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CONTENTS

NOTES

ON THE CALCULATION OF ASSOCIATION CONSTANTS OF POLAR MOLECULES

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ABSTRACT. — A previously published method of calculating dipole association constants and dipole moments from dielectric constants of dilute solutions of polar substances in a nonpolar solvent is discussed. A computer program is presented which enables simpler and more reliable treatment of the data. Some extensions of the previous method are presented and discussed.

In 1964, Treiner, Skinner, and Fuoss published a method of evaluating self-association constants and dipole moments of polar molecules in nonpolar solvents. The method involves a graphical treatment of dielectric constants and concentrations of dilute solutions of the polar substances. Further, the method assumes antiparallel dimerization of the dipoles which results in a cancellation of the contributions which the permanent dipole moments make to the dielectric constants of the solutions. The method depends upon the fact that when dipole association occurs, the total polarization increases less rapidly than when there is no such association. Thus, the rate of increase of dielectric constant with increasing concentration is indicative of the extent of solute association.

In the calculations, a rather complicated function of dielectric constant and concentration, G (ϵ, c) and derived and its reciprocal is plotted against the product of G (ϵ, c) and c. The slope and intercept of the resulting plot can be used to calculate the association constant and dipole moment of the solute (Treiner, *et al.*, 1964). The Debye-Clausius-Mosotti approximation of volume polarization was used and the volume polarization of the dimer was assumed to be twice that of the monomer.

The work described in this paper was undertaken with two main objectives. First, a computer program was developed to carry out the tedious, repeated evaluation of the function G (ϵ, c). Because the calculated values of the association constant and dipole moment are sensitive to small errors in the function G (ϵ, c), least squares evaluation of the values of the slopes and intercepts is incorporated into the program.

Second, instead of assuming that the volume polarization of the dimer is twice that of the monomer, the computer program allows a variable factor to be introduced and its effect on dipole moment and association constant to be determined. Perhaps this factor should vary as an inverse function of the strength of the forces of molecular association. The results of these extensions of the dipole association theory of Treiner, Skinner, and Fuoss are presented and discussed.

Calculations

The calculations were carried out using an IBM 360/40 computer with the program written in FORTRAN IV. The program and some general comments on its use follow.
Comments:

1. The "Fuoss G" function,

$$G = \left[\frac{1.189 \times 10^{-21}}{Es + 2}\right]\left[\frac{(E - Es)}{C\,(E + 2)} - \frac{3RHO_{solv}}{(Es - 1)\ (\beta - Mwt/1000)}\right] - \alpha 10$$

   can be reproduced from the program by substitution of the intermediate function F of S. 13 into the equation in S. 14, with $Z = 2$.

2. The notation X(I) represents the value of property X for the "Ith" solution. Other previously undefined key symbols and their meanings follow.

   $E = \epsilon =$ dielectric constant of the solution

   $ES = E_s =$ dielectric constant of the solvent

   $RHO_{solv} =$ solvent density

   $BETA = \beta =$ a factor relating solvent and solution densities as $RHO = RHO_{solv} + \beta C$. (Densities varied linearly in the range used.)

   WMOL = Mwt = molecular weight of solute

   NPTS = number of data pairs to be plotted

   $ALPIO = \alpha_{10}$ = solute monomer electronic polarization

   Notation in the "least squares" segment of the program is standard, with "s" equivalent to "Σ".

3. The output format will result in clearly labeled results. The data read by S.05 can be any identifying material, such as solute name.

4. The program recycles until a blank card is read by S.02, the parameter read instruction.

5. As listed here, the program varies Z from 1.00 to 2.50 in increments of 0.25. Removal of S.09, S.10 and S.39, and replacement of S.11 by Z = 2. will elminate this procedure.

6. The factor 298.2 in S.30 is temperature (°K) and can be changed depending on the data used.

```
0001      DIMENSION C(15),E
          (15),G(15)
0002   1  READ(1,2)NPTS,ES,
          BETA,WMOL,RHO,
          ALPIO
0003   2  FORMAT(12,2X,F5.3,2X,
          F5.4,2X,F6.2,2X,
          F7.5,2X,E14.8)
0004      IF(NPTS)3,99,3
0005   3  READ(1,4)
0006  40  FORMAT(80H
         1                    )
0007      READ(1,5)(C(I),E(I),
          I=1,NPTS)
0008   5  FORMAT(F7.6,1X,F6.4)
0009      DO 9 II=1,7
0010      AI=II
0011      Z=.75+.25*AI
0012      DO 6 I=1,NPTS
0013      F=((1.1890E-21)/(ES+
          2.))*((E(I)-ES)/(E(I)
          +2.)-(ES-1.)*
         1(BETA-WMOL/1000.)*
          C(I)/(3.*RHO))
```

```
0014        6 G(I)=F/C(I)-(Z*
          ALP10)/2.
      C LEAST SQUARES FIT
      FOR PLOT OF 1/G VS CG
      FOLLOWS
0015      SX=0.
0016      SXY=0.
0017      SY=0.
0018      SXX=0.
0019      DO 7 I=1,NPTS
0020      X=C(I)*G(I)
0021      Y=1./G(I)
0022      WRITE(3,50)X,Y
0023   50 FORMAT(1HO,2E14.7
0024      SX=SX+X
0025      SXY=SXY+X*Y
0026      SY=SY+Y
0027   7  SXX=SXX+X**2
0028      SLOPE=(SX*SY-NPTS
          *SXY)/(SX*SX-NPTS*
          SXX)
0029      CEPT=(SXY*SX-SY*
          SXX)/(SX*SX-NPTS*
          SXX)
0030      DEBYE=SQRT(3.*1.3805
          E-16*298.2/CEPT)*1.E+
          18
0031      AKEQ=SLOPE/(2.*
          CEPT*CEPT)
0032      WRITE(3,14)
0033   14 FORMAT(//////80H
          1                    )
0034      WRITE(3,4)
0035      WRITE(3,20)Z
0036   20 FORMAT(1HO,4HZ =
          ,F5.2)
0037      WRITE(3,8)CEPT,
          SLOPE,DEBYE,AKEQ
0038   8  FORMAT(1HO,12HIN-
          TERCEPT = ,E14.8/9H
          SLOPE = ,E14.8/17H
          DIPOLE MOME INT =
          ,F6.2/24H ASSOCIATION
          CONSTANT = ,E14.8)
0039   9  CONTINUE
0040      GO TO 1
0041   99 STOP
0042      END
```

SAMPLE INPUT

```
.16260   3.203
.10880   2.870
.06239   2.595
.04128   2.471
.03021   2.405
.01999   2.349
.07273   2.660
.05348   2.545
.03838   2.456
.02492   2.379
 PNITROANILINE
10 2.233  .0406  138.12  1.02796
   .15000000E-22
```

SAMPLE OUTPUT

```
          X                    Y
0.9264186E-23   0,2689928E 22
0.1390161E-22   0.2760829E 22
0.1906553E-22   0.2805061E 22
0.2544358E-22   0.2858479E 22
0.7411161E-23   0.2697282E 22
0.1084541E-22   0.2785510E 22
0.1478510E-22   0.2792000E 22
0.2187612E-22   0.2851968E 22
0.3629993E-22   0.2997250E 22
0.5170795E-22   0.3144584E 22
PNITROANILINE
Z = 2.00
INTERCEPT = 0.26292032E 22
SLOPE = 0.99281275E 43
DIPOLE MOMENT = 6.85
ASSOCIATION CONSTANT =
  O.71810728E 00
```

Discussion

Dielectric constant, density, and concentration data of Treiner, Skinner, and Fuoss (1964) for solutions of p-nitroaniline (PNA), of m-nitrophenol (MNP), and of pyridinium dicyanomethylide (PDM) in dioxane were used in the computer calculations. A comparison of the results of

these workers and the results obtained in this work is shown in Table 1. The results of the calculations for solutions of PNA, for which Treiner, *et al.*, provide the most data, show almost identical values for the association constants. The small differences are due to the use of a least squares routine for obtaining the slope and intercept of the plot of $cG(\epsilon, c)$ against $1/G(\epsilon, c)$. The values of these functions were reported by Treiner, *et al.*, for PNA solutions and the values obtained using the techniques described in this work agree closely.

For the calculations on MNP and PDM solutions the agreement is not nearly as good. In the case of MNP, the association constant calculated by Treiner, *et al.*, is too high, as is the dipole moment. For PDM, the agreement between the dipole moments is good but the association constants differ greatly.

Since the value of the dipole moment is derived from the intercept of the $cG(\epsilon,c)$ *vs* $1/G(\epsilon,c)$ plot, it is obvious that the major errors in the calculations of Treiner, *et al.*, are in the intercept for MNP and the slope for PDM. These results show the extreme sensitivity of the calculations to small errors in the determinations of the slopes and intercepts of the plots of $cG(\epsilon,c)$ against $1/G(\epsilon,c)$. Because of the small variations in these functions in some cases, it would appear that reliable results are not likely to be obtained unless very accurate data are used and the calculations carried out by means other than graphical methods. However, it appears that in the case of MNP, the intercept is so much in error that the error must be caused by a systematic numerical error in carrying out the calculations. In fact, if in the equation giving $G(\epsilon,c)$ (E_s+2) is replaced by (E_s+1) the result is an error in $G(\epsilon,c)$ which would lead to the results published by Treiner, *et al.*

Intuitively, it seems quite likely that the volume polarization of the dimer should not be exactly twice that of the monomer because of mutual inductive effects. Consequently, this assumption was tested by allowing the volume polarization of the

TABLE 1.—Calculated Association Constants and Dipole Moments for PNA, PDM, and MNP.

Compound	μ		K Assoc., (l/mole.)		Slope x 10^{-43}		Intercept x 10^{-21}	
	TSF[a]	This Work[b]	TSF[a]	This Work[b]	TSF[a]	This Work[b]	TSF[a]	This Work[b]
PNA.....	6.91	6.85	0.8	0.72	1.06	0.99	2.59	2.63
PDM.....	9.2	9.30	3	1.34	1.28	0.55	1.46	1.43
MNP.....	4.38	3.75	0.37	0.26	3.10	3.92	6.43	8.76

[a] Average values obtained from a graph, dipole moments, and association contants published by Treiner, *et al.*, (1964) when used in the equations $10^{18}\mu = \sqrt{3KT/\text{Intercept}}$ and $K = \text{slope}/2(\text{Intercept})^2$.

[b] Values obtained using densities and dielectric constants published by Treiner, *et al.*, when the computer method is used.

dimer to vary from 1.0 to 2.5 times that of the monomer. The results of these calculations are shown in Table 2. It is immediately obvious that the calculated values of the dipole moment and association constant are rather insensitive to the variable factor, Z. Taking the volume polarization of the dimer to be the same as that of the monomer or taking it to be 2.5 times that of the monomer results in a difference in the calculated value of the association constant of only a few per cent. Because of the introduction of relatively large errors when graphical methods are used in the calculations, the effect of varying the factor would probably be unclear or unnoticed unless the calculations were carried out by means of a computer.

Throughout the calculations it became increasingly clear that the most critical quantity in evaluating the function $G(\epsilon,c)$ is $(E_o\text{-}E_s)$. When the dielectric constant of the solution differs very little from that of the solvent, this quantity approaches

TABLE 2.—Calculated Values of Association Constants and Dipole Moments Showing the Effect of the Z Factor.

Z	10^{-22} x Intercept	10^{-43} x Slope	Dipole Moment	K
		Data for PNA		
1.00	0.2579	0.9285	6.92	0.6979
1.25	0.2592	0.9441	6.90	0.7029
1.50	0.2604	0.9600	6.89	0.7079
1.75	0.2617	0.9762	6.87	0.7130
2.00	0.2629	0.9928	6.85	0.7181
2.25	0.2642	1.010	6.84	0.7234
2.50	0.2655	1.027	6.82	0.7287
		Data for MNP		
1.00	0.8259	0.03252	3.87	0.2384
1.25	0.8379	0.03405	3.84	0.2424
1.50	0.8504	0.03567	3.81	0.2466
1.75	0.8632	0.03740	3.78	0.2509
2.00	0.8764	0.03924	3.75	0.2554
2.25	0.8901	0.04121	3.72	0.2601
2.50	0.9041	0.04330	3.70	0.2649
		Data for PDM		
1.00	0.1412	0.5287	9.35	1.325
1.25	0.1417	0.5334	9.34	1.329
1.50	0.1421	0.5382	9.32	1.334
1.75	0.1425	0.5428	9.31	1.337
2.00	0.1429	0.5473	9.30	1.341
2.25	0.1433	0.5520	9.28	1.345
2.50	0.1437	0.5567	9.27	1.349

zero. Thus, subtraction of α_{10} can result in a $G(\epsilon,c)$ which is about zero or even a negative quantity. This can result in a negative value for the association constant, which has no physical significance.

Treiner, *et al.*, have stated that the energy of interaction of a pair of dipoles varies as the square of the dipole moment,

$$K \approx 10^{-3}\ Na^3\ e^{-u/kT}$$

where N is Avogadro's number, a^3 is the volume occupied by a pair of dipoles, and u is the energy of interaction. Therefore, a linear relationship should exist between log K and μ^2. Their report presents such a relationship. In view of the errors in the values they reported for the association constants, it appears somewhat fortuitous that the linear relationship was obtained. The present results do not enable one to say with certainty that the relationship is linear.

ACKNOWLEDGMENT

The authors would like to acknowledge the support and cooperation of the Computer Services of Illinois State University.

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Treiner, C., J. F. Skinner, and R. M. Fuoss. 1964. Dipole Association. J. Phys. Chem., *68,* 3406-3409.

Manuscript received June 21, 1970

MICROCLIMATE, VEGETATION COVER, AND LOCAL DISTRIBUTION OF THE MEADOW VOLE

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ABSTRACT. — A comparison was made of the microclimate of the meadow vole, *Microtus pennsylvanicus,* in situations with varying amounts of cover. Substrate and surface air temperatures were higher in areas with sparse cover (not utilized by meadow voles) than in optimal habitats with dense cover. Relative humidities were higher in the optimal habitats than in situations not inhabited. There was no correlation between absolute humidity and amount of vegetation cover. Only the higher temperatures in areas with sparse vegetation have the potential of causing the voles to avoid these sites. Relative and absolute humidities were not low enough in the sites with sparse cover to place a serious physiological stress on the voles. Humidity does not appear to play a significant role in the avoidance of areas with sparse vegetation by the meadow vole.

INTRODUCTION

The local distribution of the meadow vole, *Microtus pennsylvanicus,* has been correlated with moisture (Getz, 1961, 1963; Lantz, 1907; Findley, 1954; DeCoursey, 1957) and amount of vegetation cover (Getz, 1961, 1970b; Pearson, 1959; Mossman, 1955; Eadie, 1953; Zimmerman, 1965). The actual factors involved in these correlations have not been determined, however; microclimate, especially humidity, has been suggested as a possible factor (Getz, 1963).

Data comparing the microclimate of moist and dry graminoid situations (Getz, 1965, 1970a) indicate that humidity differences between such situations are slight. Humidity apparently is not responsible for higher population densities of the meadow vole in moist marshes. The above studies compared situations in which there was dense cover in both the moist and dry habitats. Microclimate data at the level of vole runways are not available from graminoid sites with varying densities of cover. The data presented in a prior paper (Getz, 1970b) which compared areas with dense and sparse cover were all taken in a wet marsh. Humidity differences would not be expected to be great in areas with saturated substrates.

During the summer of 1968 data were obtained in southern Wisconsin which aid in evaluating the significance of microclimate on the local distribution of the meadow vole. These data were obtained in an area with varying amounts of vegetation cover; the sites included those deemed optimal for the meadow vole as well as sites which the meadow vole did not utilize (Getz, 1970a).

DESCRIPTION OF STUDY AREAS

All work was conducted in the University of Wisconsin Arboretum, Madison, Wisconsin. Five stations, all in the southern part of the Grady Tract, were selected for the main

study; other stations in this tract and elsewhere in the Arboretum were spot-checked for comparison. The five main stations were all within 100 m of each other.

Station 1 was in a stand of *Agropyron repens*. The vegetation was 30-40 cm tall; litter formed a dense mat over the surface. Light penetration through the vegetation was only 0.02% (light penetration was used as an index of amount of cover present; see below and Table 1. Station 2 was in blue-grass (*Poa pratensis*) which formed a dense mat 20-30 cm above the surface. The total cover was less than that of Station 1 (1.1% light penetration). Topographically, both Stations 1 and 2 were 20-30 cm higher than the other 3 stations. Station 3 was in a low marsh which supported a pure stand of *Carex lanuginosa*. The vegetation at this station was 40-50 cm tall and had a cover value similar to that of Station 2 (Table I). Station 4 was in an area supporting a relatively sparse growth of various species of grasses and forbs. The average height of the vegetation was 30 cm and the cover approximately 1/10th that of Stations 2 and 3. Station 5 was in an area supporting a very sparse growth of several species of grasses and forbs. The height of the vegetation was 15-20 cm. The cover was so sparse that the surface was relatively exposed in most places; light penetration was high (17.5%).

In addition to the main study area, various other sites were spot-checked. One included a mowed blue-grass area. There was a very dense growth of grass in this area; the surface was completely covered with a mat of green vegetation. Vegetation height was only 2-5 cm at the site studied. The microclimate measurements were therefore taken in the "crown" of the vegetation, i.e., within the green leaves. Spot-checks were also made under a stand of young oak (*Quercus* spp.) shrubs (2 m tall). Crown coverage of the oaks in this site was 95-100%; there was no understory of grass or forbs. The measurements were made over leaf litter 2-3 cm thick.

TABLE 1.—Soil temperature, soil moisture, and light penetration at the main study stations. See text for techniques.

	Date	Station 1	2	3	4	5	Mowed Blue-Grass	Shrubs
Soil temp. (C)	2 Aug.							
Surface		24.6	26.8	26.8	29.3	30.4	32.8	25.5
—7 cm		20.8	22.4	21.4	24.1	25.9	29.5	21.8
Soil moisture								
g/280 cm³	25 July	112	116	142	163	153	108	103
	2 Aug.	102	107	133	147	139	55	68
% Saturation	25 July	35.4	34.7	42.6	50.1	43.7	26.0	29.1
	2 Aug.	30.8	31.5	40.2	46.5	39.7	13.4	22.5
Per cent light penetration[1]	2 Aug.	0.02	1.1	1.0	10.5	17.5	61.0	.....

[1] % of full sunlight.

Some data were obtained from a dry south-facing hillside which supported a moderately dense stand of bluegrass; the vegetation was 20-30 cm tall and formed a less dense cover (4.1% light penetration) than that at Station 2. Comparative data were taken in an adjacent site (2 m distance) which was mowed. The vegetation was much more sparse in this area than in the mowed area described above; the surface was almost entirely exposed (47.5% light penetration).

A few measurements were also taken in a tall-sedge (*Carex* sp.) marsh, the typical habitat of the meadow vole in this region, for comparison with the drier sites. The sedges grew in clones approximately 10-20 cm in diameter at the base and 1.0-1.5 m tall. The bases of the clones were 30-50 cm apart; the crown coverage was 100%, however. Light penetration was 1-2%.

Observations of vole sign and live-trapping were conducted in the above described vegetation types during the summers of 1967 and 1968. These indicated that Station 3 and the tall-sedge marsh area were the optimal habitats of the meadow vole (Getz, 1970a). Late in the summer of 1968 the vole population had also extended out into areas including Stations 1, 2, 4 and the unmowed hillside. Station 5, the two mowed areas, and the shrub area were not utilized by the meadow vole. These latter areas are not considered suitable habitats for this species.

Methods

A series of microclimate measurements (1 cm above the substrate surface) were made at approximately 2-hour intervals between 0745 and 1900 on 2 August 1968. Another series were made between 1430 and 2045 on 22 July 1968. Data were not obtained during the night since these observations and other spot-checks indicated all stations had similar temperature and relative humidities between 2000 and 0800; relative humidity was 95-100% during this latter period. Five other series of spot-checks of all stations were made during July and August. All measurements were made on clear days at least 3 days after the last rain.

Surface microclimate. — A thermistor psychrometer was used to obtain dry-bulb and wet-bulb readings at the desired sites. These data gave surface air temperatures and were used to calculate relative and absolute humidities.

Three sites (within a 10 m radius) were sampled at each station; three sets of dry and wet-bulb readings were taken at each site (all within a 1 m radius); there were therefore nine sets of readings from each station each time it was checked.

Measurements were taken 1 cm above the surface. The barrel of the psychrometer was inserted through the vegetation so as to keep to a minimum disturbance to the crown cover and litter layer. All measurements were taken at the exact same place during each series of checks. The hole in the litter was closed after each set of measurements was completed. One set of dry and wet bulb readings was also taken 1 m above the surface at each site. These provided comparison of above-surface air temperatures at the various stations.

The stations were visited in the same sequence; it took 35-40 min to make the complete set of measurements. When other stations were spot-checked, all were completed within 30 min after the measurements at the regular stations were finished. See also Getz (1970a) for

other microclimate data obtained in the same study area.

Soil temperature. — A Yellowsprings telethermometer with an attached hypodermic thermistor probe was used to measure substrate temperatures. Substrate temperatures were measured at the same number of sites as described above. Three sets of surface (sensitive portion of the probe only touching the surface) and subsurface (7 cm below the surface) temperature readings were taken at each site.

Soil moisture. — a gravimetric method (Getz, 1970a) was used to estimate substrate moisture; data were obtained both in terms of per cent saturation and g water/280 cm^3 of substrate. One 280 cm^3 plug of substrate was taken at each station. Two sets of samples were taken, 25 July and 2 August 1968.

Light penetration. — Amount of light penetration through the vegetation was used as an index of total vegetation cover at each station. A previously described device (Getz, 1968) was used to measure light intensity above the vegetation and at the surface of the substrate. This device creates a minimum of disturbance to the vegetation crown and litter. One above-surface reading and 12 surface readings were taken at each station. The two most aberrant surface readings at each station were omitted from the calculations. Readings were taken 1100-1200 on a cloudless day.

Results

Main Study Area

Substrate moisture. — There were frequent showers in southern Wisconsin during the summer of 1968. As a result, substrate moisture was relatively high at all stations. There was no consistent correlation between amount of vegetation cover and substrate moisture (Table 1). In general the two areas with less vegetation had higher substrate moisture while those with the greatest amount of cover were drier. The former stations were slightly lower topographically than the latter two. Although Station 3 was the lowest of the five, the substrate moisture of this station was intermediate between that at the other stations. The relatively slight microrelief differences (combined with the frequent rains) appeared to be as important as did the amount of vegetation cover in influencing substrate moisture.

Substrate temperature.—Both surface and subsurface temperatures tended to be correlated with the amount of vegetation cover at each site; mid-day temperatures were higher and diel fluctuations greater where the vegetation was sparse. The maximum difference in substrate surface temperatures between stations was 5.8 C; maximum subsurface difference was 5.1 C (Table 1). These differences are essentially the same as those observed in the surface air temperatures (see below). Although substrate temperatures were not measured during the night, there would be less difference in substrate temperature during these times than during mid-day. Substrate temperatures would also vary little on overcast days.

Surface air temperatures. — Air temperatures 1 m above the surface did not differ significantly between any of the various stations studied. Wind currents apparently caused enough mixing of air to prevent development of different temperature profiles at each station.

As might be expected, surface air temperatures in general directly reflected the amount of vegetation cover; higher daytime temperatures

and greater diel fluctuations occurred at the sites with lesser cover than at those with greater cover. Differences in temperatures between the five stations occurred only during the period of 1000 to 1600. The maximum recorded difference was 7.5 C; observed differences were normally less than this, however (Figs. 1, 2; Table 2).

TABLE 2. — Spot checks of air temperatures (C) 1 cm above the surface; values represent an average of 9 readings at each station.

Date	Time	Station					Mowed Blue-Grass	Shrubs
		1	2	3	4	5		
21 July	1420–1510	28.5	29.4	29.6	29.9	30.0	28.9	28.2
23 July	0710–0750	14.8	15.2	14.6	15.2	15.6	16.2	14.9
25 July	1420–1450	22.4	23.2	25.4	26.9	27.3	28.4	24.9
29 July	1350–1430	22.1	25.0	22.5	25.8	26.8	24.9	23.7
26 August	1430–1540	18.6	20.3	18.7	19.7	24.0	24.1	20.3

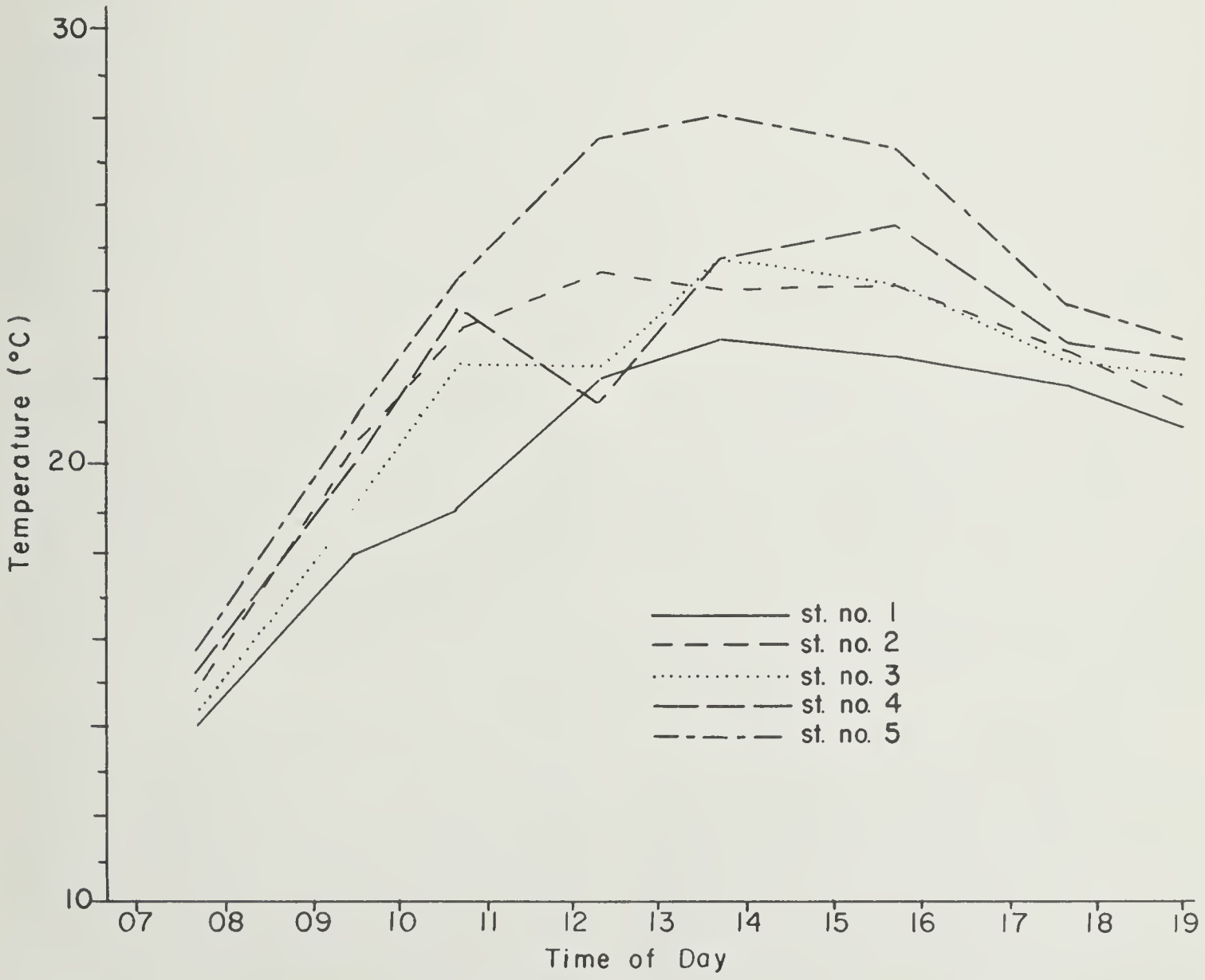

FIGURE 1. Air temperatures 1 cm above the surface, 2 August 1968.

