.* (*B.*) *bison* (Fig. 6) argues against it belonging to that species.

On the basis of horn-core indices, then, it seems likely that Specimen 165 belongs to *B.* (*S.*) *crassicornis*. The presence of Specimen 166 at the same depth at the same site would seem to favor its belonging to the same species even though its core indices do not match as well.

AGE OF THE SKULLS

Wood found in close association with Specimen 166 has been dated by the Washington laboratory of the U. S. Geological Survey as 5440 ± 200 years B. P. (W-1537). This date falls at about the middle of the post-glacial Hypsithermal interval, which according to Flint (1957, p. 377) was a time of relatively warm, dry climate lasting from about 7500 to about 4000 years ago. If the wood and skulls were buried at the same time (and there is no evidence to

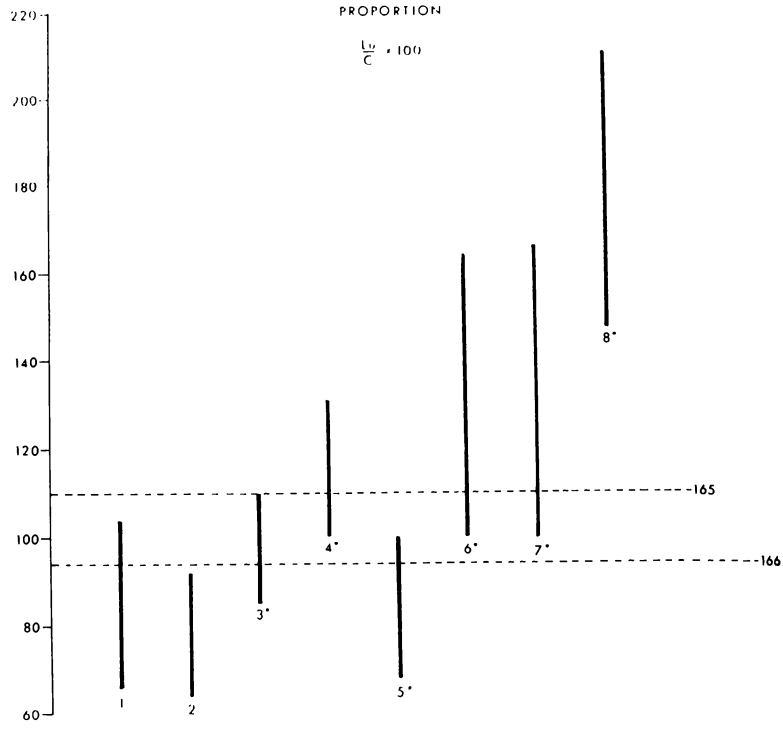


FIGURE 5—Horn-core proportion indices of Mercer County skulls (165, 166) compared with ranges of horn-core proportion indices of eight species of North American bison. (Species are numbered the same as in figure 3).

indicate otherwise) this is a surprisingly young age for *B.* (*S.*) *crassicornis.*

Skinner and Kaisen (1947, p. 155) imply that *B.* (*S.*) *crassicornis* became extinct as a result of the changing conditions introduced by the Wisconsin glacial stage, and that only *B.* (*B.*) *occidentalis* lived on to give rise to modern species. Romer (1951) however, reports a *B.* (*S.*) *crassicornis* skull from late Wisconsin gravels at Harvard, Massachusetts.

DISTRIBUTION

At the time of Skinner and Kaisen's review, *B.* (*S.*) *crassicornis* was known only from Alaska and the Yukon Territory. Since then two other finds have been made in continental United States; that of Romer (1951) in Massachusetts and one in eastern Kansas by Clemens (personal communication).