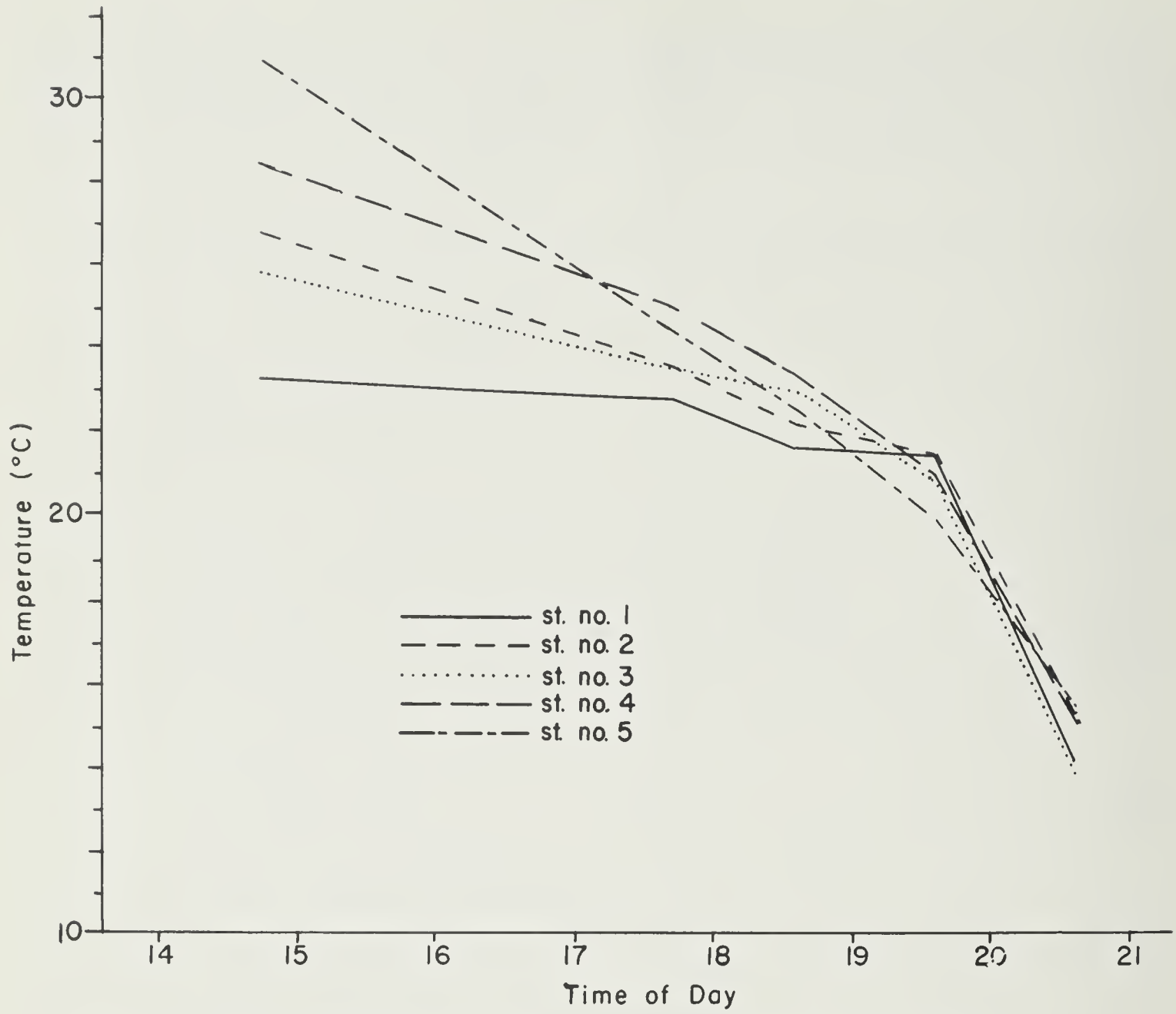

FIGURE 2. Air temperatures 1 cm above the surface, 22 July 1968.

Spot checks revealed that at night and on overcast days there was essentially no difference in surface air temperatures between the stations with sparse cover and those with considerable cover.

Relative humidity. — There was no significant difference in relative humidity 1 m above the surface between any of the stations studied.

Major differences in surface relative humidity between the five stations existed only during the period of 1000-2000; at other times differences were less than 5% (Fig. 2; Table 3). Between 2000 and 0800 relative humidity was approximately 95% at all stations.

There appeared to be more similarities between relative humidity and the amount of cover than between relative humidity and substrate moisture (Figs. 3, 4; Tables 1 & 3.) The lowest relative humidities were observed where cover was sparse and the highest where there was the most cover.

The maximum difference in relative humidity between the station with the sparsest cover and that with the most dense cover was 20%. The maximum difference between other stations was only 11%, however. Even at the stations with sparse cover, relative humidity seldom went below 60%; normally humidities

TABLE 3. — Spot checks of relative humidity (%) 1 cm above the surface; values represent average of 9 readings at each station.

Date	Time	Station					Mowed Blue-Grass	Shrubs
		1	2	3	4	5		
21 July	1420–1510	82.7	75.7	71.2	67.8	62.7	81.4	70.5
23 July	0710–0750	98.3	97.3	97.0	95.7	95.7	93.7	94.0
25 July	1420–1450	90.0	90.8	81.7	87.0	75.2	90.8	79.3
29 July	1350–1430	84.8	80.9	77.9	80.2	71.1	93.0	69.7
26 August	1430–1540	77.6	76.1	73.9	79.8	67.1	87.0	59.7

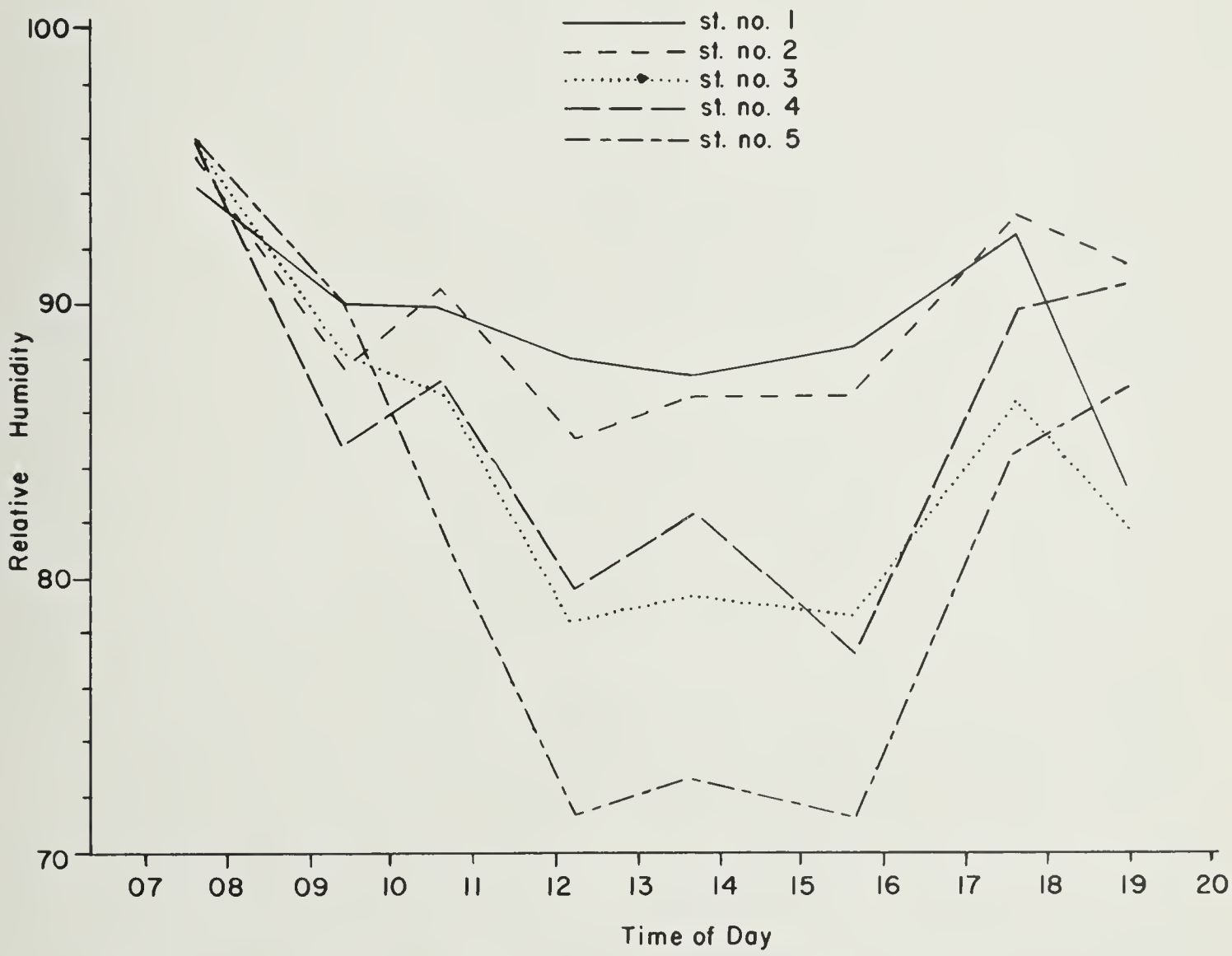

FIGURE 3. Relative humidities 1 cm above the surface, 2 August 1968.

were at least 70%. It therefore appears that differences in relative humidity between optimal habitats and situations where the meadow vole does not occur are not great.

Absolute humidity. — Absolute humidities were highest during the day. During the night and on overcast, humid or rainy days the air at the surface was essentially saturated regardless of the amount of vegetation cover. Absolute humidity at these times was essentially a function of surface air temperature. Ab-

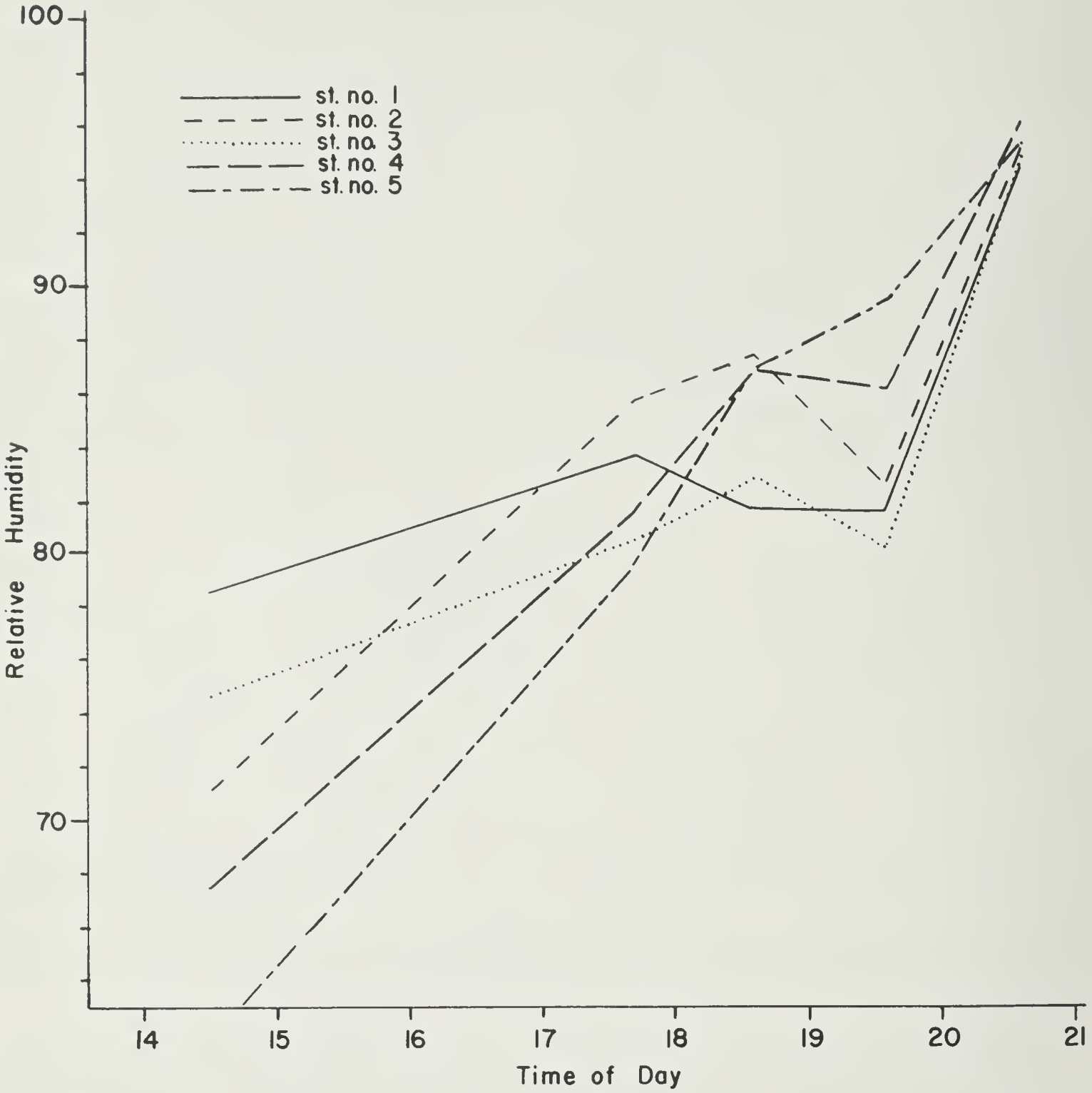

FIGURE 4. Relative humidities 1 cm above the surface, 22 July 1968.

solute humidity differences between stations existed only during the period of 0900 to 1900. The maximum difference between any two stations was 5.8 mg/l normally the differences were much less than this (Figs. 5, 6; Table 4).

Although there was no distinct correlation between absolute humidity and amount of vegetation cover, the higher absolute humidities frequently occurred at the stations with lesser cover. This is a reflection of the significance of temperature on absolute humidity; the higher midday surface air temperatures (and thus the greater capacity for the air to hold water) in the area with less cover more than compensated for the lower relative humidity at these sites.

Other Areas

Mowed blue grass. — Air temperatures 1 cm above the surface were not significantly higher than those

TABLE 4. — Spot checks of absolute humidity (mg/l) 1 cm above the surface; values represent average of 9 readings at each station.

Date	Time	Station					Mowed Blue-Grass	Shrubs
		1	2	3	4	5		
21 July	1420–1510	22.5	25.5	22.5	26.6	25.6	29.8	20.3
23 July	0710–0750	13.4	13.5	13.1	13.1	13.4	13.6	12.7
25 July	1420–1450	19.7	18.8	21.7	25.5	22.3	22.0	16.5
29 July	1350–1430	18.2	21.1	17.5	21.9	20.3	23.9	16.3
26 August	1430–1540	13.1	14.4	13.0	14.7	16.3	21.1	11.5

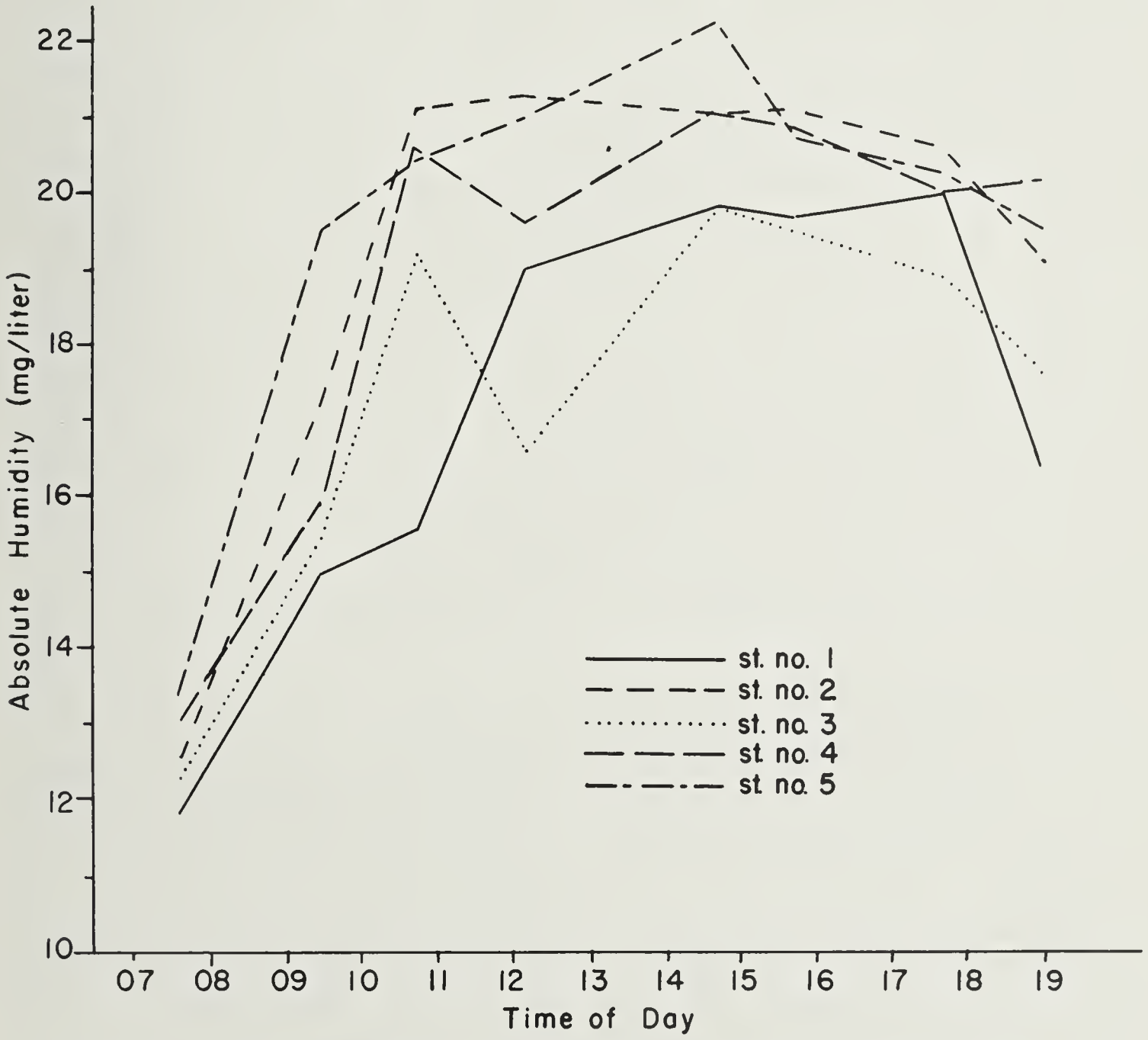

FIGURE 5. Absolute humidities 1 cm above the surface, 2 August 1968.

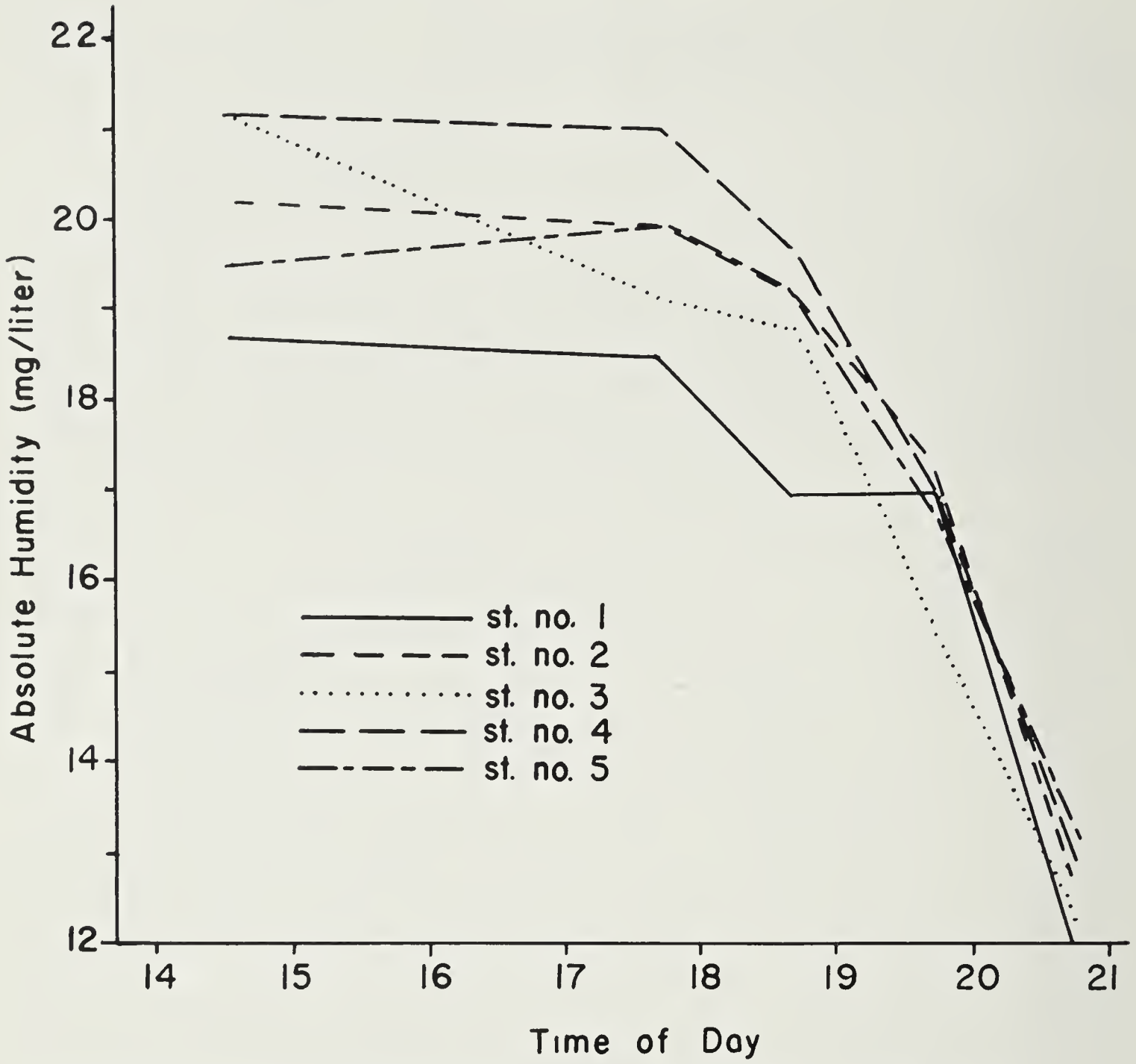

FIGURE 6. Absolute humidities 1 cm above the surface, 22 July 1968.

at other stations with more cover (Tables 1 and 2). Since measurements taken 1 cm above the surface were essentially in the vegetation "crown", transpiration cooling may be responsible for the lower air temperatures.

Relative humidity 1 cm above the surface was normally as high as or higher than (up to 10% higher) that at any station with dense cover (Table 3). The measurements were probably high owing to moisture released via transpiration. The absolute humidities in the mowed area were normally higher than those at the stations with the most cover (Table 4).

Shrub area. — Surface air temperatures were lower than most of the stations with graminoid cover (normally temperatures at the former were intermediate between the extremes of the five main stations). The crown cover of the shrubs shaded the area sufficiently to modify surface temperatures (Table 2).

Surface relative humidity was essentially the same as that recorded at Station 5 (Table 3). The combined low relative humidity and low temperatures resulted in lower ab-

solute humidities in the shrub area than were normally recorded in the graminoid areas (Table 4).

Dry hillside. — In general the surface air temperatures in the unmowed site were higher than at any of the five main stations. The relative humidities were intermediate to those observed at the other sites (except for the 26 August reading); absolute humidities were similar to those at the five main stations (Table 5).

Surface air temperatures were up to 4.2 C higher and relative humidities as much as 21% lower in the mowed site than in the unmowed area. Absolute humidities were normally 2-5 mg/l higher in the mowed area. The differences from the other mowed area undoubtedly resulted from the more sparse growth of vegetation in the hillside area. When the grass was mowed, the remaining vegetation was so sparse that transpiration was not as significant a factor in the microclimate as it was in the other mowed area.

Marsh. — Although only 3 spot-checks were made of microclimate at this site, they did not indicate significant differences from drier sites with dense vegetation (Table 5). In general, relative and absolute humidities were actually slightly less than at sites with dense cover. The green part of the leaves in this area were some distance above the surface (the parts close to the surface were dried out) so that there was little transpiration near the surface.

The more open character of the vegetation permitted drying of the soil surface; this apparently reduced evaporation from the surface. The humidity at the surface in this type of marsh vegetation is therefore lower than that at drier sites where there is dense, but low, vegetation cover. In any event, air humidity near the surface was not higher in this type of marsh (optimal habitat for the meadow vole) than in drier upland sites (marginal habitats of the meadow vole).

Discussion

As would be expected there was in general an inverse relationship between amount of vegetation cover and substrate and surface air temperatures. The greater the cover, the lesser the extremes in substrate and surface air temperatures. Relative humidity was more positively related to vegetation cover; sites with more cover had higher relative humidities.

Table 5. — Surface air temperatures and relative (RH) and absolute (AH) humidities at other sites in the vicinity of the main study area. Values represent averages of 9 readings at each site.

Date	Time	Mowed Hillside			Unmowed Hillside			Marsh		
		Temp. (C)	RH %	AH mg/l	Temp. (C)	RH %	AH mg/l	Temp. (C)	RH %	AH mg/l
25 July	1500	30.1	70.8	22.4	27.9	80.9	19.5	.....	.....	.....
29 July	1445	28.2	62.4	19.2	25.7	79.0	21.3	.....	.....	.....
2 August	1000	26.4	74.6	15.3	22.2	92.0	20.0	22.2	86.0	18.5
2 August	1620	27.9	55.9	17.9	25.6	77.2	20.1	22.5	80.0	19.7
26 August	1610	26.4	50.7	14.3	23.2	58.5	13.5	21.7	74.6	15.9

Owing to the higher air temperatures in sites with less cover, there was no direct relationship between cover and absolute humidities. The absolute humidity in the sites with the least cover were normally slightly higher than in those with the most cover, however.

The magnitudes of difference between the microclimate of the various sites included in the present study were relatively small. The vegetation types studied ranged from optimal habitats to those not inhabited by the meadow vole; the microclimate of the optimal habitats of this species is therefore only slightly different from that of other vegetated situations not normally utilized. Even when sites such as closely mowed areas are considered, microclimates do not differ greatly from situations in which the meadow vole does occur. In close-clipped, dense vegetation the microclimate actually may be similar to that in optimal habitats.

Of the factors studied, air and substrate temperatures varied the most. The 5 to 7 C differences that were recorded may be sufficient to make the sparsely vegetated sites unsuitable for the meadow vole. Data pertaining to the temperature tolerances and preferences of the meadow vole are not available, however.

Relative humidity differences between the various cover types were at the most 20% and normally in the order of only 10%. These differences do not appear to be great enough for relative humidity to place a physiological stress on the voles, if they were to attempt to inhabit situations with sparse cover. Although the water requirements of the meadow vole vary with relative humidity, such requirements are significantly higher only at relative humidities of less than 50% (Getz, 1963). The lowest humidity recorded in the present study was above 50% (normally they were above 70%).

Differences in microclimates occurred only for about a 9-10 hr period during mid-day, and then only on clear days. On overcast days and from 2000 to 1000 there was essentially no difference in the microclimate between sparse-cover and dense-cover situations. Any stress placed on the meadow vole by microclimatic differences would therefore be further reduced. It is possible, however, that the higher temperatures during mid-day on clear days would be sufficient to keep the voles from inhabiting areas with sparse cover.

Another study in same area (Getz, 1970a) also failed to find sufficient microclimate differences, humidity in particular, to account for the local distributional pattern of the meadow vole.

The results of this study and previous ones (Getz, 1965; 1970a, 1970b) make it rather obvious that relative humidity is not a significant factor in the local distribution of the meadow vole. This applies to situations involving varying amounts of cover as well as moist and dry habitats. The magnitude of habitat differences and the amount of time such differences do exist are not sufficient to place a significant stress on the voles.

Studies of temperature tolerances and preferences are required to estimate the actual significance of temperature on the local distribution of the meadow vole. It is probable that other factors such as behavioral preferences, and predation (in the case of varying cover; Getz, 1970b) may also be at least partly responsible for the association of the meadow vole with dense vegetation.

Acknowledgments

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ASSOCIATION OF PRODIGIOSIN WITH OUTER CELL WALL COMPONENTS

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Abstract. — Prodigiosin was extracted along with outer membrane glycoprotein by sodium dodecyl sulfate and sodium deoxycholate from isolated cell envelopes of *Serratia marcescens* 08. Part of the pigment was separated from the glycoprotein by organic solvent extraction and by Sephadex G-200 column chromatography.

In *Serratia marcescens* there is a tripyrrole pigment, prodigiosin, which absorbs at 535-540 mu (red) in acid medium but becomes orange in color (470 mu) in alkaline medium. Prodigiosin has been studied by many workers because of its suspected biological properties. Castro (Castro, *et al.*, 1967) noted that prodigiosin was active against various pathogenic bacteria and fungi, while Allen (Allen, 1967) found that it acted as an auto-oxidizable electron acceptor and suggested its possible role in cellular respiration. Although the biosynthesis of the pigment has been worked out to some extent (Morrison, 1966), the exact location of biosynthesis and its site of association with cell particles remains unclear. Castro (Castro, *et al.*, 1959), speculated that prodigiosin was present in the cell as the salt of a fatty acid which associated closely with the lipid of cell membrane. However, the observation that the concentration of prodigiosin paralleled that of N-acetylhexosamine in the isolated cell envelope indicated that the pigment may be associated with the cell wall (Williams and Purkayastha, 1960). However, it is not certain whether the pigment is located in the outer or inner membrane of the cell envelope (DePetris, 1967). An extracellular glycoprotein containing prodigiosin has also been isolated and characterized (Yoshida, 1967). More recently, prodigiosin was found covalently-bound to a glycoprotein of high molecular weight (Cruz-Camrillo and Sanchez-Zuniga, 1968). This report represents the study of the association of prodigiosin with the outer soft layer of the cell envelope.

Material and Methods

The cell walls were isolated from *Serratia marcescens* 08 (grown on an enriched medium) according to the procedure of Williams (Williams and Purkayastha, 1960). The cell wall preparation (fr. CW08) was extracted with dissociating reagents such as sodium dodecyl sulfate (SDS), sodium deoxycholate (SC), and guanidine hydrochloride (GH). One hundred milligrams of fr. CW08 was stirred in 50 ml of 0.5% SDS (pH 7.5) for one hour at 50°. After centrifugation at 10,000 r.p.m. for 20 minutes, the supernate and sediment fractions were separated and SDS was removed by dialysis. After lyophilization, the supernate fraction

(fr. SDS-S) and the sediment fraction (fr. SDS-P) were recovered. Similarly, extraction with 1% sodium deoxycholate yielded fractions SC-S and SC-P. The condition for guanidine hydrochloride extraction varied slightly. Three molar guanidine hydrochloride solution was used at room temperature and fractions GH-S (supernate) and GH-P (sediment) were recovered. Protein (Lowry, *et al.*, 1951), glucosamine (Rondle and Morgan, 1955), hexoses (Koehler, 1952), and uronic acids (Bitter and Muir, 1962) were determined. Total lipids were extracted with chloroform; methanol (2:1 v/v) in a soxhlet apparatus and determined gravimetrically. Other organic solvents extractions were performed in a similar manner. Presence of prodigiosin was monitored with a Beckman DK-2A UV-Visible spectrophotometer after fractions SDS-S, SC-S, and GH-S were extracted with ethanol:0.1 N HCl (9:1 v/v) mixture. Double diffusion technique in agar gel (Ouchterlony, 1962) was used to study the immunological activities of the various fractions against anti-whole cell serum. Endotoxin was isolated according to the modified method of Boivin (Tsang and Rilett, 1970).

Column chromatography of fraction SDS-S was performed in Sephadex G-200 which was equilibrated with 0.5% SDS. Thirty milligrams of the sample were dissolved in 3 ml of 0.005 M sodium phosphate buffer, pH 7.4 in 0.2% SDS. The column was eluted by the same buffer and monitored by protein determination.

Results and Discussion

The yields and the results of the chemical analyses are presented in Table 1.

Table 1.—Chemical Composition of Extracts from Isolated Cell Envelope from *Serratia marcescens* 08.

Fractions	Yield Per Cent	Protein Per Cent	Total Lipid Per Cent	Total Carbohydrate Per Cent	Hexoses Per Cent	Hexosamine Per Cent	Uronic Acid Per Cent
SDS-S.....	85	58.0	17.5	9.4	4.6	2.6	2.2
SC-S.....	65	26.3	25.0	10.3	4.3	3.4	2.6
GH-S.....	16	65.0	N.D.	8.2	3.0	3.8	1.4

It appears that the extracts were all glycoprotein in nature. Although fraction GH-S has the highest content of protein (65%) and fraction SC-S has the highest total carbohydrate content (10.3%), the extracting effectiveness of sodium dodecyl sulfate was far superior to the other two reagents. Guanidine hydrochloride proved to be a relatively poor extractant for the surface material (16%), and it also failed to remove any of the prodigiosin. On the other hand, both sodium dodecyl sulfate and sodium deoxycholate completely removed the pigment

from the isolated cell envelope (Table 2). Despite the fact that previous evidence indicated that hexosamine and prodigiosin were present in the same cellular structure, such as the cell envelope (Williams and Purkayastha, 1960), our results of analysis with the extracts (fractions SDS-S, SC-S, and GH-S) do not show such parallel correlationship of content of hexosamine with the presence of pigment (Table 2). Both fractions SDS-S and SC-S were pigmented, but their hexosamine content was lower than their corresponding colorless sediment fractions (fr. SDS-P and fr. SC-P). This observation suggested that prodigiosin was associated with certain components which could be selectively removed from the isolated cell envelope by mild dissociating reagents. These components may possibly be the glycoproteins and/or other conjugated macromolecules, such as lipoglycoprotein complexes which are present on the outer soft layer of the wall. Indeed, glycoproteins have been removed from the outer cell membrane by sodium dodecyl sulfate (Weinbaum and Markman, 1966). It is surprising, however, that guanidine hydrochloride failed to perform the same function as the other two reagents since it has also been used for removing outer surface components from walls of other Gram-negative bacteria (Kushner, 1969).

In order to demonstrate the immunochemical similarities of the extracted fractions, namely fractions SDS-S, SC-S, and GH-S and lipopolysaccharide-protein complex (endotoxin) (Tsang and Rilett, 1970) from *S. marcescens* 08, anti-whole cell serum was allowed to react with these fractions by the double diffusion technique. All fractions tested with the exception of fr. GH-S gave one precipitate line which cross-reacted with each other. It is interesting to note that antigenicity parallels with the presence of prodigiosin (Table 2). It is possible that guanidine hydrochloride extracted the non-antigenic cellular glycoprotein rather than the outer-membrane glycoprotein, while the other reagents removed glycoproteins which have at least one common antigenic component with endotoxin.

In order to study the possible linkage between prodigiosin and the cell envelope glycoproteins, fractions CW08, SDS-S, and SC-S were extracted with acetone and ethanol, as well as chloroform-methanol (2:1 v/v). After organic solvent extractions, the residues were colorless, while the extracts gave the charac-

Table 2.—Correlationship between Pigmentation, Hexosamine Content and Immunological Activities of Extracts and Residues.

Fractions	Pigmentation	Hexosamine Per Cent	Immunological Activities
SDS-S	+	2.6	Positive, 1 line
SDS-P	—	5.6	N.D.*
SC-S	+	3.4	Positive, 1 line
SC-P	—	4.6	N.D.
GH-S	—	3.8	Negative
GH-P	+	5.3	N.D.
Endotoxin	+	12.2	Positive, 1 line

* N.D. = not done

teristic spectra of prodigiosin. Cruz-Camarillo claimed that prodigiosin was conjugated with a glycoprotein to form a soluble complex which could be broken down with ethanol (Cruz-Camarillo and Sanchez-Zuniga, 1968) into a colorless component and a colored compound of low molecular weight. Our observation does not agree entirely with the implication of a possible existence of covalent linkage only between the pigment and the glycoproteins. It is likely that at least part of the pigment was associated with the macromolecule by hydrophobic bonds and/or salt bridges.

In the column fractionation experiment we obtained a single peak which gave positive protein reaction. Figure 1 shows the elution pattern. After dialysis and lyophilization, a colorless amorphous material was recovered in 90% yield. The pigment remained bound to the column, and attempts to elute it failed. A similar result was obtained when the column was equilibrated with 1% sodium deoxycholate and eluted with 0.2% sodium deoxycholate in the same buffer. The results of these experiments strengthen our belief that not all of the prodigiosin is bound to the glycoprotein.

Thus, it appears that prodigiosin is associated with the outer cell wall component which is glycoprotein in nature. This glycoprotein shares at least one antigenic component with the lipopolysaccharide-protein (endotoxin) complex. Whether the pigment is synthesized by the membrane enzymes or synthesized in the cytoplasm and transported to the outer membrane of the cell wall remains to be elucidated.

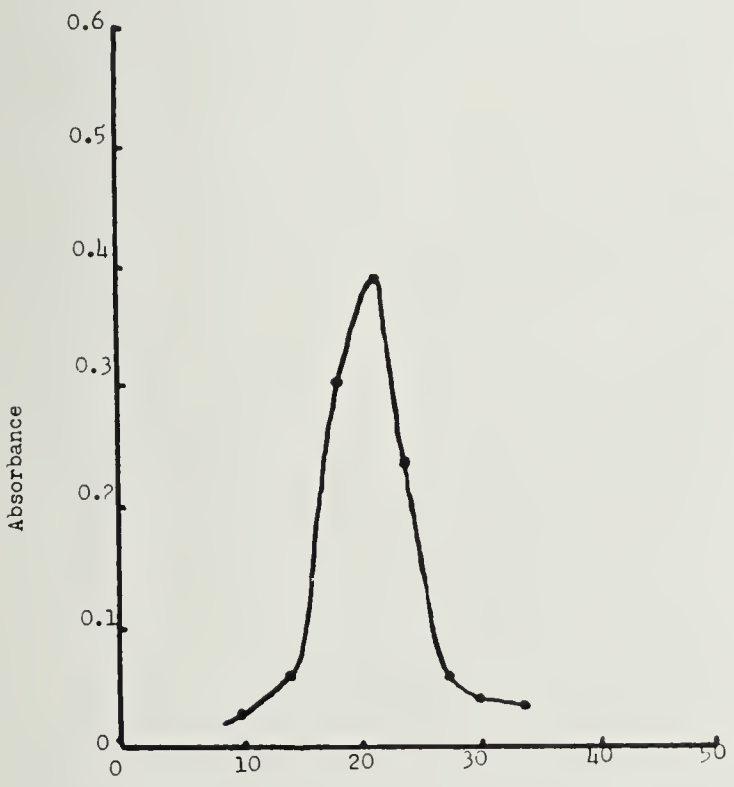

FIGURE 1. Sephadex G-200 Column Chromatography of Fraction SDS-S. Volume collected 3 ml per tube. Column was monitored by protein determination on 0.2 ml aliquots.

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WOODY VEGETATION OF HART MEMORIAL WOODS, CHAMPAIGN COUNTY, ILLINOIS

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Abstract. — The Hart Memorial Woods is one of the more xerophytic examples of upland streamside forests in the Prairie Peninsula of east-central Illinois. Upland soils are well developed and support a mixed stand of white oak (*Quercus alba* L.), black oak (*Q. velutina* Lam.), and red oak (*Q. rubra* L.). Composition and ecological trends of three physiographic units (bottomland, upland, and mixed) are discussed. Hickories appear to be becoming more important stand components, based on numbers of seedlings and saplings present. As is characteristic of other woodlands studied, the oaks are not reproducing well. Elm mortality has been extremely heavy in the bottomland and mixed physiographic units.

The Hart Memorial Woods, located along the east bank of the Sangamon River near Mahomet, Illinois, is one of the more xerophytic examples of upland streamside forests in the Prairie Peninsula of east-central Illinois. The woodland is a remnant of a much larger timbered area that was about three miles wide and extended northward for six miles along the Sangamon from Mahomet (Spaeth, 1963). Hart Woods was acquired by the University of Illinois in 1965 for its system of natural areas. These areas now complete a sequence from the wettest to the driest upland forest sites in the area (Boggess, 1964; Boggess and Bailey, 1964; Boggess and Geis, 1966 and 1967). Among the natural areas, Hart Woods is unique in that it provides a transect from moist bottomlands, formerly dominated by elm, to an upland series occupied largely by red oak, white oak, and black oak as the sites become progressively drier. The woody vegetation of this woodland and its general ecological status are discussed in this paper.

Description of Area

Hart Memorial Woods occupies the N½ and E 16 acres of the S½, NE¼, SW¼, S36, T21N, R7E, 3rd P.M. (40° 14′ N. Lat.; 88° 21′ W. Long.), Champaign County, Illinois. The area lies between elevations of approximately 672 and 703 feet above sea level and includes a level bottomland and slopes up to 30 percent. Two small streams pass through the area and empty into the Sangamon River. One is intermittent, while the other has some flow except during prolonged dry weather.

Soils

Some of the most highly developed soils in the prairie-forest border of central Illinois occur on the uplands of Hart Woods. They are recognized as Gray-Brown Podzolic soils in the classification of Thorp and Smith (1949) and as Hapludalfs in the current system detailed in the 7th Approximation (Soil Survey Staff, 1960). The Birkbeck and Camden series are the most prevalent upland soils in the woodland, and both developed in loess under the influence of forest vegetation. Birkbeck is moderately well-drained and devel-

oped in 36 to 60 inches of loess over glacial till. Camden, a shallower soil, developed in 15 to 36 inches of loess over glacial outwash and is well-drained. Horizons in both series are distinct and easily differentiated. The dark gray to brown surface layers are silt loams and grade into yellowish brown silty clay loam subsoils. Profiles are acid throughout.

One representative profile each of Birkbeck and Camden was excavated, described, and sampled. Data on selected physical and chemical characteristics are shown in Table 1. These data clearly indicate that the better upland sites are associated with the Birkbeck soils and that they support a more mesic vegetation than does Camden. Cation exchange capacity and percent base saturation is greater throughout the Birkbeck profile indicating its superior nutrient status as compared with Camden. Moisture relations are more favorable in Birkbeck due to generally higher amounts of fine-textured materials at all depths, greater amounts of organic carbon in the A horizon, and character of materials underlying the solum. Within the woodland, Camden soils are associated with stands that run heavily to black oak.

TABLE 1.—Selected physical and chemical characteristics of Camden silt loam and Birkbeck silt loam.

Horizon	Depth, Inches	Texture, %				pH	Organic Carbon, %	Cation Exch. Cap., me/ 100 gm.	Base Saturation, %
		> 2 mm.	Sand	Silt	Clay				
Camden silt loam									
A1	0– 3	0.13	8.8	71.4	19.8	4.86	2.31	9.71	36.2
A21	3– 6	0.05	9.2	69.0	21.8	4.71	0.91	8.80	39.8
A22	6–12	0.10	9.0	66.2	24.8	4.90	0.46	9.30	58.1
B1	12–18	0.11	7.0	62.0	31.0	4.97	0.25	13.47	72.5
B21	18–24	0.03	6.6	56.6	36.8	4.99	0.25	18.53	75.2
B22	24–31	0.00	11.2	53.0	35.8	4.88	0.14	18.56	75.2
II B23	31–42	0.00	21.1	50.1	28.8	4.68	0.12	17.38	71.2
II B31	42–63	0.20	22.0	53.0	25.0	4.70	0.10	13.01	66.4
II C1	63–75+	0.28	68.2	18.3	13.5	4.64	0.03	8.86	68.4
Birkbeck silt loam									
A1	0– 4	0.45	5.8	67.8	26.4	4.98	3.18	26.55	91.7
A21	4– 8	0.61	6.0	66.0	28.0	4.90	1.24	15.54	85.8
A22	8–12	0.60	4.1	65.4	30.5	4.89	0.49	14.68	85.0
B1	12–18	0.16	3.6	64.0	32.4	5.07	0.27	21.70	84.8
B21	18–26	0.04	2.5	59.5	38.0	5.10	0.14	30.10	86.0
B22	26–36	0.06	1.9	57.0	41.1	4.90	0.14	22.89	81.6
B31	36–52	0.07	8.0	60.4	31.6	4.87	0.08	21.74	84.8
II C1	52–59	2.29	13.9	60.7	25.5	4.80	0.10	20.96	92.8
II C2	59–68	9.86	24.5	48.1	27.4	4.78	0.07	13.47	97.0
II C3	68–95+	12.00	31.2	46.2	22.6	4.59	0.06	28.29	100.0

Bottomland soils include both medium-textured (loam and silt loam) and moderately fine-textured (silty clay loam) alluvial soils. Medium-textured soils are represented by Otter silt loam, and the fine-textured by Sawmill silty clay loam. Both of these soils are imperfectly drained. New soil material is added to the bottomland almost every year, as spring flods deposit soil from the intensively cultivated uplands in the surrounding area. Because of this continuing deposition, soil horizons are poorly defined. Nutrient and moisture conditions are quite favorable for tree growth.

Methods

Prior to inventory in 1965, the woodland was permanently divided into 50-meter square blocks. Inventory data were kept separately by quarter-blocks, designated by dividing each block along the diagonals. Diameters at 4½ feet above the ground (DBH) of all woody vegetation 2.6 inches and above, were measured and tallied by species. Dead-standing and dead-down trees were also measured and identified where possible. Four sets of nested circular plots (1/100 and 1/1,000 acre) were randomly located in each sample block. Small saplings (1 and 2 inches DBH) were measured on the larger plots, and seedlings on the smaller plots. Seedlings were tallied by species and height class, those less than one foot tall, and those greater than one foot in height but less than 0.6 inch DBH.

Stand data were developed by summarizing information for quarter-blocks that fell completely in the bottomland, upland, or partly in both. The latter is designated as a "mixed" topographic unit and includes areas that are transitional between the two main topographic units. Although the "mixed" category is highly artificial, it allows a more concise interpretation of the bottomland and upland data.

Results

A total of 34 woody species were tallied. These are shown, along with their density and frequency by size class, in Table 2. The number of trees and basal area per acre, relative density, relative dominance, and Importance Value for the 10 leading species in each topographic unit are shown in Table 3. As used here, Importance Value (IV) is that defined by McIntosh (1957) and is the sum of the relative dominance and relative density (Boggess and Geis, 1967). The leading dominant (species with the highest IV) is shown for each quarter-block in Fig. 1, and a breakdown for these same species into broad diameter classes is shown in Table 4.

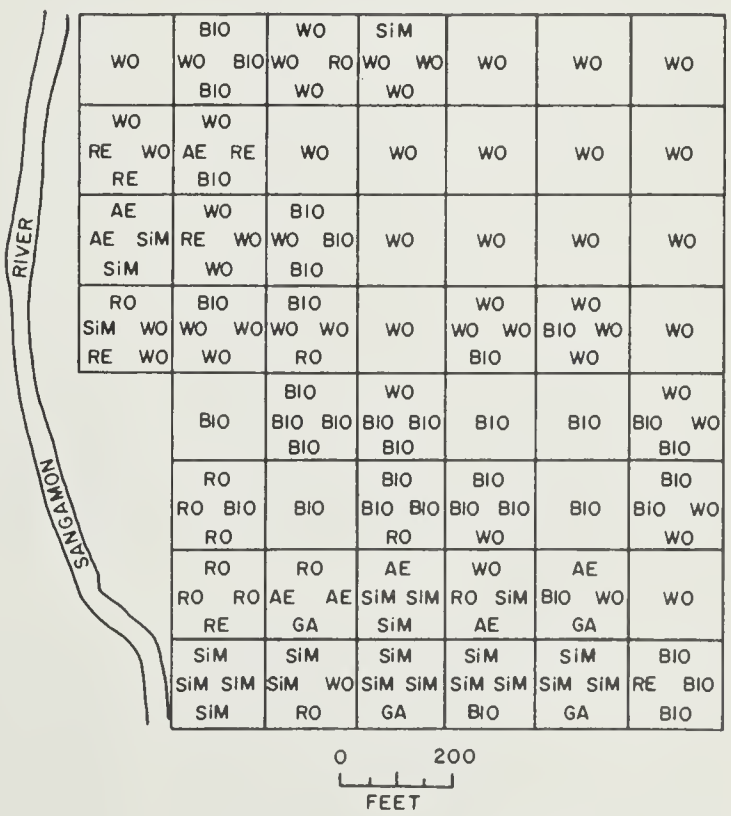

Figure 1. Diagram of woodland showing species with highest Importance Value by quarter-blocks.

TABLE 2.—Checklist of woody taxa identified and number per acre and frequency (percent) of seedlings and sapling by species and physiographic unit.

Scientific Name	Common Name	Symbol	Upland				Bottomland				Mixed			
			<0.6" DBH		1" and 2" DBH		<0.6" DBH		1" and 2" DBH		<0.6" DBH		1" and 2" DBH	
			No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
CANOPY TREES														
Ulmus rubra Muhl.	Red elm	RE	3,667	77.6	197	64.5	129	3.2	29	9.7	1,795	41.0	144	41.0
Prunus serotina Ehrh.	Black cherry	BC	2,956	66.6	67	31.2	97	6.4	.....	.....	385	20.5	8	5.1
Sassafras albidum (Nutt.) Nees.	Sassafras	Ss	862	30.4	171	37.7	.....	.....	.....	.....	51	2.6	3	2.6
Quercus velutina Lam.	Black oak	B10	507	21.0	.....	.....	.....	.....	.....	.....	26	2.6	.....	.....
Carya cordiformis (Wana.) K. Koch	Bitternut hickory	BH	312	20.3	2	2.2	.....	.....	3	3.2	513	17.9	5	5.1
Carya tomentosa (Pair.) Nutt.	Mockernut hickory	MH	377	26.8	61	33.3	.....	.....	.....	.....	.....	.....	3	2.6
Quercus alba L.	White oak	WO	290	14.5	5	2.9	.....	.....	.....	.....	.....	.....	.....	.....
Carya ovata (Mill.) K. Koch	Shagbark hickory	SH	261	19.6	16	12.3	.....	.....	.....	.....	51	5.1	.....	.....
Quercus rubra L.	Red oak	RO	58	3.6	.....	.....	32	3.2	.....	.....	333	15.4	.....	.....
Acer saccharinum L.	Silver maple	SiM	29	2.9	.....	.....	161	3.2	16	6.4	102	5.1	13	5.1
Ulmus americana L.	American elm	AE	36	1.4	17	10.9	.....	.....	6	6.4	179	10.2	62	12.8
Juglans nigra L.	Black walnut	BW	22	2.2	1	0.7	97	9.7	.....	.....	.....	.....	.....	.....
Quercus imbricaria Michx.	Shingle oak	SO	43	4.3	1	0.7	.....	.....	.....	.....	.....	.....	.....	.....
Fraxinus pennsylvanica Marsh.	Green ash	GA	.....	.....	2	2.2	.....	.....	3	3.2	26	2.6	15	7.7
Tilia americana L.	Basswood	L	7	0.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Celtis occidentalis L.	Hackberry	H	.....	.....	2	2.2	.....	.....	16	16.1	.....	.....	8	7.7
Acer negundo L.	Boxelder	BE	14	1.4	1	0.7	.....	.....	.....	.....	.....	.....	3	2.6
Gleditsia triancanthos L.	Honey locust	HL	.....	.....	1	0.7	.....	.....	.....	.....	.....	.....	.....	.....
Platanus occidentalis L.	Sycamore	S	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Quercus macrocarpa Michx.	Bur oak	BO	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE 2.—Checklist of woody taxa identified and number per acre and frequency (percent) of seedlings and sapling by species and physiographic unit. (Cont.)

Scientific Name	Common Name	Symbol	Upland				Bottomland				Mixed			
			<0.6" DBH		1" and 2" DBH		<0.6" DBH		1" and 2" DBH		<0.6" DBH		1" and 2" DBH	
			No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
UNDERSTORY TREES														
Cercis canadensis L.	Red bud	RB	116	3.6	9	4.3	.....	.....	6	3.2	51	5.1	13	7.7
Crataegus	Hawthorn	HT	51	3.6	3	1.4	.....	.....	10	6.4	.....	.....	.....	.....
Morus rubra	Mulberry	M	14	1.4	4	3.6	.....	.....	3	3.2	.....	.....	.....	.....
Ostrya virginiana (Mill.) Koch	Ironwood	IW	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15	5.8
SHRUBS														
Cornus racemosa Lam.	Gray dogwood	GD	862	15.9	1	0.7	.....	.....	.....	.....	77	5.1	3	2.6
Corylus americana Walt.	Hazelnut	HN	674	14.5	.....	.....	32	3.2	.....	.....	513	2.6	.....	.....
Rubus allegheniensis Porter	Bramble	B	717	33.3	.....	.....	.....	.....	.....	.....	102	5.1	.....	.....
Zanthoxylum americanum Mill.	Prickly ash	PA	232	8.7	.....	.....	.....	.....	.....	.....	102	7.7	.....	.....
Smilax hispida Muhl.	Smilax	Sx	36	2.9	.....	.....	32	3.2	.....	.....	590	17.9	.....	.....
Sambucus canadensis L.	Common elder	CE	22	1.4	.....	.....	258	6.4	6	6.4	231	12.8	3	5.1
Euonymus atropurpureus Jacq.	Wahoo	Wh	58	2.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ribes missouriense Nutt.	Common gooseberry	GB	22	0.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rhus glabra L.	Smooth sumac	Su	7	0.7	1	1.4	.....	.....	.....	.....	.....	.....	.....	.....
Totals			12,252		562		838		98		5,127		283	

TABLE 3.—Number of trees, basal area per acre, Importance Value Index, and average diameter for leading dominants.