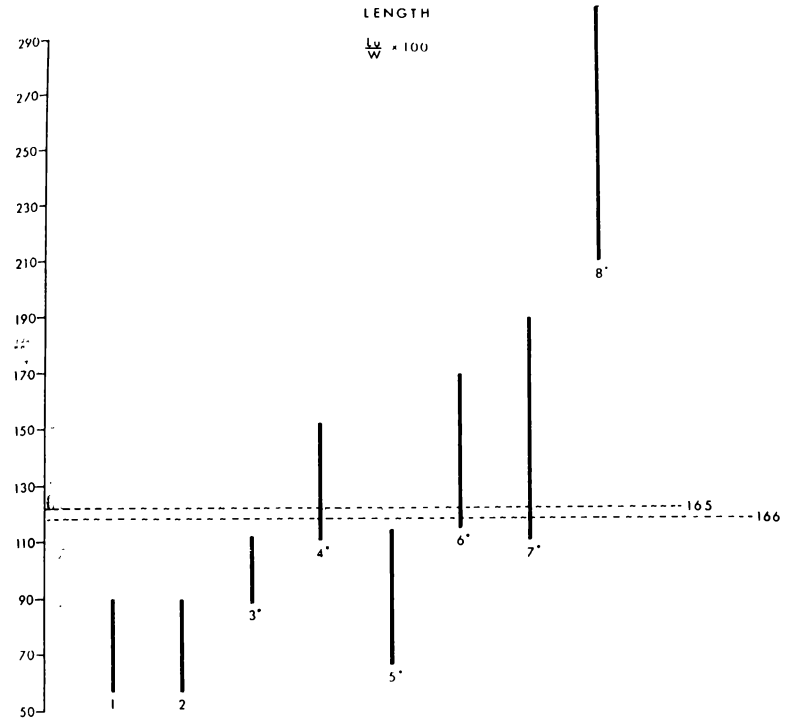


FIGURE 6—Horn-core length indices for Mercer County skulls compared with ranges of horn-core length indices of eight species of North American bison. (Species are numbered the same as in figure 3).

As far as is known, the Mercer County specimens represent the first find of *B.* (*S.*) *crassicornis* in the northern Great Plains.

TABLE I

STATIGRAPHIC SECTION (NW¼ Sec. 17, T.144N., R.89W., 3.4 miles WNW of Zap, Mercer County, North Dakota. Surface is terrace 10 ft. above flood plain of Spring Creek.)

UNIT	THICKNESS (FT.)
a. Silty, sandy alluvium, tan, bedding indistinct, no fossils seen	19
b. Sand and gravel, cross-bedded to east (downstream), contains clam shells	2 to 4
c. Interbedded sand, silt and	

clay with minor amount of gravel, beds thin, oxidized in upper 1 to 2 feet, unoxidized below, lower 2 feet contains wood (C-14 sample), shells and bones (skull #166) 4 to 6

d. Sand and gravel, stones up to boulder size ½ exposed

TABLE II
CRANIAL MEASUREMENTS (IN MILLIMETERS)

	Specimen No. 165	Specimen No. 166
Spread of horn-cores, tip to tip	820	740
Greatest spread of cores on outside curve	860	763
Core length on upper curve, tip to burr	330	253
Core length on lower curve, tip to burr	405	310
Length, tip of core to upper base at burr	280	230
Vertical diameter of horn-core at right angle to longitudinal axis (at base)	95	89
Circumference of horn-core at right angle to longitudinal axis (at base)	300	270
Greatest width at auditory openings	280	285
Width of condyles	125	145
Depth, occipital crest to lower border of foramen magnum	160	140
Transverse diameter of core at right angle to longitudinal axis (at base)	100	96
Width of cranium between horn-cores and orbits	270 (est.)	280
Greatest post-orbital width	——	330
Length, occipital crest to tip nasals	——	450
Length, occipital crest to nasal-frontal suture	——	245
Index of core curvature	140	135
Index of core compression	95	93
Index of core proportion	110	94
Index of core length	122*	118
Angle of posterior divergence of horn-cores	10°	18°

*Based on estimate of width of cranium between horn-cores and orbits.

ACKNOWLEDGMENTS

I wish to thank Dr. J. Knox Jones and Dr. William Clemens of the Museum of Natural History, University of Kansas for their help in identification and in locating references.

Thanks are also due to Mr. E. A. Panushka of the Northern

Pacific Railroad for bringing Specimen 165 to me, and to Mr. Dallman of Zap, North Dakota for permission to remove Specimen 166 from his property.

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TRICERATOPS IN NORTH DAKOTA

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ABSTRACT

Numerous fragments of leg bones, ribs and horn cores of the ceratopsian dinosaur genus, *Triceratops*, have been reported by early geologists from Late Cretaceous strata in southwestern North Dakota, but the specimen discovered late in the field season of 1963 by Charles I. Frye is the best preserved specimen found to date. It occurred in light gray bentonitic clay of the Hell Creek Formation 40 feet below the base of the Tullock Formation of Paleocene age on the southwest side of a butte in the NW¼, NE¼, Sec. 32, T. 135 N., R. 106 W. about 18 miles north of Marmarth, in Slope County, North Dakota on land leased by Lyle Tennant from the U. S. Forest Service.

A nearly complete skull, two illia, the humerus, radius, and ulna of the left front leg, a tibia, and several vertebrae and ribs were excavated during July and August, 1964 by the writers and Marshall Lambert of Ekalaka, Montana, protected by plaster of Paris casts, and transported to the University of North Dakota. The deeply excavated inferior surface of the rostral bone, configuration of the short stout supraorbital and nasal horn cores, and shape of the orbits indicate that the specimen is *Triceratops brevicornus* Hatcher.

The excavation could not have been accomplished without funds

from the North Dakota Geological Survey, the Department of Geology, a University of North Dakota Faculty Research Grant to the senior author, National Science Foundation Undergraduate Research Participation Grant No. GE-4035 and cooperation from the U. S. Forest Service and the Slope County Board of Commissioners.

SIGNIFICANCE OF STRUCTURAL FEATURES OF THE VAUGHAN LEWIS GLACIER, ALASKA

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INTRODUCTION

During the summers of 1961 and 1963, a reconnaissance investigation was made of structural features of the Vaughan Lewis Glacier, Alaska. This study was conducted under the auspices of the Juneau Icefield Research Program and the Glaciological Institute of Michigan State University (Miller, 1963).

The Vaughan Lewis Glacier, part of the Juneau Icefield, is located 38 miles north of Juneau in the Coast Range of the southwestern Alaska province (Williams, 1958). It is a valley glacier flowing from the icefield which is an area of coalesced glaciers. The glacier (figure 1) can be divided into three secors: the névé area, the icefall area, and the ablation area which extends from near the base of the icefall to the terminus. Each of these areas has one or more structural features which were caused by stresses near the glacier's surface.

Previous studies, especially the one by Meier (1960), have shown that the orientation of crevasse systems is usually related to the greatest principal strain rate. Nye (1952) relates the theoretical stresses to the formation of crevasse patterns in a glacier. It is hoped that this paper will show that information can be gained from structural features even without detailed measurements.

STRUCTURES OF THE VAUGHAN LEWIS GLACIER

Three principal types of structures were examined on the Vaughan Lewis Glacier—crevasses, icefolds, and bands. Crevasses are of three types: (1) transverse, (2) chevron, and (3) splaying. Banding structures are of two types: wave bands and ogives.

Transverse crevasses.—Transverse crevasses are perpendicular to the long axis of the glacier. They indicate a longitudinal principal tension or extending flow. Transverse crevasses in the névé area and in the icefall area of the Vaughan Lewis Glacier are long and convex upglacier indicating a steep slope downglacier (Sharp, 1960). Transverse crevasses are also present in the terminal area where the