Species	Diameter Class, Inches												Av. Diam., In.	Impor. Value
	3-6		7-12		13-24		25-36		37+		Total			
	No.	BA	No.	BA	No.	BA	No.	BA	No.	BA	No.	BA		
WO	7.97	1.20	23.18	11.46	15.91	25.20	.55	2.28	.....	.....	47.61	40.15	12.5	78.2
B10	.27	.03	2.73	1.81	14.97	26.45	.90	3.52	.....	.....	18.87	31.80	17.5	46.5
RO	.09	.01	.49	.31	3.38	6.85	.77	3.13	.03	.24	4.76	10.58	17.9	14.3
RE	14.94	.93	.28	.10	.06	.15	.....	.....	.....	.....	15.28	1.21	3.8	13.7
AE	7.61	.74	1.64	.28	.03	.08	.....	.....	.....	.....	9.28	1.11	4.7	8.0
SiM	.87	.08	.77	.36	1.45	2.48	.27	1.15	.03	.24	3.39	4.32	15.2	7.0
BC	4.10	.33	1.12	.60	.24	.37	.....	.....	.....	.....	5.46	1.28	6.5	5.6
GA	.63	.08	.61	.26	.82	1.54	.03	.14	.03	.41	2.12	2.44	14.5	4.1
Others	8.76	.83	3.73	1.76	3.42	5.46	.24	1.04	.03	.26	16.18	9.28	10.2	23.3
Total	45.24	4.23	34.55	16.94	40.28	68.58	2.76	11.26	.12	1.15	122.95	102.17		

TABLE 4.—Stand data per acre and Importance Value (IV) for the 10 leading dominants (2.6 inches d.b.h. and above) in each of three topographic units.

Species	Upland (21.31 acres)						Bottomland (4.79 acres)						Mixed (6.02 acres)					
	No. Trees	Basal Area	Rel. Density	Rel. Dom.	IV	IV Rank	No. Trees	Basal Area	Rel. Density	Rel. Dom.	IV	IV Rank	No. Trees	Basal Area	Rel. Density	Rel. Dom.	IV	IV Rank
WO	63.62	55.05	49.62	48.45	98.07	1	.....	.....	.....	.....	.....		29.57	19.20	20.27	19.84	40.11	1
B10	25.12	42.98	19.59	37.83	57.42	2	.....	.....	.....	.....	.....		12.13	17.49	8.31	18.07	26.38	3
RE	14.13	1.03	11.02	0.91	11.93	3	5.64	1.16	7.94	1.99	9.93	6	27.41	1.88	18.79	1.94	20.73	4
RO	4.41	8.69	3.44	7.65	11.09	4	.....	.....	.....	.....	.....		9.63	24.41	6.60	25.22	31.82	2
BC	6.57	1.56	5.12	1.37	6.49	5	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	
Ss	3.76	0.42	2.93	0.37	3.30	6	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	
MH	2.25	1.26	1.75	1.11	2.86	7	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	
AE	2.49	0.36	1.94	0.32	2.26	8	15.87	2.53	22.35	4.34	26.69	3	23.92	2.63	16.40	2.72	19.12	5
SH	1.88	0.72	1.47	0.63	2.10	9	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....	
BH	1.22	0.31	0.95	0.27	1.22	10	.....	.....	.....	.....	.....		3.49	2.15	2.39	2.22	4.61	9
SiM	.....	.....	.....	.....	.....		16.49	23.64	23.74	40.59	63.83	1	5.15	4.26	3.53	4.40	7.93	7
GA	.....	.....	.....	.....	.....		8.77	10.32	12.35	17.72	30.07	2	.....	.....	.....	.....	.....	
HB	.....	.....	.....	.....	.....		6.26	3.07	8.82	5.27	14.09	4	.....	.....	.....	.....	.....	
BW	.....	.....	.....	.....	.....		3.13	4.25	4.41	7.30	11.71	5	3.17	3.44	2.16	3.55	5.71	8
HL	.....	.....	.....	.....	.....		2.30	2.19	3.24	3.76	7.00	7	.....	.....	.....	.....	.....	
BO	.....	.....	.....	.....	.....		0.63	3.43	0.88	5.89	6.77	8	2.49	2.58	1.71	2.66	4.37	10
SO	.....	.....	.....	.....	.....		1.04	2.38	1.47	4.09	5.56	9	3.17	6.73	2.16	6.95	9.11	6
HT	.....	.....	.....	.....	.....		3.34	0.45	4.70	0.77	5.47	10	.....	.....	.....	.....	.....	

Upland Physiographic Unit

White oak and black oak rank first and second in importance. Red oak is in fourth position, slightly behind red elm. As a group, these three oak species comprise 70 percent of the total number of trees and 95 percent of stand basal area. Red oak and black oak have the largest diameters of all trees in the woodland, averaging 17.9 and 17.5 inches, respectively. Black oak is concentrated on the steeper slopes in the northeastern corner of the woods, where it exceeds white oak in importance. Seedling counts are 290 per acre for white oak and 507 per acre for black oak. Black oak was not represented in the 1- and 2-inch diameter classes, and there were only 5 white oaks per acre present in this size class.

Slippery elm and American elm ranked third and eighth in IV. Because of the high mortality of larger trees from phloem necrosis and Dutch elm disease, these two species have the smallest diameters of any leading dominant. Red elm comprises 30 percent of the seedlings, 35 percent of the small saplings, and almost one-third of the trees in the 3- to 6-inch diameter class. Its frequency of 77.6 percent for seedlings and 64.5 percent for small saplings exceeded that of any other species.

Black cherry, although confined largely to the smallest diameter classes, ranked fifth in IV and had the second highest number (2,956) of seedlings per acre. Unlike red elm, black cherry does not continue its high density into the 3- to 6-inch diameter class. Mortality of seedlings appears to be particularly heavy from 3 to 5 years after establishment.

In some parts of the upland, sixth-ranked sassafras forms a dense understory as evidence by the fact it accounts for almost one-third of the 1- and 2-inch trees. The 862 sassafras seedlings per acre, with a frequency of 30 percent, indicates that this species is reproducing quite well.

Three hickories—mockernut, shagbark, and bitternut—rank seventh, ninth, and tenth in IV, respectively. Collectively, there are 950 hickory seedlings per acre and 79 trees in the 1- and 2-inch diameter classes.

Other species in the upland forest, along with their IV's, are black walnut, 1.01; redbud, 0.54; shingle oak, 9.52; basswood, 0.32; red mulberry, 0.31; honey locust, 0.25; ironwood, 0.13; hawthorn, 0.06; green ash, 0.06; and hackberry, 0.04.

Mortality on the upland amounted to 6.9 square feet of basal area per acre. This included an average of about 7 elms and 8 white oaks per acre, with an occasional tree of other species.

Bottomland Physiographic Unit

Stocking in the bottomland is only one-half that of the upland, both from the standpoint of tree number and basal area. This is due to the near complete mortality of elm that once composed almost half of the bottomland stand. Dead-standing and dead-down elm comprise 65 trees and 48 square feet of basal area per acre. Total mortality (all species) in the bottomland was 70 trees and 56 square feet of basal area per acre.

Silver maple is the most important species and includes 23 percent of the trees and 40 percent of the basal area. Green ash is second in importance, followed by American elm in third place. The elms, including red elm, which is sixth in IV, are less than 6 inches in DBH. In contrast the largest tree in the entire woodland is a 49-inch green ash. Other species included in the 10 leading

dominants with their IV's are hackberry, 14.1; black walnut, 11.7; honey locust, 7.0; bur oak, 6.8; shingle oak, 5.6; and hawthorn, 5.5. Collectively the IV of the "others" category is almost as great as that of silver maple, stressing the relatively high importance of minor species in the bottomland as compared with the upland.

Regeneration of woody species in the bottomland is sparse as indicated by the tally of 83 seedlings and 98 small saplings per acre. The elms were reproducing better than any other tree species.

Mixed Physiographic Unit

Since this unit is transitional, it contains species characteristic of both the bottomland and upland areas. It also contains the highest quality sites in the woodland. While there are more trees per acre present in the upland, 146 vs. 128, the basal area of 97 square feet per acre is about 17 sq. ft. less than that of the upland. Again, elm mortality has been an important factor, amounting to 39 trees and 35 sq. ft. of basal area per acre.

White oak, with an IV of 40.1, is the leading dominant, followed closely by red oak (IV, 31.8). However, the 30 white oaks per acre have an average diameter of 10.9 inches compared with 21.5 inches for the 10 red oaks present. Black oak is third in importance and is intermediate in size (average DBH, 16.4 in.) between the white and red oak. Collectively, these three oak species comprise 35 and 63 percent, respectively, of number of trees and basal area. Two additional oak species, shingle and bur, rank sixth and tenth, respectively, in importance but constitute a minor part of the stand. Regeneration of the oaks presents about the same picture as in the upland.

Other than oaks, the elms are the second most important species, with red elm ranking fourth and American elm fifth in importance. Their IV is based on tree number rather than size, with most of the individuals falling in the 3- to 6-inch diameter class. The former position of American elm in the stand is illustrated by the fact that dead individuals have an average diameter of 12.8, compared with 4.1 for those still living. However, larger individuals of red elm persist in the stand, especially in this transitional physiographic position. Also, red elm regeneration is greater than that of any other species, comprising 35 percent of the 5,178 seedlings and 50 percent of the 283 small saplings (1- and 2-inch DBH) per acre in the stand. Amrican elm is poorly represented in both of these size classes, reflecting the lack of seed source.

Discussion

The positioning of Hart Woods upland at the xeric end of a moisture sequence for upland forests in east-central Illinois is justified by two factors: (1) the complete absence of sugar maple (*Acer saccharum* Marsh) in the stand; and (2) the greater importance of white oak and black oak, particularly the latter, compared with other woodlands studied. On more mesic sites in these woodlands, sugar maple is an extremely aggressive species and appears to be continually increasing in importance. Although sugar maple ranked only ninth in IV in the streamside forest at Allerton Park, it is an important stand component with a relatively large number of individuals in the 3- to 6-inch diameter class (Boggess and Geis, 1967).

White oak will probably increase

in importance as the larger black oaks die. There are, as replacements, 31 white oaks compared with only 3 black oaks per acre less than 13 inches DBH. Mortality data suggests this trend, as the basal area of dead black oaks in the largest diameter class (13 to 24 inches) is four times that of white oak. Although mortality of small white oaks (3- to 6-inch DBH) is relatively high, there remain more live than dead trees. The reverse is true for black oak. Then, too, back oak does not generally reproduce well in the absence of some disturbance factor (U. S. Forest Service, 1965). Any prediction of future composition must be tempered by knowledge of the recent devastation of the elm population, plus the fact that oak wilt is a threat in sections of Illinois.

Although hickories as a group ranked relatively low among the leading dominants, they were third in the number of seedlings and small saplings. On this basis, hickories are likely to increase in importance.

The future of red elm is an intriguing question, as seedlings and saplings are quite dense in parts of the upland. However, many individuals were produced from interconnecting rhizones rather than seed. There is a sharp drop in the density of red elm above 6 inches in diameter which suggests that the relatively large number of indiivduals less than this diameter may be of relatively recent origin. Competition from red elm in these smaller diameters has undoubtedly affected oak regeneration, particularly the growth of seedlings into saplings and so on. Any suggestion that red elm will form an important component of the future overstory must consider the susceptibility of this species to Dutch elm disease and phloem necrosis, even though it is less than that of American elm.

The presence of large numbers of sassafras seedlings and small saplings in the upland forest is unusual for this area. This species did not occur in Trelease Woods, Brownfield Woods, or the Funk Forest Natural Area. Although sassafras did occur on well-developed forest soils, Allerton Park numbers were quite limited compared with the Hart woodland. Sassafras along with persimmon (*Diospyros virginiana* D.) are usually the first woody plants to appear in fields abandoned from cultivation in southern Illinois. While sassafras persists for many years, it appears to drop out of the successional picture in mature oak-hickory forests of the area (Bazzaz, 1968). In sharp contrast, Eisenhauer (1967) found that seedlings of sassafras ranked second in importance in forests growing on relatively level claypan soils and on slopes in south-central Illinois. In these same stands, it ranked eleventh in IV for trees 3 inches DBH and above. Sassafras is found in Sargents and Baber woods, located on the Shelbyville Moraine approximately 50 miles southeast of Hart Woods, occurring as seedlings, small saplings, and small trees exceeding 3 inches DBH. (Ebinger, 1968; McClain and Ebinger, 1967). It ranks eleventh in importance in Baber Woods but is not included among the 10 most important species in Sargents Woods.

Hart Woods is at the northern range of sassafras in east-central Illinois, although farther to the east it extends into central Michigan. Harmon (1968) reported abundant sassafras in the dune forests of southwestern Michigan, as did Olson (1958) from the Indiana dunes.

Thus the distribution of sassafras (U. S. Forest Service, 1965) shows a distinct adjustment to the geographic occurrence of the Prairie Peninsula. Kucera and McDermott (1954) list sassafras as one of the species common to Missouri forests that drops out in the northern portions of the Prairie Peninsula. The abundance of sassafras in Hart Woods may well be related to the advanced stage of soil development that has resulted in more pronounced base removal and lower pH than is found for soils of other woodlands in the area.

The future composition of the bottomland forest is uncertain. Spring flooding, siltation, heavy soils, and a dense herbaceous cover will all affect the establishment of tree seedlings. Silver maple is now the leading dominant, but whether it will fill the niche created by loss of the elms is open to question.

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GROWTH RELATIONSHIPS BETWEEN *APHELENCHUS AVENAE* AND TWO SPECIES OF NEMATOPHAGOUS FUNGI

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Abstract. — Comparable populations of the mycophagous nematode, *Aphelenchus avenae* Bastian, were cultured for seven days upon agar cultures with two species of nematophagous fungi. In the presence of nematodes the optimum growth temperature of the fungi was usually increased 5°C and fungus colony diameters were greater than controls. Low numbers of living nematodes were recovered from fungus-sown plates after seven days at 15°C and 20°C, but at 25°C and 30°C the numbers of nematodes recovered exceeded the original inoculum. Nematode numbers at the lower temperatures suggested that nematode trapping was higher than nematode reproduction, eggs laid by the nematodes were incapable of hatching, or inhibitory fungal metabolites are stable at lower temperatures and unstable at higher temperatures.

Experimental cultures of nematode-trapping fungi have included primarily either bacterium-feeding or plant-parasitic nematode species. Mycophagous nematodes have been used only recently in such cultures even though they are probably as widely distributed in nature as are the nematophagous fungi. Recent investigations (Cayrol, 1967; Cooke and Pramer, 1968; Feder, 1963; Hechler, 1963; Monoson, 1968a) have included a study of the relationships between nematophagous fungi and mycophagous nematodes.

The purpose of the present study was to determine what effects varying numbers of nematodes have on fungus growth and what effects the fungi have on nematode populations when both are grown together.

Materials and Methods

Two species of nematophagous Moniliales were chosen. The fungi used in this study were two adhesive, network-forming species, *Arthrobotrys oligospora* Fres. and *A. musiformis* Drechs. Stock cultures of the fungi were maintained on a medium containing 20 g Difco corn-meal agar (CMA) in 1000 ml water at room temperature. The nematode *Aphelenchus avenae* Bastian was maintained on *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, and Larson which grew on a medium containing 10 g Difco potato-dextrose agar (PDA) and 15 g agar in 1000 ml water.

Three media were used in this study: 2% Difco CMA, one-quarter-strength Difco PDA (Monoson, 1968a), and one-fifth-strength V-8 agar (V-8) composed of 200 ml V-8 juice® and 20 g agar in 1000 ml water. No attempt was made to adjust the pH's of the media.

Fungus inoculations were according to the technique described by Monoson (1968a). Each of the two fungi was cultured on the three agar media for a period of four days. Fungus plugs, 7 mm diam., were cut from these four-day-old cultures and placed in the center of 9 cm petri plates that contained the same agar medium. Nematodes were extracted from stock cultures according to the following technique (Monoson.

1968b): Nematodes were separated from agar stock cultures by pouring 10-15 ml of sterile water over the surface of a plate. The nematodes readily moved into the surface water and were unable to re-enter the agar. A simple nematode extraction apparatus was formed by placing four layers of type 900-S-Kimwipe® tissues between two plastic funnel tops which rested in a Syracuse dish. The nematode extraction apparatus had been sterilized before hand by autoclaving for 15 minutes at 20 psi. Nematodes were extracted over a 1-hour-period and serially diluted to either 100 or 400 individuals per milliliter of water. Inoculation of either 100 or 400 nematodes into each experimental fungus culture was accomplished by using a sterile measuring dispenser or a calibrated 1 ml duplicating pipette. Each culture was replicated three times and appropriate controls containing fungus alone were used.

Nematophagous fungus cultures containing *A. avenae* were maintained for seven days at 15°, 20°, 25°, or 30°C. Observations and measurements of cultures began 24 hr after inoculation and continued at 24-hr intervals. The radial diameter of colonies was measured to the nearest 0.5 mm.

Upon completion of each test the agar contents of a petri dish were cut into approximately 0.5 cm squares and homoginized in a Waring blendor for 8 sec. The homogenate was poured into a nematode recovery apparatus (Monoson, 1968b) and counts of the living nematodes were made after 24 hr.

Results

Average colony growth of the two fungi in radial diameter in the presence and absence of *A. avenae* are shown in Figure 1. *A. oligospora* and *A. musiformis* had maximum growth at 25°C in the presence of the nematode. *A. musiformis* grew more at 25° than at 20°C on CMA. The growth of *A. oligospora* on PDA with *A. avenae* was as great at 20° as at 25°C. Slight growth differences were noted for both fungi on CMA in the presence of the nematode at 20° and 25°C but were not considered significant.

Neither of the fungi repelled the nematode although in two instances less than 35% of the total number introduced was recovered from an

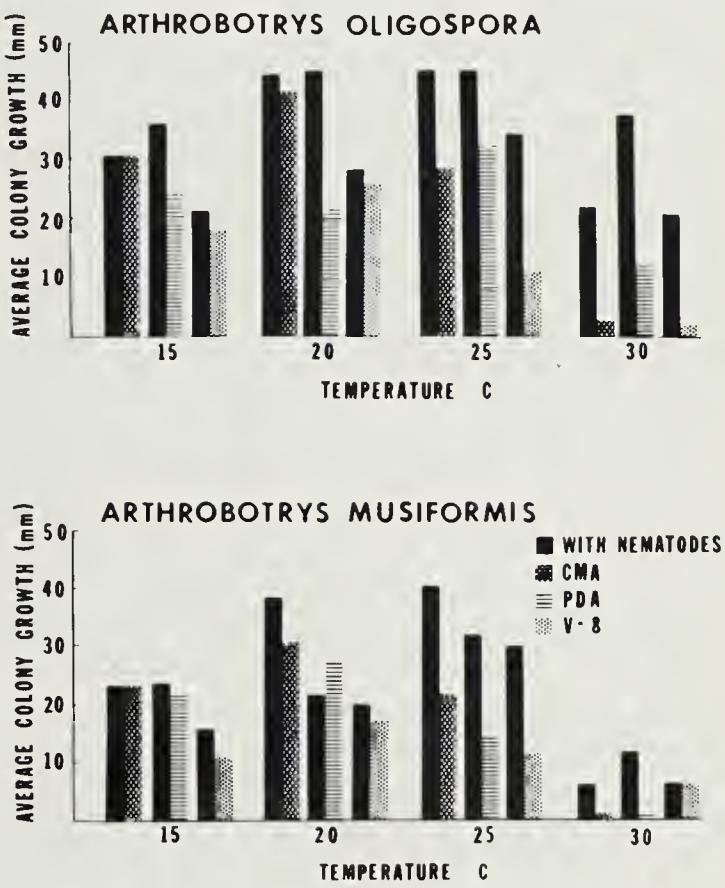

FIGURE 1. Average colony growth of two species of nematophagous fungi on three agar media after seven days of growth with and without *Aphelenchus avenae*.

The bars are arranged in pairs. The solid black is the left-hand member of the pair, and gives the average colony growth in the presence of the nematode with an initial inoculum of 100 individuals. The right-hand member of the pair gives the average colony growth in the absence of the nematode, and is appropriately shaded to designate the medium. (see key).

experimental culture (Table 1). All of the nematodes inoculated into a dish settled in the fungus plug after 12 hr. The nematodes moved freely in and around the inoculum plug prior to the 12 hr but were not observed to leave a colony once they began feeding on the hyphae. Fungal satellite colonies were never formed through the transportation of spores on the bodies of nematodes. At no time were immobile nematodes observed other than those which had been captured by a fungus.

Cultures were evaluated on the basis of nematodes recovered alive. Low numbers of nematodes were recovered from all the media maintained at 15° and 20°C, but at 25° and 30°C numbers were much higher than used as inoculum (Table 1). The number of live nematodes recovered was divided by the total area of the colony, in square centimeters,

TABLE 1.—Total numbers of *Aphelenchus avenae* recovered per plate* after being cultured seven days with two nematophagous fungi on various agar media.

Temperature	No. Nematodes Added	No. Nematodes Recovered					
		Arthrobotrys Oligospora			*Arthrobotrys Musiformis*		
		CMA	PDA	V-8	CMA	PDA	V-8
15° C	100	52	32	28	56	42	47
15° C	400	56	124	44	74	111	41
20° C	100	74	69	42	56	54	79
20° C	400	61	295	56	219	181	32
25° C	100	231	130	126	835	238	87
25° C	400	1500	445	254	1600	406	139
30° C	100	890	296	690	2600	793	834
30° C	400	5000	919	661	3900	339	427

* Numbers recorded are averages of three replicates in each case.

to yield the number of nematodes present per square centimeter of fungus (Table 2).

Discussion

The addition of *A. avenae* to fungus cultures usually resulted in more fungus growth compared to cultures without the nematode. Cultures that contained 100 nematodes as inoculum most often produced the optimum fungus growth in this study.

Monoson (1968a) reported that *A. avenae* was captured very effectively by these two fungi after four days of maintenance at 15°, 20°, 25°, and 30°C on 2% CMA, one-quarter-strength PDA, and one-fifth-strength V-8. In the present study, the low numbers of living nematodes recovered at 15° and 20°C indicated that nematode trapping might have occurred after the seven days of these tests. The data also suggested that nematode trapping was higher than nematode reproduction or that eggs laid by the nematodes were incapable of hatching.

Cooke and Pramer (1968) reported that nematode-trapping fungi displayed no predaceous activity until

TABLE 2.—Numbers of *Aphelenchus avenae* per cm² recovered after seven days of growth with two nematophagous fungi on various agar media.*

Temperature	No. Nematodes Added	*Arthrobotrys Oligospora*			*Arthrobotrys Musiformis*		
		CMA	PDA	V-8	CMA	PDA	V-8
15° C.	100	1	1	2	3	2	6
15° C.	400	2	3	3	5	6	6
20° C.	100	1	1	2	1	4	6
20° C.	400	1	5	2	7	7	2
25° C.	100	4	2	3	17	7	3
25° C.	400	24	7	4	39	22	7
30° C.	100	60	6	48	2300	202	629
30° C.	400	602	328	52	9750	484	237

* Average of three replicates.

they had completely colonized the agar substrate. In addition, they stated that the retardation of predaceous activity was due to the concentration of nematodes in a zone at or beyond the colony margin too juvenile to capture nematodes. It was of interest to note that neither of these situations were observed in the present study.

Temperature affected the amount of fungal growth in the same way both with and without nematodes (Figure 1). With the exception of *A. oligospora* on PDA fungus cultures that contained nematodes had an optimum temperature for growth five degrees above that of the controls.

Large amounts of fungus growth and high numbers of live nematodes were recovered at either 25° or 30°C on the three media. Released nematode metabolic products may have caused the stimulated growth of the fungi due to alteration of the agar media. Small shifts in the pH's of the media were noted but not enough data were available to ascribe specific effects to pH shifts.

Monoson (1968a) reported that 20-50 nematodes were recovered from CMA cultures of *A. oligospora* and *A. musiformis* after four days at 25°C when the inoculum level was 100 nematodes. Almost identical numbers of live nematodes were recovered under those same environmental conditions when the inoculum level was 400 individuals. In the present study, similar fungus cultures on CMA at 25°C with an inoculum level of 100 nematodes contained 231, 835, and 373 nematodes after seven days. These numbers were considered low when compared with those produced on *P. terrestris* (a non-predaceous fungus) after a similar maintenance period at 25°C. The number of nematodes expected on *P. terrestris* cultures after seven days was either equalled or surpassed, for the most part, during a similar period of time on *A. oligospora* and *A. musiformis,* at the two higher temperatures.

The large nematode numbers could be interpreted as being due to: nonfunctioning of the trapping mechanism after a seven-day period at high temperatures—i.e., 25° and 30°C, (2) increase of nematode reproduction over the rate of nematode trapping, or (3) decrease in generation

time of the egg to the larval and adult stages because of cultural conditions. Microscopic observation of whether or not a trap still functioned after seven days was considered impossible because of the amount of fungal growth.

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TRANSVERSE AND CRESCENT CRACKS IN SOYBEAN COTYLEDONS ASSOCIATED WITH IMBIBITION

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Abstract. — Transverse and crescent cracks in cotyledons and cotyledon fracture were induced in soybean seeds by placing them in water (room temperature) prior to planting. These injuries resulted in reduced rates of seedling growth or non-emergence, depending on location and severity of the cotyledon damage. The cracks also were induced by allowing seeds to swell for 12 hours on wet filter paper at 10 degree intervals from 10 to 60°C. The highest percentage of cotyledon cracking (100%) was observed at the lowest temperature and the lowest percentage (19%) at the highest temperature. These cotyledon cracks were apparently caused by uneven swelling during imbibition.

In a study of cell death in pith tissue of soybean (*Glycine max* L.) seedlings, Liu (1966) often observed transverse and crescent cracks in the upper surface of cotyledons of young seedlings in all 24 varieties studied. By anticipating a small percentage of damaged seedlings in each experiment and overplanting to permit selection of uniform seedlings having no obvious cotyledon damage, Liu was able to complete the study of parenchyma cell death in pith tissue of normal seedlings. This type of cell death also occurred in seedlings with injured cotyledons. For future studies of the cell death process in soybeans, more uniform stands are desired. Thus, an explanation of the cotyledon injury was required.

We thought that cotyledon injury could be due to injuries to the seed during harvesting (Bainer and Borthwick, 1934). More recent studies suggested that soybean cotyledons could be injured by preharvest moisture conditions and during storage (Metzer, 1967; Tachibana, *et al.*, 1968). Earlier literature indicated that cracked cotyledons could result due to rough harvesting and cleaning treatments (Humphrey, 1958; Moore, 1957). Cracks in cotyledons reduced field stands due to weakened plants even when the seeds were treated with fungicides and planted under favorable field conditions. Internal injuries in those reports included bruised and fractured seed leaves, roots, and plumules with complete or partial fractures noted in many plant parts or at the point of attachment of one part or another. Other studies also had shown that transverse cotyledon cracks reduced germination, seedling growth and yield (Atkins, 1958; Waters and Atkins, 1959).

In an attempt to reduce seedling growth rate differences, we soaked seeds and removed seed coats after various stages of imbibition in shallow water and observed further growth stages after planting in sand and peat mixtures. Transverse cotyledon cracks were observed more frequently after soaking. When cotyledons from swollen seeds were examined before planting, it was obvious that the cracks had occurred during imbibition and not after

planting. Individual cotyledons on wet filter paper (either with lower or upper epidermis in contact with the water) developed crescent cracks, beginning in the boundaries of the wet and dry tissue. Complete fractures often occurred.

We did find literature to suggest that seeds of beans, peas, and corn were damaged due to differences in seed coat permeability, rate of water imbibition, and uneven swelling (McCollum, 1953; Shull and Shull, 1932). Resuhr (1941) described injury to soybean seeds due to 24 hours of soaking, the injuries being spotting and split cotyledons. The purpose of this paper is to present selected data from a number of similar studies designed to test the hypothesis that the transverse and crescent cracks (partial or complete fractures) occur in soybean seeds during imbibition.

Materials and Methods

In each of six replicates, 30 seeds of variety Wayne and Shelby were selected for uniform size and absence of visible damage. Each 30-seed sample was divided into two groups of 15 seeds, one group being placed in a 9 cm Petri dish containing a 9 cm filter paper and 15 ml of water (room temperature) for 30 minutes of soaking. The seeds of both groups were planted at a depth of 5 cm in unsterilized sand and peat (equal volume, 6″ diameter plastic pots) watered to saturation. Seedlings grew at room temperature for 14 days.

One four-replicate study of the effect of temperature was conducted using seeds of Wayne. Sixty seeds, four Petri dishes with filter paper, and 100 ml of tap water were placed in incubators at 10, 20, 30, 40, 50, and 60°C. When the water reached the desired temperature, 15 seeds and 10 ml of water were placed in each Petri dish and the swollen seed examined 12 hours later.

Results and Discussion

Typical results from one replicate are presented for the study of water soaking effects on planted seeds. Five days after planting, all of the 15 untreated seeds of Shelby and 14 of Wayne had emerged. The last seedling of Wayne emerged on the seventh day. Of these, one cotyledon of Shelby and four of Wayne showed some small transverse cracks. On the eleventh day after planting, seedlings were uniform in height in both varieties.

Of the seed soaked in water before planting, seven seeds of Shelby and seven of Wayne had emerged five days after planting. Five additional seedlings of Shelby and four of Wayne emerged on the seventh day. On the ninth day, another seedling of Wayne emerged. None of the other seeds germinated in either variety. Of the 12 seedlings of Wayne, 22 cotyledons were counted, 14 with one or more cracks. Two seedlings had one cotyledon as a result of complete fracture near the point of attachment to the hypocotyl. Only three seedlings had both cotyledons without cracks. Of the 12 seedlings of Shelby, 24 cotyledons were counted, all with one or more cracks. On the eleventh day after planting, seedlings of both varieties were irregular in height. The non-emerged seeds of both varieties had severe cracks or complete fractures near the plumule.

Each experiment with our seeds of these varieties resulted in more cotyledons of Shelby with cracks than those of Wayne. We would prefer more trials with several sources of

seed before suggesting that this is due to varietal differences to cotyledon cracking. We conclude that uneven swelling caused a small percentage of seedling damage in our previous experiments. Whether these cracks predispose the seedlings to pathogens or alters the cell death pattern in pith tissue remains to be studied.

The amount of damage to seeds of Wayne (average of four replicates) during 12 hours of soaking in water at various temperatures was as follows: 10°C, 100% of the seeds with one or both cotyledons cracked; 20°C, 92%; 30°C, 93%; 40°C, 80%; 50°C, 55%; and 60°C, 19%. These observations are in agreement with those of McCollum (1953) for snap beans germinating in water or soil; as temperature increases (from 10 to 30°C), cracking decreases, especially in soil conditions. We believe, as did Shull and Shull (1932) and McCollum (1953) for their seeds, that cracks in soybeans occur simultaneously with water uptake as a result of uneven swelling producing a tension crack in the dry interior portion or along the boundry between wet and dry tissue. It may be that the rate of penetration of water into the seed tissue at higher temperatures creates moisture gradients less conducive to cracking. Orphanos and Heydecker (1968) reported that oxygen deficiency in the interior of soaked snap beans resulted in injury and killing. Although we did not see ungerminated seeds without severe cracks or complete fractures in cotyledons, it may be that similar oxygen deficiencies exist in soybeans also under prolonged soaking. Further study on this problem is required to discover the extent of seed damage due to soaking in wet fields and the relationship of this type of seed damage to vigor, disease resistance, and yield.

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A PROPOSED BIOCHEMICAL MECHANISM OF THE TOXIC ACTION OF DDT

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ABSTRACT. — DDT, 5.9 x 10^{-4} grams per ml of reaction medium, inhibited oxygen uptake by bluegill liver mitochondria in the presence of succinic acid. DDT increased the hydrolysis of adenosinetriphosphate in the presence of magnesium and manganese ions. A biochemical mechanism of the toxic action of DDT is suggested.

DDT (1,1,1, -trichloro -2,2 bis (P-chlorophenyl) ethane) has been widely used as an insecticide and apparently has spread throughout the world. DDT has been the subject of much research in efforts to explain its mode of action on insects, the primary target organisms. More recent research has attempted to explain its effects on non-target organisms, such as fish and birds. Metcalf (1955) discussed the mode of action of DDT, and later O'Brien (1967) indicated that after 12 years of intensive research the mechanism of the insecticidal properties of DDT has not been elucidated. However, the general agreement was that the primary target of DDT appeared to be the nervous systems of both vertebrates and invertebrates.

This view is untenable, as it does not explain the diverse biological effects, such as the effects on fish and bird reproduction, which have been shown to be related to or caused by DDT. However, some of the observed results, such as increased activity, may be due to the effects of DDT on the nervous system, but there is evidence that DDT affects fundamental biochemical processes in other tissues. The involvement of the production of cellular energy and the effects of DDT on these processes was dismissed by Metcalf (1955) as of little importance in explaining the mode of the toxic action of DDT. The suggested effects of DDT on the nervous systems, extensively reviewed by O'Brien (1967) does not offer a suitable explanation of the effects of DDT on the reproductive processes in birds and fishes, while the effects of DDT on basic biochemical enzymatic processes may explain both phenomena.

We have been investigating the effects of possible pollutants including pesticides on energy production by the mitochondria of the liver of the bluegill sunfish, *Lepomis macrochirus,* (Hiltibran, 1967b) and have found that compounds which were very toxic to fishes, such as rotenone, antimycin A, cyanide, isopropyl ester of 2,4-D altered the oxygen or phosphate uptake by bluegill liver mitochondria (Hiltibran, 1967b). The previous investigations of Sacktor (1950), Johnston (1951), and Anderson *et al* (1954) indicated that DDT altered the oxygen uptake by various tissues. The results of our investigation of the effects of DDT on the oxygen and phosphate metabolism of bluegill liver mitochondria is reported.

Methods

Native, wild bluegills were held in the laboratory aerated aquaria at 25° C and all enzymatic assays were conducted at that temperature. Procedures for the preparation of the mitochondria, for estimating the oxygen and phosphate, for estimating the rate of release of inorganic phosphate from adenosinetriphosphate, ATP, and for estimating the nitrogen content of the mitochondrial preparations have been previously reported (Hiltibran and Johnson, 1965). The data are the average changes observed from three or more experiments and have been corrected for endogenous activity and the effects of the solvent. Reference samples of DDT were used, and redistilled ethyl alcohol or acetone were used as solvents. DDT concentrations are expressed as grams of DDT per ml of reaction medium.

Results

The effects of DDT on oxygen and phosphate uptake by bluegill liver mitochondria in the presence of succinate and alpha-ketoglutarate as substrates are summarized in Table 1. In the presence of succinate, 5.9 x 10^{-4} g of DDT per ml of reaction medium completely inhibited the uptake of oxygen, and at a concentration of 5.9 x 10^{-6} g oxygen uptake was inhibited approximately 40 percent. Lower levels of DDT were not as effective on oxygen uptake. Usually when there is severe inhibition of oxygen uptake there is an increase in the inorganic phosphate content of the reaction medium. However, in the presence of DDT there was not an increase in the inorganic phosphate content of the reaction medium, when oxygen uptake is altered.

DDT inhibited oxygen uptake in the presence of alpha-ketoglutarate

Table 1.—Effects of DDT on Oxygen Uptake by Bluegill Liver Mitochondria.

g/ml of Reaction Medium	Average Change in μ 10_2/hr/mg N		Average Change in μ moles $P0_4$/hr/mg N		Percent Inhibition of 0_2 Uptake
Succinate					
5.9 x 10^{-4}	(—)	118	(±)	16	100
5.9 x 10^{-5}	(—)	89	(±)	18	80
5.9 x 10^{-6}	(—)	39	(±)	9	40
5.9 x 10^{-8}	(±)	30	(±)	4	
Alpha-Ketoglutarate					
5.9 x 10^{-4}	(—)	31	(+)	8	20
5.9 x 10^{-5}	(—)	58	(+)	20	45
5.9 x 10^{-6}	(—)	37	(+)	13	25
5.9 x 10^{-7}	(+)	41	(+)	3	30 (increase)

as substrate, but to a lesser extent. There was no effect of DDT on phosphate uptake in the presence of alpha-ketoglutarate. DDT did not appear to uncouple the phosphate uptake from the oxidation of either substrate, therefore, the primary effect of DDT appeared to be on the utilization of oxygen.

The effects of DDT on the hydrolysis of ATP by the bluegill liver mitochondria are summarized in Table 2. In the presence of cadmium, DDT inhibited the hydrolysis of ATP at all concentrations of DDT used, and in the presence of zinc, DDT inhibited the hydrolysis of ATP at the highest concentration of DDT. The hydrolysis of ATP was not greatly altered in the presence of either manganese or calcium, but in the presence of magnesium, DDT increased the hydrolysis of ATP from approximately 60 percent at a DDT concentration of 1.5 umoles of DDT per ml of reaction medium to 150 percent at a DDT concentration of 2.5 umoles.

The data suggests that DDT can alter the activities of various enzyme complexes from the bluegill liver mitochondria and that the observed effects appear to be related to the concentration of DDT. The data also suggests a specific effect of the DDT molecule on each liver enzyme complex. When the DDT concentration was increased, complete inhibition of oxygen uptake occurred and this is consistent with the report that as the DDT poisoning of insects increased, the oxygen utilization decreased, until the death of the organisms occurred.

Discussion

Soon after the large scale use of DDT for the control of insect pests was begun, it became evident that bird populations (Robbins *et al*, 1951) declined in the DDT treated areas. Some of the observed changes appeared to be due to the effects of DDT on the viability and hatchability of eggs (Mitchell *et al*, 1951). More recent data have suggested that the wide-spread use of DDT and other organochloro insecticides may be responsible for the decline of populations of the golden eagle, *Aquila chrysaetas* (Lockie and Ratcliffe, 1964), Osprey, *Pandion haliaetus* (Ames, 1966), herring gull, *Larus*

Table 2.—Effects of DDT on Hydrolysis of ATP.

Metal	g of DDT per ml reaction medium		
	5.3×10^{-4}	8.9×10^{-4}	17.7×10^{-4}
	Average Change in μ moles ATP/hr/mg N		
Cadmium	(—) 34	(—) 16	(—) 7
Zinc	(±) 13	(±) 9	(—) 19
Manganese	(+) 40	(+) 45	(±) 19
Magnesium	(+) 16	(+) 36	(+) 23
Calcium	(±) 7	(±) 11	(±) 33

argentatus (Paynter, 1949), and the peregrine falcon, *Falco peregrinus* (Hickey and Anderson, 1968).

Lake trout (*Salvelinus namaycush*) sac fry, hatching from eggs of females from Lake George, New York, did not survive. However, when the sperm from males from Lake George were used to fertilize eggs of females from another watershed, fry survival was normal. The watershed of Lake George had received applications of DDT for the control of gypsy moth. Burdick *et al* (1964) investigated this phenomenon, described the syndrome produced in the lake trout sac fry, and pinpointed the time of its development as late in the yolk-sac utilization period and just before the fry began to feed. This time appeared to be correlated with the period of maximum utilization of the phospholipid content of the yolk-sac. Further, comparison of the affected fry with normal fry did not reveal any histological or pathological differences. They noted that fry hatched from eggs which contained 2.95 ppm of DDT developed the syndrome, whereas fry from eggs with a DDT content of 2.67 ppm did not.

Allison *et al* (1963) reported that mortality among sac fry appeared to be highest in those cutthroat trout (*Salmo clarki lewisi*) which received high concentrations of DDT. Maeck (1968) reported similar observations with brook trout (*Salvelinus fontinalis*) which had received repeated sub-lethal concentrations of DDT.

The recent work reported by Ames (1966) on the Osprey in Connecticut and the work of Paynter (1949) on the herring gull in Lake Michigan indicated that DDT is the causative agent in the decrease in populations of these two species and that the effect was on the development of the embryo. It has been suggested that in the eagle (Lockie and Ratcliffe, 1964) and peregrine falcon (Hickey and Anderson, 1968) DDT caused a reduction in egg shell thickness. However, the effects of DDT on the reproduction of the quail (Dewitt, 1956) and the pheasant (*Phasianus colchicus*) (Genelly and Rudd, 1956) do not fit this pattern, for their chicks die several weeks after hatching.

DDT can cause mortality among fishes and birds. However, repeated sub-lethal doses of DDT did not cause mortality. Further, sub-lethal doses of DDT to fishes and birds did not cause any reduction in the number of viable eggs produced. In fishes, normal sac-fry were produced from females from the Lake George watershed which apparently were not susceptible to the effects of DDT until late in the sac-fry stage. The fish affected during sac-fry stages did not show any histological or pathological damage. Bird embryos, however, appeared to die in embryonic life. Thus, it would appear that DDT must be altering some fundamental biochemical or physiological processes common to all the organisms and to the various situations discussed. Energy production would be such a common denominator.

The increased oxygen consumption and the associated random, awkward body movements observed in insects treated with DDT suggest a neuromuscular involvement. It is generally agreed that the increased oxygen consumption is the result of increased activity of the organisms (Metcalf, 1955, O'Brien, 1967). However, Riker (1946), Jandorf *et al* (1946), and Laug and Fitzhugh (1946) found that the oxygen consumption of liver slices of animals in sub-acute and chronic DDT poisoning had an increased oxygen consumption, and

that the oxygen consumption from animals in advance stages of DDT poisoning was decreased. The changes in body coordination could be considered as a partial impairment of functions due to the decreased ability of the nervous system to function normally, a possible result of reduced energy output.

Part of the reaction of the organisms would appear to be a response to stress. It was observed that as the poison symptoms became more severe, oxygen uptake declined (O'Brien, 1967), which is consistent with the observed effects of DDT on oxygen uptake as described above. The first system to be involved might be the nervous system because of its high fat content. This point will be discussed later.

Rotenone, which has been used as an insecticide but more recently has been widely used as a fish toxicant, has been shown to decrease oxygen uptake by insects (Tishler, 1935) and was thought to cause damage to the tissues (Danneel 1933). Fukami and Tomizawa (1956) reported that rotenone inhibited the oxidation of glutamate, and later it was shown that this compound did not cause tissue damage (Oberg, 1955) but that its primary effect was the inhibition of the transfer of electrons from the substrate to the cytochrome chain (Lindahl and Oberg, 1961). This has been the only biochemical effect shown for rotenone. It should be pointed out that in rotenone poisoning, excessive, random movements of fishes are observed, but rotenone has not been considered a nerve poison (O'Brien, 1967). The effects of rotenone on the oxygen uptake by bluegill liver mitochondria have been reported (Hiltibran and Johnson, 1965).

Cyanide and antimycin A have been used as fish toxicants (Bennett, 1962), (Walker *et al,* 1964) and appear to block the uptake of oxygen by bluegill liver mitochondrial systems (Hiltibran, 1965, 1967a). They interrupt the electron flow from the substrate to the electron acceptor, oxygen (Chance, 1956). The data strongly suggest that when the oxidative pathways are blocked, energy cannot be produced, various cells cannot function, and if some regulatory cells or organ would be affected, for example the respiratory center, the organism cannot maintain its integrity and dies. This has been suggested previously (Hiltibran, 1971), (Skidmore, 1965).

It is well known that some metals are extremely toxic to bluegills (McKee and Wolf, 1963). Recently we observed that cadmium and zinc at relatively low levels (Hiltibran, 1965; 1967a) severely inhibited the uptake of oxygen by bluegill liver mitochondria. We found also that calcium and manganese can interrupt energy production, but the effect was primarily on phosphate metabolism, whereas the primary effect of cadmium and zinc was on the uptake of oxygen (Hiltibran, 1971).

Previously we had been intrigued by the fact that certain derivatives of 2,4-D were more toxic to small bluegills than were others (Hiltibran, 1967c). Therefore, we investigated the effects of nine derivatives of 2,4-D and found that the most toxic 2,4-D derivatives used in the study, the butyl and isopropyl esters, altered the uptake of oxygen and phosphate by the bluegill liver mitochondria. Thus it would appear that the primary effect of these derivatives was on oxygen uptake and that their toxic action could be produced via their effects on energy production (Hiltibran, 1969a; 1969b). It appears, then, that the

effects of DDT on the oxygen uptake cited above would be of paramount importance in explaining the mode of action of DDT, since we have shown the similarity of the effects of DDT, rotenone, cadmium, zinc, the butyl and isopropyl esters of 2,4-D, and other electron flow inhibitors and oxidative phosphorylation uncoupling agents.

Thus I believe that the interruption of the production of energy is of primary importance in explaining the toxic action of DDT. I suspect that similar cases can be developed for some of the other organochloro insecticides on the basis of available data (Hiltibran unpublished data; Colvin and Phillips, 1968).

DDT is very soluable in or has a high affinity for the tissue fat. Further, it is assumed that the incorporation of DDT into the tissue fat is a passive process and is not an active ''detoxification'' process. It does not appear that definitive data are available to determine whether this incorporation is a biological active or a biological passive process.

Exposure of target or non-target organisms to lethal levels of DDT would result in a DDT uptake which would exceed the rate at which DDT could be incorporated into the tissue fat and/or exceed the DDT storage capacity of the total body fat content. This amount of DDT would be in addition to that which would be metabolized. The remaining ''unbound DDT'' or ''circulating DDT'' would be able to exert its toxic action by blocking the oxygen uptake in the various tissues. Repeated light doses of DDT to fishes and birds would give time for the storage of the DDT in the tissue fat, and this could account for the large buildup of DDT without apparent damage.

Apparently the DDT is transferred to the eggs of fishes or birds which would account for the high levels of DDT in these eggs. In fishes, most of the DDT would remain bound throughout the development of the fish embryo, and DDT could not exert its toxic action until the late stage of the development when the yolk fat was mobilized, and the DDT again became ''circulating'' or ''unbound''. If only small amounts of DDT were involved, the effects on fry would not be lethal. This is consistent with the data in Table 1 and with the observations of Burdick *et al* (1954). The DDT content of bird eggs which did not develop normal embryos has not been as well documented as has the DDT content of fish eggs; the effects of DDT on bird reproduction is not as clearly defined as with the fishes. However, the developing bird embryos apparently were severely affected by high levels of ''circulating DDT''.

There are intraspecies differences in the quail and pheasant that complicate the picture, and these will remain a mystery until the comparative biochemistry of the organisms involved is known. These differences might help to explain why quail and pheasant chicks are the susceptible unit to the lethal effects of DDT. It is suspected that the ''circulating DDT'' is the causative agent, but apparently the stress develops later which may coincide with the production of feathers or using the remaining portion of the yolk, which may contain large quantities of DDT.

Data to support this hypothesis comes from the work of Johnston (1951), Anderson (1954), Riker (1946), Jandorf *et al* (1946), and Laug and Fitzhugh (1946). Judah (1949) could not demonstrate any effect of DDT on the succinic oxidase,

but Johnston could. Judah had administered the DDT in his experiments in an oil emulsion, whereas Johnston had administered the DDT in acetone. Further, when Johnston administered DDT in an oil emulsion, he could not demonstrate any effect of DDT on the succinic oxidase. This demonstrates the protective effect of the oil or fat on the DDT. It is not surprising, therefore, that with the high fat and phospholipid content of the nervous tissue that nervous systems might be one of the first tissues affected.

It has been suggested that DDT altered the production of the egg shell by the peregrine falcon (Hickey and Anderson, 1968) and the golden eagle (Lockie and Ratcliff, 1964), and this effect of DDT was confirmed in experiments with the American sparrow hawk, *Falco sparverius* (Porter and Wiemeyer, 1969). The synthetic formation of the egg shell, primarily calcium carbonate, would suggest that its synthesis is an energy-requiring process for the mobilization of the required calcium and for the production of the carbonate. Torda and Wolff (1959) demonstrated that DDT inhibited the carbonic anhydrase enzyme complex, which has been suggested as one enzyme complex involved in the formation of the egg shell (Sturkie, 1954). Further, the effects of organochloro pesticides on the metabolism of endogenous steroids is not known (Kupfer, 1967), but recently Welch *et al* (1969) reported that a DDT isomer was converted to an estrogentic hormone metabolite which would indicate that organochoro insecticides, particularly DDT, may have some effect on the steroid hormones involved in calcium metabolism.

Johnston (1951) found that DDT did not alter the succinic dehydrogenase but that the succinic oxidase complex was affected. The data in Table 2 indicate that DDT did not appreciably alter the oxidation of alpha-ketoglutarate. These observations indicate that DDT inhibited the flow of electrons at the flavoprotein transferring site between the succinic dehydrogenase and the cytochrome chain. Matsumura and O'Brien (1966) have isolated complexes of DDT and tissue components and suggested that the complexes involved were of the charge-transfer type. These data would further support the biochemical hypothesis suggested, since DDT could form a complex with the flavoprotein of the electron transferring site between succinic acid and the cytochrome chain. Such action could block the flow of electrons, which would block the production of energy via the oxidative pathways. DDT has been found to have an effect similar to rotenone, cyanide, and antimycin A, and it is suggested that this is the primary effect of DDT. DDT also has been shown to have an effect on phosphate metabolism, as indicated by its effect on the hydrolysis of ATP, which rotenone, cyanide, and antimycin A did not alter. The data suggest that the primary effect is the inhibition of electron flow from succinic acid to the cytochrome chain.

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SURFACE TENSIONS OF BINARY SOLUTIONS OF NITROPARAFFINS IN CARBON TETRACHLORIDE

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Abstract. — The surface tensions of binary solutions of nitromethane in carbon tetrachloride and nitroethane in carbon tetrachloride were determined by the ring method at 30°, 35°, and 45°C. Assuming perfect solutions, the surface excesses and the surface entropies and enthalpies were calculated. The surface entropies indicated increasing ordering in the solutions. Surface entropies and enthalpies of mixing were also calculated. These calculations indicated that nitroethane formed a more ideal solution than nitromethane. Nitromethane showed evidence of association as well as disassociation. The deviations for these solutions are within the limits of an empirical mixture law.

This investigation is a continuation of the series of simple physical measurements upon binary solutions of nitroparaffins in carbon tetrachloride. The immediate purpose of such measurements is to place into the literature accurate solution data concerning commercial chemicals that have important applications in automotive fuels. Although data involving pure nitroparaffins, particularly nitromethane is plentiful, solution data is sparse (cf. Timmermans (1959)). The current series of papers indicates the degree to which solution values, for engineering purposes, can be obtained by interpolation and/or extrapolation of existing data and by prediction from empirical formulae.

A second major objective of the current investigations is to obtain inferential information concerning the structure of these binary solutions. First, are the solutions ideal, regular, or irregular? Second, if the solutions are irregular, what is the nature of the irregularity? Gunter *et al.* (1967) by means of density measurements have shown that these binary solutions are irregular with respect to volume effects. Wettaw *et al.* (1969) by means of viscosity measurements have shown that these binary solutions are irregular with respect to entropy effects. The present paper shows the nature of irregularities determined by surface tension measurements. Since inferences concerning the bulk solution may differ from inferences concerning the surface layer, this work has the advantage of providing two sets of conclusions with one set of measurements. Third, how do solutions of homologous members of the nitroparaffin series differ? The previous sets of measurements indicated that the first member of the series is quite different from the second member. Whether or not the same conclusion is true for each different physical property is determined only by making the measurement.

This paper discusses the surface tension, the surface excess, the surface entropy and enthalpy, and the excess surface entropy and enthalpy

of mixing as functions of concentrations, and their relation to the molecular structure of the pure nitroparaffins. In order to acquire a proper perspective between older and more modern investigations of regularity, one empirical relationship, the parachor, is examined.

Experimental

Fisher certified and spectro grades of carbon tetrachloride, Fisher certified grade nitromethane, high purity research samples of nitromethane and nitroethane (Commercial Solvents), and highest purity nitroethane (Brothers Chemical Co.) were used without further purification. The solutions were prepared by mixing volumes of pure components. The estimated cumulative transfer error was ± 0.005 mole fraction.

The solutions, in closed, ground glass containers, were brought to equilibrium in a Precision Scientific Co. bath (No. 66580) and regulated (± 0.02°C.) with a Merc to Merc Model PS-62510-D1 thermoregulator. Temperature readings, precise to ± 0.01°C., were obtained with a thermometer calibrated with a National Bureau of Standards thermometer. The precision in the preparation of solutions dictated rounding of the temperature readings by 0.1°C. or less to integral values.

The apparent surface tensions were obtained, after rapid transfer of the solutions from the bath, by the ring method using a Fisher Surface Tensiometer, Model 20. True surface tensions were calculated with the formula for the correction factor,

$$\gamma = .7250 + \sqrt{\left[\frac{.04534 - 1.679}{(R_{wire}\ R_{ring})}\right] + \left[\frac{.01452\ D_{Av}}{C^2\ \ (D_{liq}\ \ - D_{vap}\ )}\right]} \quad (1)$$

where D_{Av} is the average dial reading; γ is the true surface tension; R_{wire} and R_{ring} are the radii of the suspending wire and the platinum ring, respectively; C is the circumference of the ring; and D_{liq} and D_{vap} are the densities of the solution and the vapor in equilibrium with the solution, respectively. Vapor densities were estimated, by linear interpolation, for use in the correction calculation; liquid densities were taken directly from or estimated from Gunter *et al.* (1967).

Results

The number of readings and the average deviation of each set of readings are given in Table 1. These averages sometimes represent readings taken on different days; sometimes they represent readings taken on different sample grades; and sometimes they represent readings on repetitive trials taken to ascertain temperature loss in the transfer process. There is no explanation for the readings with the greatest deviation, i.e. 0.8 and 0.9 $CH_3\ NO_2$, except lack of experimental technique. Nevertheless, even these readings are acceptable for an instrument whose scale gradations are given in 0.1 units. Each set of readings was processed by a computer program which calculated the average deviation and the probable error of an individual reading, tested each individual reading for discard as being beyond normal experimental error, and, if necessary, recalculated the averages and the deviations. The discard judgment was based on Chauvenet's criterion (Worthing and Geffner [1943]). Insomuch as

Table 1.—Surface Tensions of Carbon Tetrachloride-Nitroparaffin Solutions.