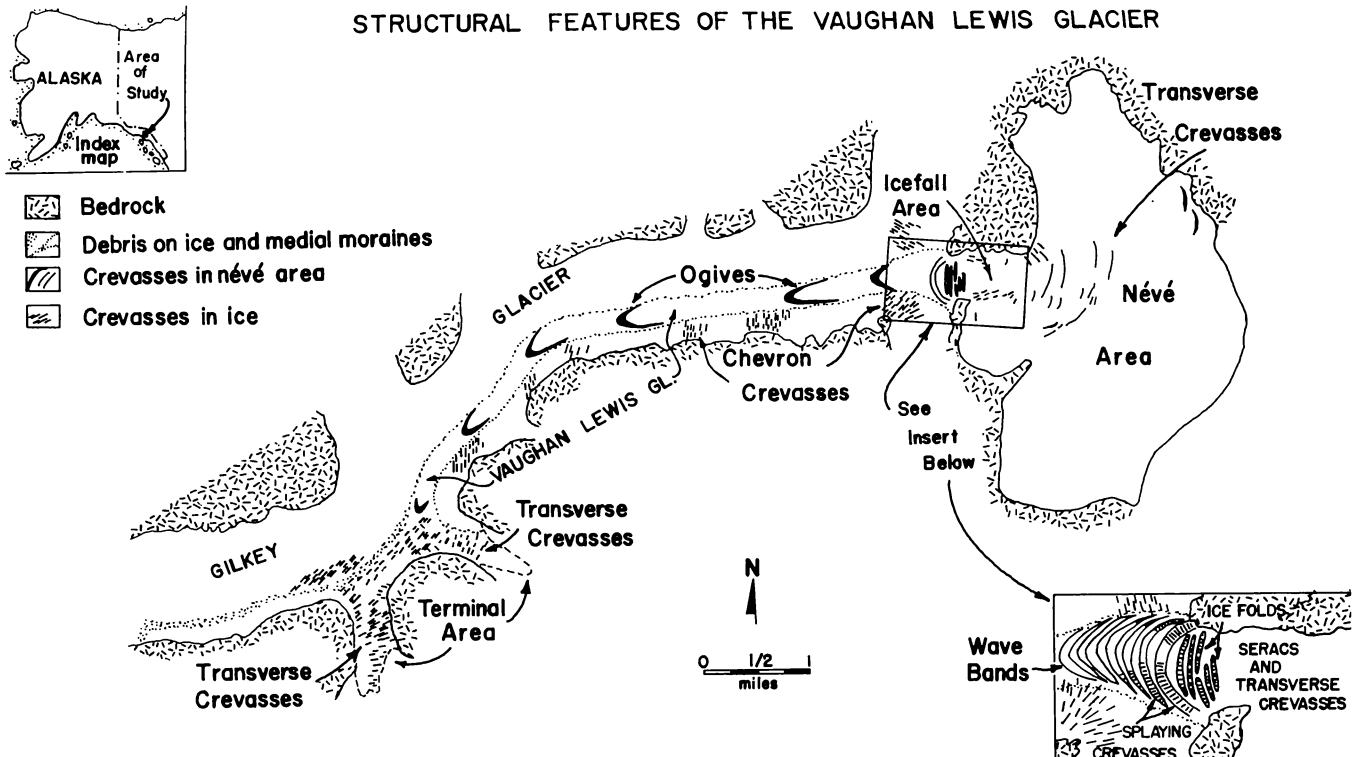

FIGURE 1—Planimetric representation of the Vaughan Lewis Glacier and its structures.

glacier ends in two side valleys (figure 1). Whereas transverse crevasses are usually the result of decreasing elevations on the bedrock surface downglacier, the opposite is true here. In the area where the glacier terminates in the two side valleys, the bedrock surface actually rises. The transverse crevasses are caused in this area by the upper part of the glacier flowing into the side valley; at the same time, the lower part continues to flow in the main valley.