Mole Fraction (RNO_2)	30°C				35°C				45°C			
	γ (dyne/cm)	Probable Error (dyne/cm)	No. Read.	Av. Dev. Dial Read.	γ (dyne/cm)	Probable Error (dyne/cm)	No. Read.	Av. Dev. Dial Read.	γ (dyne/cm)	Probable Error (dyne/cm)	No. Read.	Av. Dev. Dial Read.
Nitromethane												
0.0	26.70	.01	40	.08	25.77	.01	26	.04	24.65	.01	26	.09
0.1	26.88	.00	10	.08	25.90	.00	10	.00	24.77	.01	19	.10
0.2	27.08	.02	10	.07	26.01	.01	15	.04	24.95	.01	14	.04
0.3	27.13	.02	15	.11	26.29	.00	15	.04	25.22	.01	13	.03
0.4	27.35	.01	30	.09	26.42	.02	15	.08	25.36	.02	15	.10
0.5	27.51	.01	20	.03	26.50	.01	20	.10	25.81	.01	12	.04
0.6	27.72	.00	20	.01	27.08	.01	15	.05	26.15	.01	12	.05
0.7	28.37	.01	20	.08	27.79	.01	15	.07	26.76	.01	12	.05
0.8	29.70	.02	20	.08	29.34	.03	40	.21	27.96	.01	12	.06
0.9	31.76	.01	15	.09	31.46	.04	20	.22	29.84	.01	12	.05
1.0	35.44	.01	15	.03	34.00	.01	12	.07	32.74	.02	9	.07
Nitroethane												
0.0	26.70	.01	40	.08	25.77	.01	26	.04	24.65	.01	26	.09
0.1	26.62	.00	35	.05	25.87	.01	25	.09	24.79	.01	9	.06
0.2	26.73	.00	20	.02	26.08	.01	15	.06	24.89	.02	9	.06
0.3	27.11	.01	10	.03	26.40	.01	25	.09	25.17	.01	9	.04
0.4	27.23	.01	10	.03	26.56	.01	15	.07	25.50	.02	9	.08
0.5	27.55	.00	10	.02	26.84	.01	15	.06	25.83	.02	9	.07
0.6	27.96	.00	10	.00	27.42	.00	15	.04	26.19	.02	9	.09
0.7	28.59	.01	5	.02	28.01	.02	5	.08	26.72	.02	9	.06
0.8	29.24	.01	5	.02	28.62	.00	10	.00	27.41	.01	9	.04
0.9	29.86	.02	5	.06	29.39	.01	5	.04	28.22	.01	9	.05
1.0	30.66	.01	20	.05	30.16	.02	5	.04	29.11	.02	9	.08

this particular criterion was used for decision making, the standard deviation of each set of readings was not recovered in the computer output. The standard deviation, ascertained by trial calculations, is approximately 1.1 to 1.2 the average deviations tabulated in Table 1.

The probable error of the true surface tension calculated with Equation (1) was calculated in the usual formulas for propagation of errors (cf. Daniels *et al.* (1962)) using the errors given by Gunter *et al.* (1967) for D_{liq} , the average deviations of D_{Av} , and zero error in R_{wire} , R_{ring} , C, and D_{vap} . The probable errors are given in Table 1 along with the true surface tensions calculated from Equation (1). The D_{liq} determination involved the compounding of errors from several sources which makes it, relatively, less determined than D_{Av} which involved a single, accurate measurement. The probable errors shown in Table 1 reflect the weighted importance of the errors in D_{liq} .

Individual measurements at 30°C. showed no significant variation for the different grades of carbon tetrachloride and nitromethane. The results are in good agreement with the nitroparaffin values interpolated from the ring method data of Boyd and Copeland (1942). The results are lower than the data measured by and/or the data calculated from the non-ring methods used by Snead and Clever (1962) and Thompson, Coleman, and Helm (1954). The greatest 30° deviation from the literature involves CCl_4 and is probably due to slight evaporation loss during transfer. On the other hand, the deviation found in $C_2H_5NO_2$ is undoubtedly due to the impurities inherent in the grade of chemical used. Although of superior quality, the $C_2H_5NO_2$ is not of the great purity as the CH_3NO_2 and CCl_4. No literature values were available at 35°C or 45°C. One may surmise that the present data are of quality comparable to that of the 30°C data with somewhat greater experimental error due to evaporation at the higher temperature. A comparison of the rapid ring method with a more accurate technique, e.g. the maximum bubble pressure method, is obtained by comparing the values from the literature found in Table 6. No literature data for the solutions are available but the present data can be construed to be of the same excellent quality as the data for our pure compounds.

The relative surface adsorption (surface excess) of the nitroparaffin (component 2) with respect to the carbon tetrachloride (component 1).

$\Gamma_{2,1}$ was calculated from the equation for perfect solutions (Defay *et al.* (1966)),

$$\Gamma_{2,1} = -\frac{x_2}{RT}\left(\frac{\partial \gamma}{\partial_2 x}\right)_{T,P} \qquad (2)$$

where x_2 is the mole fraction of the nitroparaffin, R is the ideal gas constant, T is the absolute temperature, and P is the ambient pressure. The derivative in Equation (2) was obtained analytically from a least squares fit of the surface tension data to a quadratic function of the mole fraction. The choice of a quadratic function was arbitrary but limited by the criteria of a good representation of the input data and statistical reliability for the number of degrees of freedom. The zero degree and first degree polynomials are eliminated by the first criterion while any polynomial of quintic degree or higher is eliminated by the second criterion. Consideration of the errors inherent in determining a derivative through the use of data fit plus the errors in assuming the

validity of Equation (2) suggested that cubic degree or higher polynomials were an over determination. The absolute surface adsorption of the nitroparaffin, Γ_2, was calculated assuming a dividing surface under an inhomogeneous monolayer and a mixture surface tension which is a linear function of the surface adsorption mole fraction, $\Gamma_2/_2 + \tau_1$), (van Rysselberghe (1938)). The results are given in Table 2. In general, the results illustrate the principle that the substance of lower surface tension is concentrated at the surface. For example, the negative values of $\Gamma_{2.1}$ reflect the increasing deficiency of RNO_2 with respect to CCl_4 (the substance of lower surface tension). The values of Γ_2 are consistent with the size of the nitroparaffin molecule. The amount of excess is proportional to the difference in surface tensions of the components of the solution. The more negative values of $\Gamma_{2.1}$ for CH_3NO_2

TABLE 2.—Surface Adsorptions of Carbon Tetrachloride-Nitroparaffin Solutions.

Mole Fraction (RNO_2)	Stated in Units of Q cm^{-2} x 10^{10}					
	$\Gamma_{2,1}$			Γ_2		
	30°C	35°C	45°C	30°C	35°C	45°C
Nitromethane						
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.190	0.143	0.107	—0.0462	—0.0241	—0.0154
0.2	0.152	0.0776	0.334	—0.0340	—0.0107	—0.00601
0.3	—0.115	—0.196	—0.220	0.0159	0.0369	0.0470
0.4	—0.610	—0.678	—0.653	0.0836	0.0997	0.110
0.5	—1.33	—1.37	—1.27	0.152	0.149	0.254
0.6	—2.29	—2.27	—2.06	0.221	0.327	0.368
0.7	—3.47	—3.37	—3.03	0.392	0.545	0.537
0.8	—4.88	—4.69	—4.18	0.735	1.11	0.872
0.9	—6.51	—6.21	—5.51	1.18	2.06	1.37
1.0	—8.38	—7.94	—7.03	.....	.....	.....
Nitroethane						
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	—0.0219	—0.0416	—0.0313	—0.00316	0.0113	0.0121
0.2	—0.112	—0.147	—0.128	0.00451	0.0642	0.0379
0.3	—0.271	—0.317	—0.291	0.00989	0.207	0.129
0.4	—0.498	—0.550	—0.519	0.150	0.271	0.283
0.5	—0.794	—0.847	—0.814	0.301	0.405	0.455
0.6	—1.16	—1.21	—1.17	0.521	0.807	0.634
0.7	—1.59	—1.63	—1.60	1.02	1.32	0.947
0.8	—2.09	—2.12	—2.09	1.68	1.82	1.43
0.9	—2.66	—2.67	—2.65	2.08	2.98	2.15
1.0	—3.30	—3.29	—3.27	.....	.....	.....

mirror the greater differential in the γ-values of CH_3NO_2 and CCl_4 as compared to $C_2H_5NO_2$. Although the specific values of Γ_2 depend upon the mode of calculation (the choice of surface), these qualitative conclusions remain invariant (Guggenheim and Adam (1933)).

The surface entropy per unit area, s_σ, was obtained from a least squares fit of surface tension data to a linear function of temperature. The linear function was chosen because of the well-known behavior of surface tension as a function of temperature (Partington, 1951). This fortuitous functional behavior results in a particularly simple calculation whereby the slope of the least squares fit is simply $(d\gamma/dT)_{P,A}$ (A being the surface area). The identification of this slope as s_σ results from the alternate view of surface tension as surface free energy and the application of the usual thermodynamic equations to the surface layer. The heat of extension of the surface per unit area (latent heat per unit area), l_σ, was calculated from s_σ and, then, the enthalpy of extension of the surface per unit area, h_σ, was calculated from l_σ and γ. That is, the latent heat is obtained by a temperature multiplication of the slope of the least squares linear plot. The enthalpy then follows immediately from the first law of thermodynamics, that is, from the conservation of energy. The values of s_σ and h_σ are given in Table 3.

The surface entropy per mole, s_m, was calculated from the slope of a Ramsay-Shields (1893) plot determined by the method of least squares. This calculation proceeds exactly as that of the preceding paragraph but the independent variable is not γ but $\gamma(MV)^{2/3}$, where M is the molecular mass of the compound and V is the molar volume of the compound. For solution data, M was obtained by the assumption that mass of the solution is additive with respect to the mole fraction of the components,

$$M = x_1M_1 + x_2M_2 \qquad (3)$$

where x_1 and x_2 are the mole fractions of CCl_4 and nitroparaffin, respectively; M_1 and M_2 are the molecular masses of CCl_4 and nitroparaffin, respectively; and M is the molecular mass of the solution. The molar volumes were taken from Gunter *et al.* (1967). Whereas γ is the surface free energy, the quantity $\gamma(MV)^{2/3}$ is the free molecular surface-energy. As is true for

$$\gamma, \gamma(MV)^{2/3}$$

fits well a linear function of temperature, if the temperature is given in absolute terms. In a manner analogous to the calculation of l_σ and h_σ, the heat of extension of the surface per mole (latent heat per mole), l_m, and the enthalpy of extension of the surface per mole, h_m, were calculated from s_m. The entrophies and enthalpies are tabulated in Table 3.

The values of s_σ and h_σ for pure nitroparaffins are in good agreement with those given by Snead and Clever (1962) while the pure CCl_4 values, like those of Vogel (1948) are higher than most values which can be obtained from literature data. The fact, that the values for CH_3NO_2 are slightly higher than the best literature values while the $C_2H_5NO_2$ values are equivalent to the best literature values implies that the slope of the CH_3NO_2 $\gamma - t$ function is changing more rapidly than usually accepted; the conclusion is not inconsistent with the possibility of slightly more evaporative loss at higher temperatures for the lower boiling CH_3NO_2. A greater variation is

TABLE 3.—Surface Thermodynamic Quantities.

Mole Fraction (RNO_2)	Entropy		Enthalpy					
			h_σ (ergs/cm²)			$h_m \times 10^{-10}$ (ergs/mole)		
	s_σ (ergs/cm²-deg)	$s_m \times 10^{-7}$ (ergs/mole-deg)	30°C	35°C	45°C	30°C	35°C	45°C
Nitromethane								
0.0	0.133	20.0	66.9	66.7	66.9	10.8	10.8	10.8
0.1	0.137	20.3	68.4	68.2	68.4	10.8	10.8	10.8
0.2	0.137	19.6	68.6	68.2	68.5	10.5	10.4	10.5
0.3	0.124	16.6	64.8	64.6	64.8	9.46	9.44	9.46
0.4	0.128	16.5	66.3	66.0	66.2	9.33	9.28	9.32
0.5	0.107	11.8	59.8	59.4	59.7	7.76	7.68	7.75
0.6	0.103	10.4	58.9	58.8	58.9	7.19	7.17	7.19
0.7	0.107	10.1	60.8	60.8	60.8	7.05	7.03	7.04
0.8	0.120	11.1	66.0	66.2	66.0	7.34	7.36	7.34
0.9	0.133	12.5	72.0	72.4	72.1	7.85	7.89	7.87
1.0	0.172	17.8	87.7	87.1	87.6	9.71	9.64	9.70
Nitroethane								
0.0	0.133	20.0	66.9	66.7	66.9	10.8	10.8	10.8
0.1	0.120	17.3	63.1	62.9	63.0	9.94	9.99	9.93
0.2	0.122	17.4	63.7	63.7	63.7	9.89	9.89	9.89
0.3	0.128	18.2	65.9	65.8	65.9	10.1	10.1	10.1
0.4	0.114	15.2	61.7	61.6	61.7	9.15	9.10	9.14
0.5	0.113	14.7	61.8	61.6	61.7	8.95	8.91	8.94
0.6	0.119	15.6	63.9	64.0	63.9	9.21	9.22	9.21
0.7	0.125	16.5	66.5	66.6	66.5	9.47	9.48	9.48
0.8	0.122	15.5	66.1	66.1	66.1	9.20	9.20	9.20
0.9	0.111	13.5	63.4	63.4	63.4	8.59	8.59	8.59
1.0	0.104	12.0	62.2	62.2	62.2	8.13	8.14	8.13

found for the molar thermodynamic quantities because of the variations in the density data. Both s_σ and s_m for nitromethane exhibit a minimum and, thus, indicates that the surface molecules of some nitromethane solutions are more ordered than the surfaces of either of the components. A possible explanation for the greater ordering would be the formation of a complex between CCl_4 and CH_3NO_2, a possibility diametrically opposed to the suggestions of de Maine *et al.* (1957) that a monomer-dimer nitroparaffin equilibrium occurs in these binary solutions. More likely, this effect is the demonstration previously unreported, for surface layers of the entropy of mixing effect, previously demonstrated for the bulk solutions of CCl_4 and CH_3NO_2 (Wettaw, *et al.* (1969)).

The surface entropies and enthalpies of mixing were calculated for each solution by taking the differ-

ence between a value in Table 3 and its corresponding value predicted for perfect solutions based upon additivity of components with respect to mole fraction. That is, the perfect solution value is found by replacing M_1 and M_2 in Equation (3) with the appropriate thermodynamic values, e.g. $(s_m)_1$ and $(s_m)_2$ to obtain s_m^M. Molar excess surface entropies of mixing, s_m^E, were calculated by addition of the terms

$$R \sum_{i=1}^{i=2} X_i \ln x_i$$

to the molar surface entropies of mixing, s_m^M. Scratchard (1949) has shown, for bulk solutions, that excess volume of mixing, V^E, can be fitted to a function of the form

$$V^E = a_0 x_1 x_2 + a_1 x_1 x_2 (x_1 - x_2) + \ldots\ldots\ldots \quad (4)$$

where a_0 and a_1 are constants, and

TABLE 4.—Surface Mixing and Excess Thermodynamic Quantities.

Mole Fraction (RNO_2)	Entropy			Enthalpy					
	M s_σ (ergs/cm²-deg)	M $s_m \times 10^{-7}$ (ergs/mole-deg)	E $s_m \times 10^{-7}$ (ergs/mole-deg)	M h_σ (ergs/cm²)			M $h_m \times 10^{-10}$ (ergs/mole)		
				30°C	35°C	45°C	30°C	35°C	45°C
Nitromethane									
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.0001	0.52	3.2	—0.58	—0.54	—0.57	0.11	0.12	0.11
0.2	—0.0038	0.04	4.0	—2.5	—2.6	—2.5	—0.08	—0.17	—0.08
0.3	—0.021	—2.7	2.3	—8.3	—8.2	—8.3	—1.0	—1.0	—1.0
0.4	—0.021	—2.6	3.0	—8.9	—8.9	—9.0	—1.0	—1.0	—1.0
0.5	—0.046	—7.1	—1.3	—18.	—18.	—18.	—2.5	—2.5	—2.5
0.6	—0.053	—8.3	—2.7	—20.	—20.	—20.	—3.0	—2.9	—3.0
0.7	—0.053	—8.4	—3.3	—21.	—20.	—21.	—3.0	—3.0	—3.0
0.8	—0.044	—7.1	—3.1	—18.	—17.	—17.	—2.6	—2.5	—2.6
0.9	—0.035	—5.5	—2.8	—14.	—13.	—13.	—2.0	—1.9	—1.9
1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitroethane									
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	—0.010	—1.9	0.80	—3.3	—3.4	—3.4	—0.59	—0.63	—0.60
0.2	—0.0052	—1.0	3.0	—2.3	—2.1	—2.3	—0.38	—0.38	—0.38
0.3	0.0037	0.60	5.7	0.41	0.45	0.41	0.10	0.098	0.10
0.4	—0.0074	—1.6	4.0	—3.3	—3.3	—3.3	—0.58	—0.63	—0.59
0.5	—0.0055	—1.3	4.5	—2.8	—2.8	—2.8	—0.52	—0.56	—0.52
0.6	0.0034	0.40	6.0	—0.18	0.0	—0.18	0.012	0.016	0.012
0.7	0.012	2.1	7.2	2.9	3.0	2.9	0.54	0.54	0.55
0.8	0.012	1.9	5.9	3.0	3.0	3.0	0.54	0.53	0.54
0.9	0.0041	0.70	3.4	0.73	0.75	0.73	0.19	0.18	0.19
1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

x_1 and x_2 are the mole fraction of CCl_4 and nitroparaffin, respectively. For binary solutions, the first term on the right hand side of Equation (4) is a quadratic term in either x_1 or x_2 while the second term is a cubic term. Other molar excess quantities are expected, for bulk solution, to have the same functional form as Equation (4). Gunter *et al.* (1967) have shown for the bulk solutions of CH_3NO_2–CCl_4 that only a_0 is non-zero while for bulk solutions of $C_2H_5NO_2$–CCl_4 only a_1 is non-zero. The surface enthalpies of mixing, which are identical with the excess surface enthalpies of mixing, shown in Table 4 indicate exactly the same functional information. That is, the cubic functional forms of the nitroethane values represent less deviation from ideality than the quadratic functional form of the nitromethane values. The first example known to the present authors of the usual treatment of perfect solutions and excess functions to surface layers rather than to bulk solutions is given by Bloom, *et al.*(1960). Their application to molten salts is rather

TABLE 5.—Parachors of Carbon Tetrachloride-Nitroparaffin Solutions.

Mole Fraction (RNO_2)	Calculated from Solution Surface Tensions			Calculated from Component Surface Tensions			Calculated from Atomic and Bond Parachor Values
	30°C	35°C	45°C	30°C	35°C	45°C	
Nitromethane							
0.0	222.0	221.3	221.8	222.0	221.3	221.8	229.8
.1	212.7	211.8	212.2	213.1	212.4	212.9	220.1
.2	203.4	202.5	202.9	204.3	203.6	204.0	210.3
.3	193.7	193.4	194.0	195.4	194.7	195.1	200.6
.4	184.5	184.0	184.9	186.5	185.8	186.3	190.9
.5	174.7	174.2	176.2	177.7	177.0	177.4	181.2
.6	165.2	165.2	167.2	168.8	168.1	168.5	171.4
.7	156.2	156.3	158.4	159.9	159.2	159.6	161.7
.8	147.9	148.3	149.9	151.1	150.4	150.8	152.0
.9	140.1	140.5	141.5	142.2	141.5	141.9	142.2
1.0	133.3	132.7	133.0	133.3	132.7	133.0	132.5
Nitroethane							
0.0	222.0	221.3	221.8	222.0	221.3	221.8	229.8
.1	216.2	215.9	216.6	216.9	216.2	216.7	224.1
.2	210.8	210.6	211.0	211.8	211.2	211.7	218.3
.3	205.8	205.4	205.7	206.6	206.2	206.7	212.6
.4	200.2	198.6	200.6	201.5	201.2	201.6	206.9
.5	195.0	193.2	195.4	196.4	196.1	196.6	201.2
.6	189.8	190.0	190.1	191.2	191.1	191.6	195.4
.7	185.1	185.2	185.1	186.1	186.1	186.6	189.7
.8	180.4	180.3	180.5	181.0	181.0	181.5	184.0
.9	175.6	175.4	175.9	175.9	176.0	176.5	178.2
1.0	170.7	171.0	171.5	170.7	171.0	171.5	172.5

remote from the type of solutions discussed in this paper. The only other example of this treatment to surface layers known to the authors was published by Suri and Ramakrishna (1969) after the conclusion of the present work. However, the agreement with the conclusions of Gunter, *et al.* (1967) is a validation of the application to surface layers rather than an assumption that surface layers obey the same physical laws as the bulk solution.

The negative s_m^E nitromethane values indicate (Scatchard and Raymond (1938))that some CCl_4 molecules are associating with the clusters of nitromethane molecules, thus, substantiating the conclusion of ordered surfaces derived from the s_σ and s_m values. The positive s_m^E values indicate dissociation of the nitroparaffins. In one sense, both of the conclusioss suggested by Table 3 are valid, if a complex is interpreted as a simple association of molecules. Reid and Sherwood (1966) suggest the use of the parachor for the estimation of surface tensions of nonaqueous mixtures. Table 5 compares the values of the parachor calculated directly from solution densities and surface tensions and those calculated from the parachors of the mixture components. The maximum deviations of 2.3% (CH_3NO_2) and 1.5% ($C_2H_5NO_2$) from the linear mixture law indicate that estimation from parachor values is appropriate for these solutions. The greater deviations for the nitromethane solutions is attributed to the greater difference in surface tension of the components as first observed by Hammick and Andrew (1929). The discrepancy between the observed CCl_4 parachor value and the value obtained by addition of atomic parachors is attributed to the accumulation of negative groups (Mumford and Phillips (1929)) and suggests that a better estimation of surface tensions of carbon tetrachloride-nitroparaffin binary solutions would result from the use of an experimental CCl_4 parachor.

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TABLE 6.—Summary of Literature Values.

Surface Tension 30°C, γ (dyne/cm)	Entropy		Enthalpy	Parachor
	s_σ (ergs/cm²-deg)	$s_m \times 10^{-7}$ (ergs/mole-deg)	h_σ (ergs/cm²)	[P]
Nitromethane				
34.26[2]	0.1387[7]	31.59[7]	81.1[4]	131.8[14]
35.47[3]	0.150[4]	15.8[5]	86.4[3]	132.6[3–8]
35.48[4]	0.1678[3]	14.4[8]	81.3[7]	132.7[7]
35.50[1]	0.160[5]	20.3[3]	84.4[5]	
35.9[5]	0.1464[8]		79.8[8]	
32.11*[2]				
Nitroethane				
31.5[3]	0.1090[7]	12.83[7]	69.8[3]	171.0[7–8]
	0.1255[3]	14.1[9]	65.7[9]	171.1[3]
	0.1163[9]	14.9[8]	67.1[7]	171.2[15]
	0.1218[8]	18.4[3]	67.9[8]	
Carbon Tetrachloride				
25.54[6]	0.119[4]	17.6[12]	61.7[4]	219.3[16]
25.57[4]	0.1259[10]	17.8[11]	63.6[10]	219.6[17]
	0.1327[8]	18.1[6]	66.5[8]	220.0[15]
	0.1173[11]	18.7[13]	60.5[11]	221.0[8]
		20.3[8]		
*45.1°C				

1. Suri and Ramakrishna (1969)
4. Hennaut-Roland and Lek (1931)
7. Boyd and Copeland (1942)
10. Pugachevich *et al.* (1963)
13. Morino (1932)
16. Ray (1934)
2. Morgan and Stone (1913)
5. Thompson *et al.* (1954)
8. Vogel (1948)
11. Ramsay and Aston (1894)
14. Hammick and Andrew (1929)
17. Mumford and Phillips (1950)
3. Snead and Clever (1962)
6. Harkins and Cheng (1922)
9. Ramsay and Shields (1893)
12. Renard and Guye (1907)
15. Mumford and Phillips (1929)

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COMPUTERIZED CURVE FITTING: AN ALTERNATIVE TO GRAPHICAL INTERPRETATION

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Abstract. — A comparative study is made of the use of statistical techniques in determining the degree of an empirical polynomial in a problem with experimental error. Each problem requires information on error limits and the magnitude of the dependent and independent variables for establishing a statistical criterion. Data for densities of binary solutions of nitromethane or nitroethane in carbon tetrachloride are used for examples. Concentration, temperature, and excess functions are examined.

The purpose of this paper is to investigate criteria, determining the degree of an empirical polynomial, which appeal to chemists (as well as other scientists whose data contains experimental error). Particular emphasis is placed upon first degree (linear) functions because of their common occurrence in chemical problems.

The availability of high speed computers suggests the feasibility of an alternate to the "eye-ball" graphical techniques long used by chemists in analysis of laboratory data. Graphical analysis involving vagaries such as choice of scale, artistic care, etc., is as much an art as a science while a computer alternative removes the subjective element from the analysis. An ideal criterion has two attributes, *viz* it can be routinely programmed for use on a computer and it is based upon concepts familiar to chemists. Proposed criteria are related to formalized statistical techniques.

The various techniques are applied to density data of binary mixtures of nitromethane and nitroethane which were reported by Gunter, et. al. (1967). These data are neither complete enough nor accurate enough to warrant extensive research treatment but they are sufficient for pedagogical purposes. Consequently, the reader is cautioned that the chemical conclusions are only indicative, not definitive.

The Quintic Concentration Equation

For each temperature reported by Gunter, et. al. (1967), the best coefficients, a_i, for density as a function of concentration

$$d = \sum_{i=0}^{i=5} a_i c^i \qquad (1)$$

were determined by a least squares procedure (Daniels, et. al., 1962) contained as part of a standard regression program (Purcell, 1965) available at the Data Processing and Computing Center of Southern Illinois University at Carbondale, (Nitromethane at 45°C was not included in this experiment due to insufficient data). Three different representations of concentration were used, x_2, weight fraction of nitroparaffin, *viz.* mole fraction of nitroparaffin,

w_2, and volume fraction of nitroparaffin, v_2. In each case, the nitroparaffin was assumed to be a monomer. These calculations were performed on an IBM 7044 computer using FORTRAN IV as the computer language.

The choice of a quintic degree was arbitrary but with the expectation that the degree was sufficiently high as to contain terms of no statistical significance. The coefficients are given in Table 1. The value of the mixture density at zero concentration is the carbon tetrachloride density at the specified temperature, irrespective of the concentration representation or the nature of the second solution component. The a_0 values are in close agreement with each other as well as with experiment except for $C_2H_5NO_2$ at 35°C which agrees somewhat less well with experiment. The slightly poorer 35°C $C_2H_5NO_2$ a_0 value is clearly due to the data, a conclusion which can be verified by an examination of the data and their errors reported by Gunter, et. al. (1967). In Table 1, the values of the mixture density calculated for unit concentration are also listed. These values are the nitroparaffin density values at the specified temperature. The exceptional agreement between sets and between calculated and experimental values indicates that any faults lie with the data near zero concentration. Every value reported in Table 1 has four or five significant figures

TABLE 1.—Calculated Constants for Quintic Concentration Equations.

Temp. (°C)	Nitromethane						
	a_0 (g/ml)	a_1 (g/ml-conc)	a_2 (g/ml-conc^2)	a_3 (g/ml-conc^3)	a_4 (g/ml-conc^4)	a_5 (g/ml-conc^5)	d(conc=1) (g/ml)
			d *vs.* x_2				
30	1.5749	—0.2624	—0.1400	0.04214	—0.1049	0.007572	1.1173
35	1.5662	—0.2448	—0.2820	0.4054	—0.4713	0.1378	1.1113
45	.....	.....	.....	.....	.....	.....	.....
			d *vs.* w_2				
30	1.5750	—0.6757	0.3752	—0.3013	0.2139	—0.06987	1.1172
35	1.5667	—0.6789	0.3613	—0.1868	0.04097	0.007873	1.1112
45	.....	.....	.....	.....	.....	.....	.....
			d *vs.* v_2				
30	1.5749	—0.4767	0.02663	0.007180	—0.03319	0.01837	1.1172
35	1.5665	—0.4684	—0.07702	0.3432	—0.4280	0.1751	1.1114
45	.....	.....	.....	.....	.....	.....	.....

Temp. (°C)	Nitroethane						
	a_o (g/ml)	a_1 (g/ml-conc)	a_2 (g/ml-conc^2)	a_3 (g/ml-conc^3)	a_4 (g/ml-conc^4)	a_5 (g/ml-conc^5)	d(conc=1) (g/ml)
			d *vs.* x_2				
30	1.5748	—0.4000	—0.1681	0.2476	—0.3825	0.1628	1.0346
35	1.5670	—0.5201	0.8910	—2.5015	2.4683	—0.8753	1.0294
45	1.5455	—0.4251	0.09944	—0.4724	0.4413	—0.1719	1.0168
			d *vs.* w_2				
30	1.5749	—0.8377	0.5220	—0.3867	0.1983	—0.03624	1.0346
35	1.5660	—0.8859	1.3898	—3.4098	4.0286	—1.6599	1.0288
45	1.5452	—0.8286	0.6076	—0.6634	0.5452	—0.1892	1.0168
			d *vs.* v_2				
30	1.5749	—0.5471	—0.004345	0.1398	—0.2630	0.1344	1.0347
35	1.5673	—0.6922	1.4641	—4.1008	4.5409	—1.7504	1.0289
45	1.5454	—0.5618	0.2168	—0.4943	0.5024	—0.1916	1.0169

depending upon whether the absolute value is less or greater than unity. Gunter, et. al. (1967) indicate an experimental uncertainty in x_2 of 5×10^{-3}, which, for 30°C CH_3NO_2, leads to

$$d = d \pm 10^{-3}(1.31 + 1.40\ x_2 - 0.63\ x^2_2 + 2.10\ x^3_2 - 0.189 x^4_2) \quad (2)$$

where the expression following the $\pm$ is the error due to the error in x_2. For $x_2 = 0$, an error of 0.001 results and for $x_2 = 1$, an error of 0.004 results. The conclusion is that the number of significant figures in Table 1 are useful only to prevent rounding errors during calculation and that all final calculated densities should be rounded to four significant figures. From this viewpoint, it is clear that at each temperature all a_0 values are in *perfect* agreement. In other words, the experimental scientist would not consider the 35°C $C_2H_5NO_2$ value as slightly poorer but merely a deviation within the range of experimental error. Since, as stated in the introduction a criterion for chemists is sought, the latter viewpoint must prevail.

Many chemists have made little use of statistical tools excepting means and standard deviations. The standard program (Purcell, 1965) produced the variance after the addition of each power of the independent variable. The familiar standard deviation was derived by taking the square root of the variance divided by the degrees of free-

dom (number of observations less the number of constants determined by least squares). These results are presented in Table 2. In both the x_2 and w_2 representations, an error results in the second decimal place if only the linear term is used thereby indicating that the linear term is insufficient. For 30°C CH_3NO_2 the use of x^2_2 gives a standard deviation equal to the maximum error due to experimental error in the mole fraction while x^3_2 gives a standard deviation equal to the minimum error. Depending upon the experimenter, either the quadratic or cubic term is sufficient. In either case, it is clear that an individual error analysis is required for each compound, at each temperature. In the present research, the decision to accept the maximum error due to experiment was made. The data in Table 2 indicates that the quadratic term is sufficient in the mole fraction and weight fraction representations and that the linear term is sufficient in the volume fraction representation. Figures 1 through 3 graphically depict the density as a function of volume fraction. Every chemist would agree that both sets of nitroparaffin data are well represented by a linear function thereby verifying the sufficiency of linear terms. The fact that density is a linear function of concentration, expressed in volume units, indicates the solutions are ideal (Weissberger, 1959).

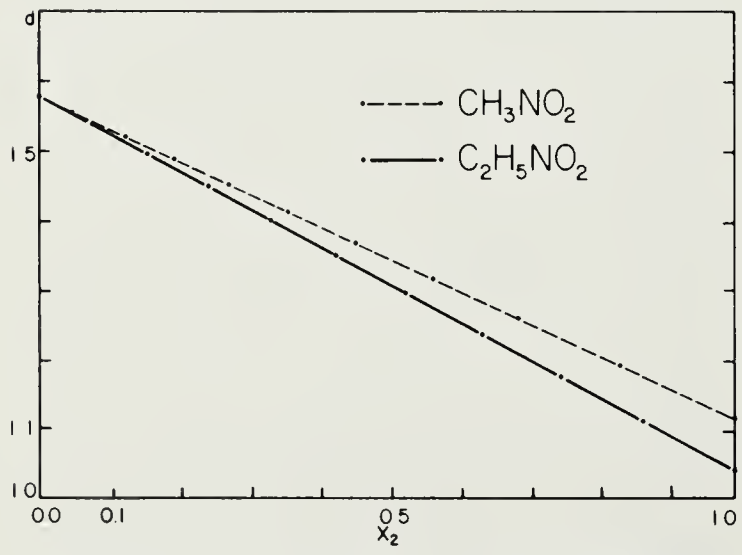

FIGURE 1. Density of Nitroparaffinic Binary Solutions as a Function of Volume Fraction of Nitroparaffins at 30°C.

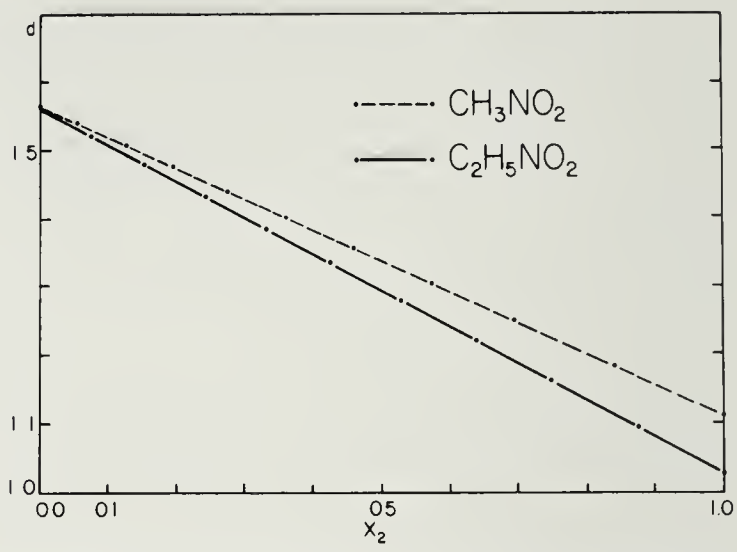

FIGURE 2. Density of Nitroparaffinic Binary Solutions as a Function of Volume Fraction of Nitroparaffins at 35°C.

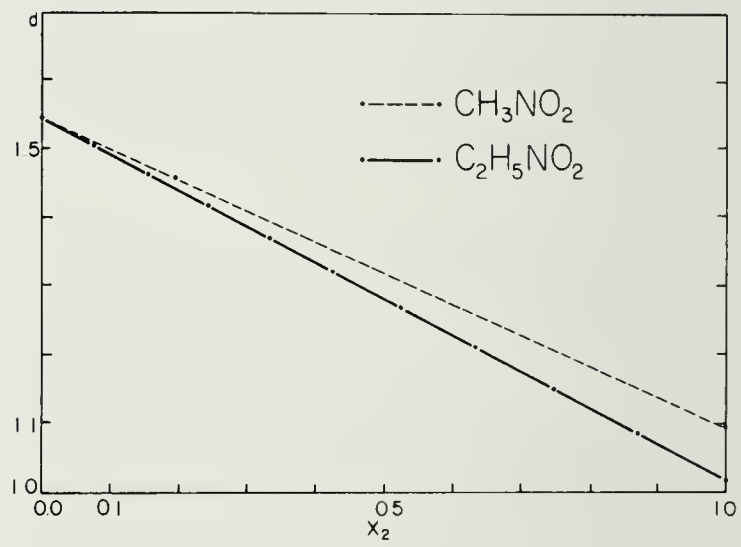

FIGURE 3. Density of Nitroparaffinic Binary Solutions as a Function of Volume Fraction of Nitroparaffins at 45°C.

This linearity also substantiates the conclusions of MacFarlane and Wright (1933) that volume fraction is the most suitable independent variable in a density function.

The per cent deviations suggested by Foley, et. al. (1964) were calculated by dividing each entry of Table 2 by the means of all observations of that nitroparaffin at the temperature of that entry. The results

TABLE 2.—The Standard Deviation.[a]

Temperature (°C)	Nitromethane					Nitroethane				
	x	x^2	x^3	x^4	x^5	x	x^2	x^3	x^4	x^5
				d *vs.* x_2						
30	0.0244	0.00417	0.000967	0.000764	0.000836	0.0158	0.00172	0.000568	0.000581	0.000520
35	0.0238	0.00428	0.00113	0.000617	0.000601	0.0183	0.00407	0.00427	0.00397	0.00389
45	0.0351	.....	.....	.....	.....	0.0157	0.00213	0.000580	0.000618	0.000556
				d *vs.* w_2						
30	0.0166	0.00208	0.000775	0.000760	0.000825	0.0220	0.00276	0.000925	0.000506	0.000550
35	0.0168	0.00242	0.000769	0.000701	0.000769	0.0204	0.00396	0.00417	0.00451	0.00375
45	0.0190	.....	.....	.....	.....	0.0211	0.00308	0.000921	0.000734	0.000717
				d *vs.* v_2						
30	0.00154	0.000755	0.000774	0.000831	0.000900	0.000736	0.000739	0.000750	0.000572	0.000553
35	0.00194	0.000862	0.000710	0.000767	0.000759	0.00518	0.00475	0.00488	0.00500	0.00395
45	0.00306	.....	.....	.....	.....	0.00107	0.00102	0.000658	0.000673	0.000607

[a] All entries stated in units of g/ml.

given in Table 3 show that the per cent deviations corresponding to terms judged of sufficient degree by the criterion of standard deviation are of $0(10^{-1}\%)$. Consequently, an error analysis, as in the case of standard deviation, is used to establish a criterion for judging sufficiency of degree. Another observation is that the percent deviation (or the standard deviation) does not change, perceptibly, if terms of no *statistical* significance are added. For example, with 30°C CH_3NO_2, cubic, quatric, and quintic terms in x_2 give a per cent deviation of 0.06% indicating the cubic term is sufficient, but as noted, the use of *experimental* error determined the quadratic term is sufficient. The difference between the experimentalist and non-experimentalist is further emphasized. In the case of 35°C $C_2H_5NO_2$ the two viewpoints merge, indicating a quadratic term in x_2 is sufficient since per cent deviation remains constant at 0.3%. the proper value considering experimental error. Of course, the fact that even the use of a quintic term does not reduce the per cent deviation again shows the difference in quality of this particular set of data.

The standard computer program also yielded the value of R^2, the square of the multiple correlation coefficient (Baten, 1938) after the addition of each power of the independent variable. These values are given in Table 4. The values of R^2 are also the sum of the proportions of variance through the term being added (Musulin and Musulin, 1967). Consequently a value of 1 indicates that all variance has been accounted for within the number of significant figures yielded by the standard program, i.e. five significant figures for R^2. An examination of Table 4 indicates that the 35°C nitroethane data is generally not comparable to the data at 30°C and 45°C, again emphasizing the experimental errors in 35°C data. Excepting this 35°C $C_2H_5NO_2$ data from a statistical viewpoint, the mole fraction representation recovers all the variance with terms through the cubic degree, as is true for the weight fraction representation with nitroethane. For the volume fraction representation, quadratic terms are sufficient for nitromethane and linear terms for nitroethane. Once again, the viewpoint of the experimentalist must be introduced for the use of statistics alone results in equations of greater refinement than can be warranted by experimental error. Figures 1 through 3 show that the requirement of quadratic v_2 terms suggested by statistical methods for the CH_3NO_2 data is too stringent.

Foley, et. al. (1964) have suggested that a criterion for linearity, within 6%, is $|r| \geq 0.995$. It is proposed to generalize this criterion for use with polynomials of any degree by establishing a lower bound for R^2 (this particular standard program yields R^2 but a lower bound on the absolute value of R would serve as well). The sum of squared deviations (and hence, the per cent deviation) is proportional to variance of the dependent variable and to $(1 - R^2)$ (Baten, (1938)). Since the range of the dependent variable is essentially the same for each nitroparaffin, the average criterion $R^2 \geq 0.998$ can be established for determining the proper polynomial degree to be used in an empirical equation. This criterion provides the same results as were obtained with the standard deviation and the per cent deviation, i.e. quadratic equations are appropriate with x_2 and w_2, and linear equations with v_2.

An alternate statistical technique to determine degree of the inde-

TABLE 3.—The Per Cent Deviation.

Temp. (°C)	Nitromethane					Nitroethane				
	x	x^2	x^3	x^4	x^5	x	x^2	x^3	x^4	x^5
				d *vs.* x_2						
30	1.76	0.301	0.0698	0.0551	0.0603	1.19	0.129	0.0427	0.0437	0.0391
35	1.73	0.311	0.0821	0.0448	0.0437	1.38	0.307	0.323	0.300	0.294
45	2.57	.....	.....	.....	.....	1.20	0.163	0.0444	0.0474	0.0426
				d *vs.* w_2						
30	1.20	0.150	0.0559	0.0549	0.0596	1.66	0.208	0.0696	0.0381	0.0414
35	1.22	0.176	0.0559	0.0510	0.0559	1.54	0.299	0.315	0.341	0.283
45	1.39	.....	.....	.....	.....	1.62	0.236	0.0706	0.0562	0.0549
				d *vs.* v_2						
30	0.111	0.0545	0.0559	0.0600	0.0656	0.0554	0.0556	0.0564	0.0430	0.0416
35	0.141	0.0627	0.0516	0.0558	0.0552	0.391	0.359	0.369	0.378	0.298
45	0.224	.....	.....	.....	.....	0.0820	0.0782	0.0504	0.0516	0.0465
				V_M *vs.* x_2						
30	0.0975	0.0474	0.0505	0.0546	0.0593	0.0424	0.0450	0.0410	0.0366	0.0303
35	0.127	0.0514	0.0542	0.0527	0.0482	0.333	0.301	0.319	0.294	0.286
45	0.220	.....	.....	.....	.....	0.0691	0.0714	0.0447	0.0462	0.0410
				V_M *vs.* w_2						
30	4.96	1.24	0.294	0.0831	0.0610	2.06	0.406	0.0484	0.0312	0.0328
35	4.93	1.21	0.290	0.0951	0.0686	2.23	0.621	0.315	0.322	0.272
45	6.16	.....	.....	.....	.....	2.10	0.378	0.0703	0.0498	0.0516
				V_M *vs.* v_2						
30	3.12	0.490	0.0855	0.0541	0.0577	0.866	0.0616	0.0361	0.0302	0.0288
35	3.09	0.472	0.0876	0.0561	0.0549	1.04	0.298	0.274	0.280	0.226
45	4.29	.....	.....	.....	.....	0.890	0.0539	0.0470	0.0482	0.0472

TABLE 4.—The Multiple Correlation Coeffiecient.

Temp. (°C)	Nitromethane					Nitroethane				
	x	x^2	x^3	x^4	x^5	x	x^2	x^3	x^4	x^5
				d *vs.* x_2						
30	0.9763	0.9994	1.0000	1.0000	1.0000	0.9930	0.9999	1.0000	1.0000	1.0000
35	0.9771	0.9993	1.0000	1.0000	1.0000	0.9905	0.9996	0.9996	0.9997	0.9998
45	0.9890	1.000	.....	.....	.....	0.9927	0.9999	1.0000	1.0000	1.0000
				d *vs.* w_2						
30	0.9890	0.9998	1.0000	1.0000	1.0000	0.9864	0.9998	1.0000	1.0000	1.0000
35	0.9886	0.9998	1.0000	1.0000	1.0000	0.9882	0.9996	0.9996	0.9996	0.9998
45	0.9968	1.0000	.....	.....	.....	0.9868	0.9997	1.0000	1.0000	1.0000
				d *vs.* v_2						
30	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
35	0.9998	1.0000	1.0000	1.0000	1.0000	0.9992	0.9994	0.9995	0.9995	0.9998
45	0.9999	1.0000	.....	.....	.....	1.0000	1.0000	1.0000	1.0000	1.0000
				V_M *vs.* x_2						
30	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
35	1.0000	1.0000	1.0000	1.0000	1.0000	0.9990	0.9993	0.9993	0.9995	0.9996
45	1.0000	1.0000	.....	.....	.....	1.0000	1.0000	1.0000	1.0000	1.0000
				V_M *vs.* w_2						
30	0.9371	0.9965	0.9998	1.0000	1.0000	0.9600	0.9986	1.0000	1.0000	1.0000
35	0.9377	0.9966	0.9998	1.0000	1.0000	0.9534	0.9968	0.9993	0.9994	0.9996
45	0.9758	1.0000	.....	.....	.....	0.9594	0.9988	1.0000	1.0000	1.0000
				V_M *vs.* v_2						
30	0.9750	0.9994	1.0000	1.0000	1.0000	0.9930	1.0000	1.0000	1.0000	1.0000
35	0.9753	0.9995	1.0000	1.0000	1.0000	0.9899	0.9993	0.9995	0.9995	0.9997
45	0.9883	1.0000	.....	.....	.....	0.9927	1.0000	1.0000	1.0000	1.0000

pendent variable which is required in the empirical equation is the Analysis of Variance (Bennett and Franklin (1954)). In order to confirm the statistical conclusions drawn from Table 2, an analysis of variance was performed on the 30°C CH_3NO_2 data. At the 1% level, the F-ratio test indicated x^3_2, w^2_2, and v^2_2 were significant. This information is identical to that derived from Table 4. Statistically the analysis of variance is equivalent to the use of R^2 made in this study but insertion of the experimentalists viewpoint would require redefining F-ratio test values at the various levels of significance.

In an ideal mixture, the molar volume, V_M, is a linear function of the mole fraction of the solute, x_2, (Rowlinson, 1959). The plots of V_M *vs.* x_2, Figures 4 through 6, are linear. In order to verify the validity of the criteria which have been suggested, the experiment of substituting V_M for the dependent variable in Equation (1) was performed. The per cent deviation and the values of R^2 are given in Tables 3 and 4, respectively. An error analysis for 30°C CH_3NO_2 indicates $0.39\% \geq$ per cent deviation $\geq 0.23\%$ for $0 \leqq x_2 \leqq 1$. Thus the maximum per cent deviation is approximately the same as the maximum per cent deviation for density even though the values of V_M are approximately 50 times greater than the values of d reaffirming the contention of Foley, et. al. (1964) with regards to per cent deviation.

From the per cent deviation, linear equations are appropriate if x_2 is the independent variable and quadratic equations are appropriate (barely so for CH_3NO_2) if v_2 is the independent variable. With w_2 as the independent variable, the per cent deviation indicates quadratic equations with nitroethane and cubic

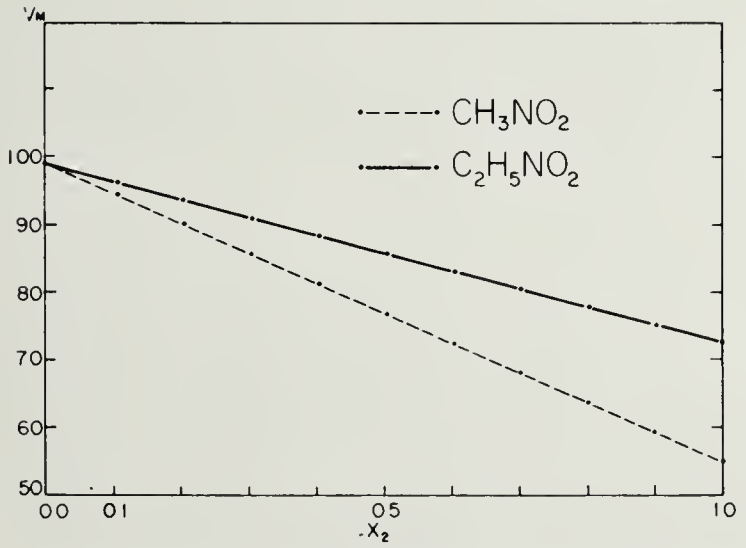

FIGURE 4. Molar Volume of Nitroparaffinic Binary Solutions as a Function of Mole Fraction of Nitroparaffins at 30°C.

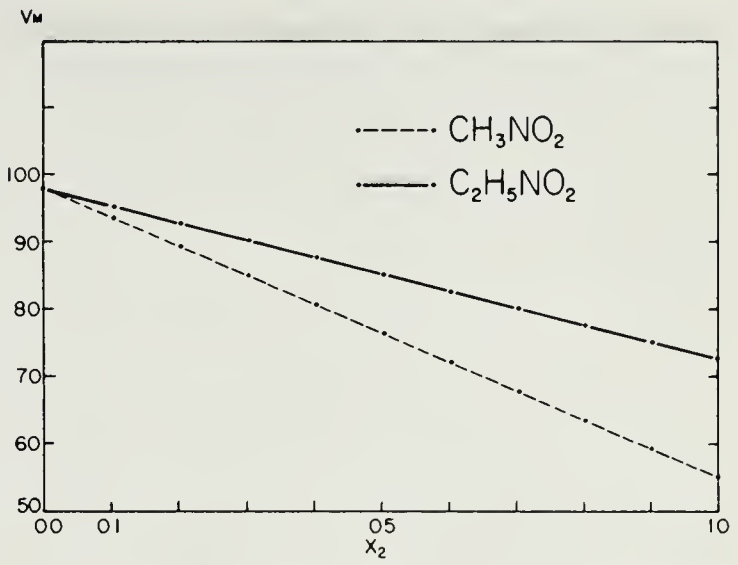

FIGURE 5. Molar Volume of Nitroparaffinic Binary Solutions as a Function of Mole Fraction of Nitroparaffins at 35°C.

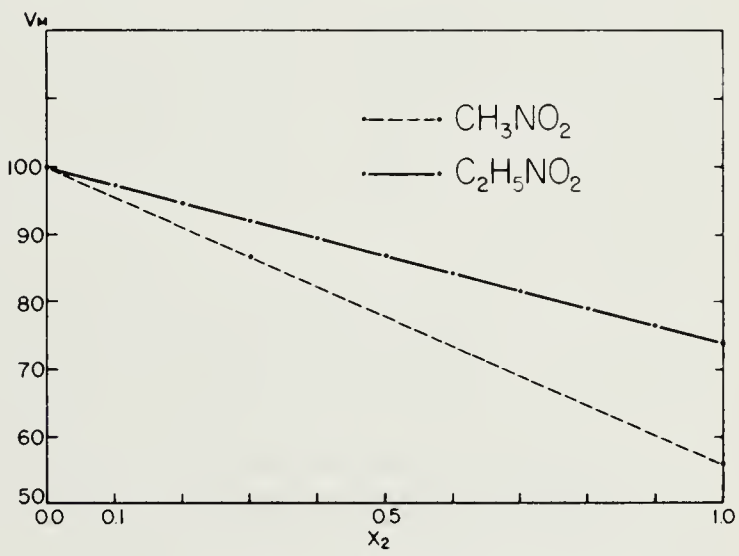

FIGURE 6. Molar Volume of Nitroparaffinic Binary Solutions as a Function of Mole Fraction of Nitroparaffins at 45°C.

equations with nitromethane. As usual, 35°C $C_2H_5NO_2$ is slightly poorer than the other data. For this choice of dependent variable the range of the dependent variable is rather different for the two nitroparaffins resulting in a standard deviation for nitromethane 1¾ times larger than the standard deviation of nitroethane. The difference in standard deviation leads to two different exact criteria, *viz.* $R^2 \geq 0.999$ (CH_3NO_2) and $R^2 \geq 0.998$ ($C_2H_5O_2$). These separate criteria reproduce exactly the information given by per cent deviation.

An exact analysis of variance for 30°C nitromethane indicates that quadratic terms in the x_2 representation, quatric terms in the w_2 representation, and cubic terms in the v_2 representation are required. As with the density data, terms of degree one higher are required by the use of pure statistics than are required by an experimentalist desiring correctness within the experimental error defined by Gunter, et. al. (1967).

Final Concentration Equations

The least squares procedure was repeated with the density and molar volume data, the degree of each empirical polynomial being that degree determined by the criteria established in this work. The coefficients which result are slightly different than those presented in Table 1 since the minimization is accomplished with less variables. The new coefficients are given in Table 5. The values of the standard deviation, per cent deviation, and R^2 given in Tables 2, 3, and 4 for each degree of freedom are invariant and desired information of this type may be read directly from the appropriate column of the suitable table. Entries have been made in Tables 2, 3, and 4 from the quadratic and linear fits of the three point 45°C CH_3NO_2. The R^2 value does not have the same significance as adjudged by the Student t-test (Baten (1938)). Of course, the resulting minimization yields predicted values which are not as good, mathematically, as those obtained by the quintic equations but the range of a_0 values and the values of the functions at unit concentration (also listed in Table 5) are within the appropriate tolerance ranges.

In those cases where quadratic coefficients are listed, the value of a_2 indicates the amount of curvature, i.e. the lack of linearity. Further, extrema of the quadratic functions lie outside the region of physical significance, i.e. $0 \leqq$ conc. $\leqq 1$. For density as a function of v_2, the second derivative of a quadratic fit, in every case, is positive which indicates the curvature of each plot is convex downward. It should be noted that the magnitude of the second derivative also shows that the amount of such curvature is very small. In such plots, Usol'tseva (1960) attributes a curvature which is convex downward to an associated compound dissociating into another component. Usol'tseva's conclusion is in accord with the concept of a nitroparaffin dimer dissociating, slightly, into a monomer.

Temperature Functions

For each mole fraction of each solution, the density was fitted, by a least squares procedure, to a linear function of temperature (Equation (1) with the temperature, t, as the independent variable and the upper limit i = 1). The linear form was chosen for two reasons; the tem-

TABLE 5.—Calculated Constants for Final Concentration Equations.

Temperature (°C)	Nitromethane a_o (g/ml)	Nitromethane $-a_1$ (g/ml-conc)	Nitromethane a_2 (g/ml-conc^2)	Nitromethane d(Conc =1)	Nitroethane a_o (g/ml)	Nitroethane $-a_1$ (g/ml-conc)	Nitroethane a_2 (g/ml-conc^2)	Nitroethane Per Cent d(conc =1)
			d *vs.* x_2					
25[a]	1.5796	0.1977	—0.2447	1.1372	.....	.....	.....	.....
30	1.5702	0.2009	—0.2462	1.1231	1.5727	0.3755	—0.1608	1.0364
35	1.5619	0.2042	—0.2404	1.1173	1.5629	0.3504	—0.1829	1.0296
45	1.5456	0.2378	—0.2101	1.0977	1.5426	0.3637	—0.1592	1.0197
			d *vs.* w_2					
30	1.5723	0.6217	0.1686	1.1192	1.5709	0.7572	0.2236	1.0373
35	1.5636	0.6207	0.1706	1.1135	1.5628	0.7378	0.2053	1.0303
45	1.5456	0.6504	0.2025	1.0977	1.5407	0.7346	0.2140	1.0201
			d *vs.* V_2					
30	1.5728	0.4577	.....	1.1151	1.5746	0.5398	.....	1.0348
35	1.5637	0.4549	.....	1.1088	1.5687	0.5370	.....	1.0317
45	1.5437	0.4465	.....	1.0972	1.5446	0.5259	.....	1.0187

[a] Brown & Smith (1955)

Temp. (°C)	Nitromethane a_o (ml/g)	Nitromethane $-a_1$ (ml/g-conc)	Nitromethane a_2 (ml/g-conc^2)	Nitromethane $-a_3$ (ml/g-conc^3)	Nitromethane V_M (Conc =1)	Nitroethane a_o (ml/g)	Nitroethane $-a_1$ (ml/g-conc)	Nitroethane a_2 (ml/g-conc^2)	Nitroethane V_M (Conc =1)
				V_M *vs.* x_2					
30	97.776	43.049	.....	.....	54.727	97.697	25.175	.....	72.522
35	98.332	43.290	.....	.....	55.042	98.097	25.327	.....	72.770
45	99.625	43.990	.....	.....	55.635	99.599	25.894	.....	73.705
				V_M *vs.* w_2					
30	97.395	96.548	87.748	34.071	54.524	97.210	42.034	17.734	72.910
35	97.911	96.533	86.927	33.482	54.824	97.712	43.236	18.909	73.385
45	99.527	96.850	52.916	.....	55.593	99.117	43.393	18.413	74.137
				V_M *vs.* v_2					
30	97.153	66.252	24.135	55.036	.....	97.624	32.549	7.536	72.611
35	97.685	66.478	24.111	55.318	.....	98.192	33.872	8.766	73.086
45	99.527	72.811	28.877	55.593	.....	99.533	33.592	7.889	73.830

perature range used was narrow and the maximum of three temperature points would allow for compensation of experimental error. The results are given in Table 6. The per cent deviation is also tabulated. Where only two temperature points were available, no error per cent is given. These and succeeding calculations were performed on an IBM 1620 computer with 40K storage using an IBM PR 025 monitor. The programs were written in FORTRAN II (IBM, 1962).