Chevron crevasses.—Chevron crevasses are probably the most common type of crevasses in a valley glacier. They are found along the margins of glaciers; and when first formed, they point upglacier at an angle of about 45° (Nye, 1952, p. 91). Movement of the glacier rotates chevron crevasses until they are nearly perpendicular to the valley walls. The chevron crevasse pattern is caused by a shearing action due to drag of the glacier on the valley walls or on an adjacent glacier. Chevron crevasses on the Vaughan Lewis Glacier are found in the icefall and along the entire length of the ablation area.

Splaying crevasses.—Splaying crevasses are usually found in the center of and parallel to the long axis of a glacier. Sometimes the downglacier ends of the crevasses curve out toward the glacier's margin. Splaying crevasses indicate a transverse principal tension and longitudinal compressive flow (Sharp, 1960, p. 52). A transverse tension results when a glacier emerges from a constriction in the valley (Meier, 1960, p. 61) or when a glacier expands laterally in the bulbous terminal area. Splaying crevasses on the Vaughan Lewis Glacier (figure 1) are found in the lower one-third of the icefall area and in the first mile below the icefall. They are caused by the transverse tension that results as the glacier emerges from the narrow part of the valley containing the upper part of the icefall.

Icefolds.—Icefolds are symmetrical anticlinal folds of ice with axes transverse to the long axis of the glacier. They vary in length from several hundred feet to nearly one mile and in width from 10 to 100 feet. They appear to be associated with longitudinal compressive flow which is indicated by the presence of splaying crevasses. Compressive flow alone does not cause icefolds, but when it is accompanied by a rapid deceleration of the glacier's movement and thin ice, as found in the icefall, folds will develop. A straight transverse line will become distorted by surface movement, and the shape of the distorted line is called a transverse velocity curve (Sharp, 1960, p. 35). Icefolds are initially straight transverse features, but they can be deformed as the glacier moves. The icefolds are only slightly deformed as they move downglacier. This probably indicates a plug-flow type movement.

Wave bands.—Wave bands occur just below the base of the icefall. They are curved transverse swells and swales that are convex downglacier. Initially, wave bands have a smooth hyperbolic shape; but as they move downglacier they assume parabolic shapes. The

transverse velocity curve indicated by the trace of the wave bands reveals that the velocities at opposite points on the glacier's margins are nearly equal. This suggests that the Vaughan Lewis Glacier's velocity in the area of wave bands is relatively unaffected by the tributary glaciers.

Ogives.—Ogives are similar to wave bands but they do not have any surface amplitude. The first ogive is about two miles downglacier from the foot of the icefall. Ogives are englacial features which extend into the glacier to an unknown depth. As was the case with the wave bands, ogives reflect the transverse velocity curve on the glacier's surface. Measurements made from aerial photographs reveal a radius of curvature of about 900 feet for the first wave band at the foot of the icefall. About seven miles downglacier, the minimum radius of curvature for the ogives is 1.25 miles (6,600 feet). This great elongation of the bands suggests streaming-flow movement. About four miles from the base of the icefall, the north ends of the ogives become shorter than the south ends. This indicates that the Gilkey Glacier is exerting an influence on the Vaughan Lewis Glacier.

SUMMARY

The structural features of the Vaughan Lewis Glacier reveal the types of flow and stresses at the glacier's surface. In the névé area above the icefall, large transverse crevasses indicate extending flow. The highly transverse-crevassed upper two-thirds of the icefall indicates a longitudinal principal tension or extending flow. Icefolds and splaying crevasses in the lower one-third of the icefall reveal transverse principal tension or compressive longitudinal flow. In the ablation zone, from the foot of the icefall to the terminal area, the crevasse patterns and shapes of the bands suggest continuous longitudinal compressive flow. Transverse crevasses in the terminal area indicate extending flow.

In a reconnaissance study such as the one conducted on the Vaughan Lewis Glacier, detailed measurements of the structural features are difficult to obtain. However, interpretation of the orientation, position, and shape of the structures can reveal a considerable amount of information of the near-surface glacier mechanics.

ACKNOWLEDGEMENTS

Sincere thanks is given to Professor Maynard M. Miller, Director of the Glaciological Institute for guidance during all phases of the field work. Thanks is also given to Mr. John P. Bluemle of the North Dakota Geological Survey for critically reading the manuscript and offering many useful suggestions.

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UNIVERSITY OF NORTH DAKOTA GEOLOGICAL RESEARCH IN ALASKA

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ABSTRACT

For the past three summers (1962-1964) research teams from the Department of Geology, University of North Dakota, have been undertaking investigations of the Martin River Glacier area in south-central Alaska. Work during the first summers centered on a study of the relationships of the glacier to associated lakes, moraines, vegetation and animals, while this past summer was spent investigating the effects of the Good Friday earthquake on the glacier and adjacent areas.

The Martin River Glacier, although unusual in the sense that it does not portray the "ideal" alpine glacier so commonly pictured in text books, has many of the physical characteristics that we now associate with the ice sheets that covered most of North Dakota as recently as 12,000 years ago. The glacier features include large masses of ice buried beneath a veneer of superglacial debris (ablation till), abundant vegetation growing on this debris, numerous ice-walled and ice-bottomed lakes, and evidence of former superglacial lakes both on the glacier as well as adjacent to it.

From these investigations has come much information and satisfaction. Among the 11 papers in 5 professional journals published on data collected during these summers are observations on the glacier cycle, rapid water-level fluctuations of impounded glacier lakes, mode of flow of ice, propagation of fauna in ice-walled and proglacial lakes, and design and use of equipment to core the lake bottoms.

One Master's thesis on physical limnology of the largest of the impounded lakes has been completed and a Ph.D. dissertation on the faunal ecology of the proglacial and the ice-walled lakes is in progress. At least 13 additional papers are in varying stages of completion. Of prime importance was the greater appreciation of glacial activity and a clearer understanding of the genetic implications of certain glacial features common in North Dakota. A 20-minute movie depicting the field environment and activities of the personnel has been prepared and is available from the writers.

The Public Image of Science

Invited Comment from the Historian

In the last paragraph of **The First Fifty Years** (1) I stated:— "If free nations are to survive, their citizens no longer can remain indifferent to science. It is not enough to train more technicians or even to educate more scientists and engineers. We must make science an integral part of our modern culture."

Eight more years have passed since that was written. Today that accomplishment seems even more urgent; for the public image of science is a dangerously distorted image. Science is confused with technology, and scientists themselves often are pictured as heartless people who have not hesitated to create a frightful Frankenstein monster now bent on destroying man himself and all of his accomplishments in one final nuclear holocaust.

Much of this misunderstanding may be due to the fact, as recently stated by a brilliant commencement speaker, that modern society utterly has failed to develop the "discriminating mind." In this respect it has lagged fully 200 years behind the development of the "scientific mind." But, fundamentally it results from a lack of communication between the creative scientist and the masses. As active members of a State Academy of Science it would seem that individually and collectively we cannot escape the responsibility of acting as interpreters as well as investigators, correcting as best we can public misunderstandings and fallacies.

In their panic, many are urging that science be made to take a moratorium and wait until social and spiritual activities can catch up. Of course this is by no means the first time that the world has heard the clamor that science cease and desist. Even if such a thing were possible, history does not encourage us to believe that they would catch up. Again it is equally pointless to say that science and technology are destroying the world. Obviously, they enormously have increased man's power; but they have not created or increased man's folly. They merely have made this folly enormously more dangerous. If man is enslaved it is not his machines; but his own spirit that is responsible.

In this age of extreme and growing specialization, it is important to realize that science alone offers no complete panacea for human ills. It becomes effective only in harmonious cooperation with other human agencies. Humanity benefits enormously from the knowledge contributed by science; but needs even more a dominating will to be good and to direct the creative forces of science toward human benefits and idealistic ends. If science is to become an integral part of our modern culture, I believe that the scientist himself must take the initiative and try to establish the necessary communication.

Dr. G. A. Abbott, Historian
April 5, 1966

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