Except for 0.4 and 0.5 nitroethane, all per cent deviations are $0(10^{-2})$. This order of magnitude indicates a better fit than is warranted by the data. It further substantiates the validity of choice of linear form. Although the exceptional data have per cent deviations appropriate to this study, these two sets are not of the same quality. Combining this information with the information from the concentration data, the two slightly poorer data points are 0.4 and 0.5 35°C $C_2H_5NO_2$. Various literature values are presented in Table 8. Intercepts and slopes calculated from several sets of density data in the literature have been included in Table 8.

Excess Functions

Scatchard (1949) has found that the excess molar volume of mixing, V^E, can be fitted to a series,

$$V^E = \sum_{i=0}^{i=n} a_i x_1 x_2 (x_1 - x_2)^i \qquad (3)$$

where the a_i are constants. The V^E values calculated by Gunter, et. al. (1967) were fitted by a least squares procedure to a two-term equation of the form given in Equation (3) ($i = 0$ to 1). Least squares fits were also made of single term equations ($i = 0$ and $i = 1$, corresponding to quadratic and cubic equations, respectively, in x_2). A discard criterion (Worthing and Geffner, 1943) was applied in the fitting procedures. The coefficients for each case are summarized in Table 7.

The per cent deviation is also

Table 6.—Calculated Constants for Linear Temperature Equations.

Mole Fraction (RNO_2)	Nitromethane			Nitroethane		
	a_o (g/ml)	$-a_1 \times 10^3$ (g/ml-°C)	Per Cent Deviation	a_o (g/ml)	$-a_1 \times 10^3$ (g/ml-°C)	Per Cent Deviation
0.0	1.6344	1.9686	0.03	1.6344	1.9686	0.03
0.1	1.5943	1.5740	.....	1.5939	2.0073	0.03
0.2	1.5679	1.6940	.....	1.5476	1.9096	0.05
0.3	1.5424	1.9333	0.02	1.4971	1.7567	0.06
0.4	1.4980	1.7180	.....	1.4553	1.8293	0.37
0.5	1.4661	1.9180	.....	1.4030	1.7731	0.41
0.6	1.4067	1.4580	.....	1.3378	1.5503	0.01
0.7	1.3585	1.5220	.....	1.2743	1.3794	0.01
0.8	1.2989	1.4160	.....	1.2096	1.2877	0.03
0.9	1.2296	1.2620	.....	1.1410	1.1883	0.09
1.0	1.1566	1.3066	0.02	1.0703	1.1841	0.01

TABLE 7.—Calculated Constants for Volume of Mixing Equations.

Temp. (°C)	Nitromethane			Nitroethane		
	a_o (ml/mole-(mole fraction)^2)	$-a_1$ (ml/mole-(mole fraction)^3)	Error Per Cent	a_o (ml/mole-(mole fraction)^2)	$-a_1$ (ml/mole-(mole fraction)^3)	Error Per Cent
			Two Term Equation			
30	0.6715	0.009510	30.3	0.01983	0.3995	86.5
35	0.8251	—0.04203	30.2	0.1183	0.5076	109
45	.....	.....	.....	0.08515	1.0004	55.2
			Quadratic Term Only			
30	0.6715	0.0000	28.6	0.01983	0.0000	142
35	0.8251	0.0000	28.5	0.1457	0.0000	144
45	1.0502	0.0000	.....	0.08515	0.0000	141
			Cubic Term Only			
30	0.0000	0.009510	131	0.0000	0.3995	82.7
35	0.0000	—0.04203	136	0.0000	0.5378	114
45	0.0000	—2.6255	.....	0.0000	1.0004	59.8

given for each fitted equation in Table 7. A slight modification was necessary in calculating the per cent deviation for nitroethane binary solutions. At all temperatures, the mean value of V^E for each of these solutions was approximately zero. For tabulation purposes, and to eliminate anomalous values due to sign cancellation, the per cent deviation was calculated using the mean of the absolute values of V^E.

The error columns of Table 7 verify the conclusions drawn by Gunter, et. al. (1967) that the V^E values of nitromethane are of the quadratic form obtained by discard of all but the leading term of Equation (3) and the V^E values of nitroethane are of the cubic form obtained by discard of all but the second term of Equation (3). For nitromethane, the two-term fit reduces the error by a minimal amount compared to the use of only the quadratic term. In a like-manner, for nitroethane, the two-term fit is only slightly better than the use of only a cubic term. In every case, the relative smallness of one coefficient in the two-term fit also indicates the validity of a single term fit. (The magnitude of the V^E values also changes the base used to calculate the error per cent which, in turn, explains the differences in magnitude of that quantity in Table 7.) The fact that CH_3NO_2–CCl_4 solutions are less nearly ideal than $C_2H_5NO_2$–CCl_4 solutions is also sub-

TABLE 8.—Summary of Literature Coefficients for Temperature Equations.

a_o (g/ml)	$-a_1 \times 10^3$ (g/ml-°C)	$a_2 \times 10^6$ (g/ml-(°C)^2)	Temperature Range (°C)
		Nitromethane	
1.1574	1.356		20-25[6]
1.1637	1.346		0-45.1[32]
1.1639	1.340		0-25[24]
1.1639	1.342		0-50[23]
1.1642	1.323		0-50[27]
1.1643	1.361		17.3-60.9[14]
1.16448	1.351		20-30[5]
1.1645	1.337	—1.15	0-100[1]
1.1646	1.377		16.4-96.3[26]
1.1648	1.366		25-45[16]
1.1649	1.346		0-30[25]
1.1652	1.384		20-50[29]
1.1657	1.341	—4.94	0-85[30]
1.1657	1.377		20-30[7]
1.1657	1.744	—5.49	—21.5-101.4[3]
1.16576	1.383		25-60[2]
1.1668	1.358	—0.55	20-101[4]
		Nitroethane	
1.06823	1.202		25-60[2]
1.0707	1.210		18.6-108.5[26]
1.0724	1.170		20.2-87.3[14]
1.0743	1.187		20-30[7]
1.0743	1.207		20-50[29]
1.0750	1.206		16.6-79.6[17]
		Carbon Tetrachloride	
1.5239	—0.4607	—11.2	20-283[31]
1.6287	1.763	—2.09	.....[8]
1.6296	1.956		24.2-54.0[10]
1.6306	1.949		11.8-78.0[28]
1.6314	1.870		20.1-59.8[14]
1.6321	1.877	—1.26	.....[21]
1.6325	1.920		0-40[11]
1.63255	1.9110	0.690	0-40[1]
1.6326	1.920		20-25[12]
1.6327	1.936		20-25[6]
1.6329	1.927	—0.0469	15-75[22]
1.6329	1.927	0.563	10-80[18]
1.6331	1.945	0.392	15-75[20]
1.6334	1.961		11.8-68.0[9]
1.6335	1.955	0.705	15-75[19]
1.6337	1.960		19.35-58.2[13]
1.6347	2.006		25-45[16]
1.6468	2.759		15-44[15]

1. Washburn (1928)
2. Boyd & Copeland (1942)
3. Jaeger & Kahn (1915)
4. Williams (1925)
5. Thompson, Coleman, & Helm (1954)
6. Dreisbach & Martin (1949)
7. Toops (1956)
8. Pugachevich, Nisel'son, Sokolova, & Anurov (1963)
9. Renard & Guye (1907)
10. Morgan & Higgins (1908)
11. Cowley and Partington (1936)
12. Mumford and Phillips (1950)
13. Patterson & Thomson (1908)
14. Vogel (1948)
15. Souvek (1938)
16. Brown & Smith (1962)
17. Ramsay & Shields (1893a)
18. Washington & Battino (1968)
19. Wood & Brusie (1943)
20. Wood & Gray (1952)
21. Fried and Schneier (1968)
22. Gibson & Loeffler (1941)
23. Walden (1909)
24. Walden (1906)
25. Timmermans & Hennaut-Roland (1932)
26. Friend & Hargreaves (1943)
27. Walden & Birr (1933)
28. Ramsay & Aston (1894)
29. Geiseler & Kessler (1964)
30. Philip & Oakley (1924)
31. Ramsey & Shields (1893b)
32. Morgan & Stone (1913)

stantiated by the tests for linearity given in Tables 3 and 4.

Nitromethane at 45° does not fit into the pattern of these calculations. The data which was obtained at 45° is such that the system of equations in the least squares procedure degenerates into a single equation. The result is that only the single constant, shown in Table 7, is derivable. In all other cases except one, the coefficient of the single term equation is the same as the corresponding term of the two-term equation. This identity results from the facts that the terms occurring in the least squares procedure are symmetric in x_1 and x_2 and that the input data of the independent variables are symmetric in x_1 and x_2. The single exception occurs when two data points are rejected, by the usual criterion (Worthing and Geffner, 1943), which destroys the symmetry of the input data.

Prigogine (1957) provides a theoretical basis for Equation (3) through the use of the average potential model. The coefficients of Equation (3) can be estimated from the critical constants of the components of the binary solution. The critical constants of nitromethane (Weissberger, 1955) were used to make an estimate which could be compared with the results in Table 7. Since the method depends upon which component of the solution is taken as the reference substance, the calculation was made in both frames of reference. The results are

$$V^E = x_1x_2\,[1.2898 + 0.001876(x_1 - x_2)]\quad CH_3NO_2 \text{ as reference}$$

$$V^E = x_1x_2\,[2.0960 + 0.003416(x_1 - x_2)]\quad CCl_4 \text{ as reference}$$

Both estimates clearly show the same behavior as the results in Table 7, i.e. the quadratic term completely dominates the cubic term. Further, the order of magnitude of the quadratic coefficient is the same as those obtained in Table 7 (the estimation process is temperature independent). Insufficient critical data for nitroethane prevented a similar estimation.

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ON VARIOUS METHODS USED IN THE CALCULATIONS OF INELASTIC ELECTRON-ATOM AND ELECTRON-MOLECULE COLLISIONS

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Abstract. — Various approximate methods used in the calculation of inelastic electron-atom and electron-molecule collisions are classified into six different categories, namely, Born approximation, Born-Oppenheimer approximation distorted wave Born approximation, distorted wave Born-Oppenheimer approximation, the method of integral equation, and variational method. The nature of these six different methods are described and their advantages and disadvantages are presented. The difficulties in the calculations of inelastic electron-molecule collisions are discussed. Some recommendations are made for future progress in the calculation of inelastic electron-molecule collisions.

The theory of electron-atom and electron-molecule collision processes has been developed very rapidly in the last few years and has attained a very important practical meaning. This is because the best way to learn something about the forces acting between elementary particles is to observe their interaction with one another. Most of what we know about the microworld has been established by means of studying collision processes. Besides, the understanding of a great many facets of chemical kinetics, spectroscopy, the physics of gaseous discharges, astrophysics, radiative transfer in flames and in the atmosphere, atomic physics, physics of the upper atmosphere and solid state physics depends on our knowledge of the elementary interaction processes of electrons with atoms, molecules and other elementary particles. The calculations of inelastic electron-atom and electron-molecule collisions are highly desirable. Because for many applications it is necessary to have reliable information on the rates of the various processes involving excitation of atoms and molecules by electrons. Many of the processes concerned are not readily investigated experimentally, so it is essential to rely to a considerable extent on theoretical prediction. Various approximate methods have been used in the calculations of the inelastic scattering cross sections for electron-atom collisions. These methods can be roughly classified into six different categories, namely, Born approximation, Born-Oppenheimer approximation, distorted wave Born approximation (D.W.B.), distorted wave Born-Oppenheimer approximation (D.W.O.B.), the method of integral equations, and variational methods.

Born Approximation

The Born approximation assumes that the coupling of the incident electron with the atom is weak (all matrix elements of the interaction between the electron and atom, whether diagonal or non-diagonal are small). Therefore the calculation of the scattering cross-section can be carried out by a first-order perturbation method. The effect of

electron exchange between the incident beam and the atom is ignored in this approximation. The cross section is then proportional to the square of the matrix element of the interaction between initial and finial states in which the free electron wave functions are plane waves and the overall wave function is simply an unsymmetrical product. The inapplicability of Born approximation for electron energies near the threshold is well known (Bates, Fundaminsky and Massey, 1950). In low energy collisions the processes become quite complicated owing to various threshold effects which arise during the excitation and ionization of atoms and ions. Some transitions which are quite strong near threshold become completely forbidden in this approximation, because the effect of electron exchange is ignored in the approximation.

Born-Oppenheimer Approximation

If we include the effect of electron exchange between the incident beam and the atom but still assumes that all matrix elements are small, then we obtain the so called Born-Oppenheimer approximation to the scattering amplitude or, in short, Born-Oppenheimer approximation (see, for example, B.L. Moiseiwitsch, Revs. Mod. Phys. *40*, 238, (1968). However, we should distinguish this approximation from the Born-Oppenheimer approximation to the separation of electronic and nuclear motions). The overall wave functions used to calculate the probability amplitude are properly summetrized combinations of plane wave and atomic wave functions of the form

$$\Psi_o(r_a)e^{ik_o\cdot r} \quad \Psi_n(r_a)e^{ik_n r}$$

where Ψ_o and Ψ_n are the wave functions of the initial and final atomic states respectively and r_a represents the aggregate of the coordinates of the atomic electrons. The relative motion of the colliding electron is represented by undisturbed plane waves of wave length $2\pi/k_o$ before the collision and $2\pi/k_n$ after the collision.

However, the Born-Oppenheimer approximation, apart from the inclusion of the possibility of electron exchange, is still only a first-order perturbation formula. From an analysis of the observed data it appears that this approximation often overestimates the importance of exchange to a very serious extent. This failure is particularly serious in the calculations of the cross section near the threshold of excitation of one level from another if both belong to the same electron configuration and in other cases in which there is no change of azimuthal quantum number of the atomic electron concerned in the process (e.g. when an s-s transition is involved). Marriott (Marriott, 1958) has shown that the Born-Oppenheimer approximation cannot be relied on at all at low electron energies whether the coupling between initial and final states is weak or not. Unfortunately, for many applications it is the cross-section near the threshold which is required. Ochkur (Ochkur, 1963) thinks that the deficiencies of the calculations using the Born-Oppenheimer formula primarily result from an incorrect extrapolation into the domain of low-energies. By using the Born-Oppenheimer formula as a basis, Ochkur obtains a new simple formula. The excitation functions have been calculated for the 2^3S and 2^3P level in helium by using this new formula. The results are in reasonably good agreement with the experimental data. Rudge (Rudge, 1965)

pointed out that Ochkur's formula is not rigorous in the sense of being obtainable from a variational principle and gave an alternate expression obtainable from a variational principle. Nevertheless, Ochkur's formula has been successfully used in several other inelastic atomic collision calculations and has been applied to the excitation of the hydrogen molecule by Khare (Khare, 1966a, 1966b, 1967). Khare's results are in fair accord with experimental results and other theoretical estimates.

Distorted Wave Born Approximation

The distorted wave Born approximation assumes that only the non-diagonal matrix elements are small. The plane waves which represent the initial and final free electron wave functions are then replaced by waves distorted by the mean interaction with the atom in the initial and final states respectively. It was first pointed out by Mott (Mott, 1932) that the contribution to the cross section for an inelastic collision between two interacting systems which arises from impacts in which their relative angular momentum is $\{l(l+1)\}^{1/2}\hbar$ can never exceed $(2l(l+1)\lambda^2/_4$ where λ is the wavelength of the initial relative motion. Bates et al. have shown that the calculations for $O(2^3P, 2^1S)$, O^+ $(2^4S, 2^2D)$ and $O^{2+}(2^3P, 2^1D$ and $2^1S)$ using D.W.B. approximation yields cross sections in excess of the possible maxima (Mott, 1932). So it is also not a good method for the calculation of inelastic cross sections for electron-atom collisions.

Distorted Wave Born-Oppenheimer Approximation

The distorted wave Born-Oppenheimer approximation also assumes that only the non-diagonal matrix elements are small. The plane waves which represent the initial and final free electron wave functions are then replaced by waves $F_o(r)$, $F_n(r)$ distorted by the mean interaction with the atom in the initial and final states respectively. $F_o(r)$ is the wave function which represents a plane wave $e^{ik_o r}$ and an outgoing spherical wave scattered by atom in its ground state, not allowing for the possibility of any inelastic collisions but including exchange effects as far ts they effect elastic scattering. $F_n(r)$ is the corresponding wave function representing a plane $e^{ik_n r}$ and an outgoing spherical wave scattered by the atom in the nth excited state. The effect of electron exchange in producing distortion is allowed for in this approximation. Erskine and Massey (Erskine and Massey, 1952) first applied this approximation to the excitation of the 2S level of hydrogen from the ground state. They find that for electron energies near the threshold the cross section for the excitation is given by

$$Q = 4_{\pi} k_1 |\beta|^2 / k_o^3 \qquad (1)$$

k_o and k_1 are the wave numbers of the initial and final motion of the election relative to the atom. $|\beta|$ arises from requiring the solution of a pair of integro-differential equations have the asymptotic form

$$f \sim \beta \exp(ik_1 r) \qquad (2)$$

Since the maximum possible value (Mott, 1932) for Q is π/k_o^2, we may call $4\pi k_1 |\beta|^2 k_o$ the probability of the particular inelastic collision concerned.

The integro-differential equation expressed in atomic units is of the form

$$[\frac{d^2}{dr^2} - 2V_{oo}(r) + k_o^2]f_o + \int_o^\infty K_{oo}(r,r')f_o(r')dr' = 2V_{o\,1}(r)f_1(r) - \int_o^\infty K_{o\,1}(r,r')f_1(r')dr$$

$$[\frac{d^2}{dr^2} - 2V_{11}(r) + K_1^2]f_1 + \int_o^\infty K_{11}(r,r')f_1(r')dr' = 2V_{o\,1}(r)f_o(r) - \int_o^\infty K_{1o}(r,r')f_o(r')dr' \quad (3)$$

and the solutions must be proper functions satisfying the asymptotic conditions (2) and

$$f_o \sim \mathrm{Sin}\, k\, r + \alpha \exp(ik_o r) \quad (4)$$

In these equations V_{oo} and V_{11} are the interactions between the electron and the atom arranged over the initial and final states of the atom, K_{oo} and K_{11} are interaction kernels which represent the contribution of electron exchange to the mean interaction in each case, $V_{o\,1}$ is the non-diagonal matrix element of the interaction and K_{o1}, K_{1o} are the corresponding contributions from exchange effects. If $V_{o\,1}$, $K_{1\,o}$ and K_{1o} are zero the probability of the transition vanishes. If equation (3) is solved exactly the resulting cross section should be accurate, provided that the wave numbers k_o and k_1 are sufficiently small so that incident electrons with angular momenta greater than zero can be ignored and provided that the influence of other excited states is also negligible. It is quite clear that almost all of the violations of the conservation law (Mott, 1932) occur for electron energies near the threshold in which case the main contribution comes from head on collisions. The neglect of excited states is equivalent to ignoring dynamic polarization effects. At the election energies concerned these are probably not very important, although it is difficult to estimate their order of magnitude. In any case the exact solution of equation (3) will certainly give cross sections which obey the conservation laws. It is only when the equation is solved by approximate methods that violation of these laws can occur. All previous methods solve equation (3) by successive approximations on the asumption that $V_{o\,1}$, $K_{o\,1}$ and K_{1o} are small. The distorted wave Born-Oppenheimer method makes no further assumptions, but the Born-Oppenheimer approximation assumes that V_{oo}, V_{11}, K_{oo} and K_{11} are also negligible and the Born approximation neglects K_{1o} and $K_{o\,1}$ as well. It is to be expected that the D.W.B.O. method will give accurate results if $V_{o\,1}$, $K_{o\,1}$ and K_{1o} are small but this is not sufficient to justify the Born-Oppenheimer approximation. In fact, for excitation of atoms by electrons with energy near the threshold the distortion introduced by V_{oo} and V_{11} is very marked. Erskine and Massey found their results for the excitation of the 2s level of atomic hydrogen were considerably different from those obtained by use of the Born-Oppenheimer approximation in which distortion is neglected. Whereas the latter approximation gives cross-sections at energies close to the threshold which are greater than the maximum possible value, the D.W.B.O. method gives values always less than this. Nevertheless, it approaches within 30% of this value at low energies. The coupling between the motion in the initial and final states is quite strong in this case. The D.W.B.O. approximation, which depends on this coupling being weak, cannot be expected to yield very good results under these conditions. Further evidence in support of this was obtained in a calculation, by a variational method, carried out by Massey and

Moisewitsch (Massey and Moisewitsch, 1953), which did not require the coupling to be weak.

The next application of the D.W.B.O. method was to the excitation of the 2^3S and 2^1S states of helium by Massey and Moiseiwitsch (Massey and Moiseiwitsch, 1954). In the 2^3S calculation they found a resonance effect very close to the threshold, leading to a sharp peak in the cross section, and the absolute magnitude was much smaller than given by the Born-Oppenheimer approximation. This was due to the fact that the distortion effectively annihilated the partial cross-section for excitation by incident electrons of zero angular momentum. Their results are in agreement with the experimental data, and represent an important success of the method. It is noteworthy that in this case the cross sections are all well below the maximum possible values showing the coupling to be weak. In the 2^1S state, experimental results show that a sharp peak in the excitation function near the threshold also occurs. This does not appear according to their calculations probably because the close coupling between the 2^3S and 2^1S state is ignored. Evidence for this has been afforded by accurate numerical calculations by Marriott on the cross-section for the deactivation of 2^1S helium atoms by slow electrons which produce transitions to 2^3S states. The first application of the D.W.B.O. method to an s-p transition was made by Khashaba and Massey (Khashaba and Massey, 1958) for the excitation of the 2p level of hydrogen. They calculated the total cross-section and the polarization of the radiation excited by the impact. Their results did not differ very much from those given by the Born-Oppenheimer approximation either for the total cross-section or for the polarization, but this was partly coincidental. Thus the distortion virtually annihilates the partial cross-section q_0 (for scattered electrons with zero angular momentum) but largely compensates this by increasing q_1 (for scattered electrons with angular momentum $\sqrt{2}\hbar$). The usual tests of coupling indicate that it is not very strong (the distorted wave partial cross-section q_1 never exceeds one-quarter of the maximum possible value $3\pi/k^2$). Massey and Moiseiwitsch carried out a calculation of the cross-section for excitation of the 2^3P state of helium by D.W.B.O. method on similar lines to those of Khashaba and Massey (Khashaba and Massey, 1958) for the 2p of hydrogen. The triplet state calculation is of great interest because excitation can only come through exchange which limits the significant contributions to the first two partial cross-sections for which the effects of distortion are much more marked. In other words, there is less dilution of these effects from the higher order partial cross-sections which are little affected by distortion. Their results have been compared with the corresponding results given by the Born-Oppenheimer approximation. Although there are considerable differences at electron energies below 100eV, these are much less marked than for the excitation of the 2^3S state (Massey and Moiseiwitsch, 1954). The D.W.B.O. method gives cross-sections in closer agreement with observation but substantial discrepancies still remain. The coupling between the 1^1S and 2^3P states is nowhere as strong as judged by the ratio of their results for the partial cross-sections to the maximum possible value. Thus their calculated values for either q_0 or q_1 is never as great as 0.25 of the

maximum and is usually much less. Effects due to coupling between 2P and 2S states are likely to be smaller than for atomic hydrogen owing to the larger energy differences. The cross-section for excitation of the 2p state of hydrogen by electron impact, calculated by Khashaba and Massey (Khashaba and Massey, 1958) using the exactly similar D.W.B.O. method, is in considerably closer agreement with the observations of Fite and Brackmann (Fite and Brackmann, 1958). On the other hand, in the hydrogen case, the atomic wave functions are accurately known and no account has to be taken of the coupling between 2^3P and 2^1P states. It is difficult to judge how important these two factors are. It is also difficult to estimate the accuracy of Massey and Moiseiwitsch's results with any certainty because there are inconsistencies in the observed data. In any case, one would have expected the D.W.B.O. method to give very good results if the coupling between the motion in the initial and final states is weak.

The Method of Integral Equations

The method of integral equations had been developed by Drukarev (Drukarev, 1953). Using this method, the excitation of the sodium atom by slow electrons has been calculated (Veldre, 1956). In this calculation only the s-wave of the incident beam was taken into account. For a certain value of incident electron energy the exchange and strong coupling have been included. The elastic scattering of slow electrons by lithium atoms with the inclusion of exchange was calculated by the Drukarev approximation (Veldre, Gailitis, Damburg and Stepinsh, 1956). In the incident beam only the s-wave was taken into account. The question of the choice of radial wave functions of atomic electrons was investigated (Veldre, 1959) and it was shown that the behavior of the radial wave function near the zero does not have an important influence of the magnitude of the effective cross-section. In the inelastic cases the main advantage of the method of integral equations is that it can obtain analytical expressions for the desired atomic wave functions of different states for all r, and not merely their asymptotic forms. This feature can be used to obtain appropriate classes of variation functions associated with the calculation of electron scattering by variational methods. Matora (Matora, 1960) applied the Drukarev method to calculate the elastic scattering of electrons and the excitation functions of the 2^3S and 2^1S states of helium by electrons with energies from 0 to 40 eV. However, Massey and Moiseiwitsch's (Massey and Moiseiwitsch, 1954) result for 2^3S calculated by D.W.B.O. methods corresponds more closely to the experimental data than the result obtained by Matora using the method of integral equations. Damburg et al., pointed out that the method of integral equations has slow convergence. The effective cross-section for the elastic scattering of electrons by hydrogen atoms, calculated by the second approximation of the method of integral equations, agrees with the effective cross-section calculated by the second Born approximation. However, calculations by the second Born approximation are less complicated than the method of integral equations.

Variational Method

As mentioned before, it seems D.W.B.O. method is a reasonably good approximation in inelastic scattering of electrons by atoms and molecules. Owing to the labor involved in calculating the distorted waves F_o and F_n the method cannot be applied widely. However, variational methods greatly facilitate D.W.B.O. calculations, for both the functions F_o and F_n may be obtained in a convenient analytical approximation for both the antisymmetric and symmetric cases.

The application of variational methods to bound state problem has proved to be very fruitful, especially for obtaining approximations to the lowest eigenvalue of the energy. Although in principle still applicable to the approximate determination of the energies of excited states, the method becomes much less convenient because of the volume of analytical and computational work required. Variational methods for dealing with atomic collision problems were first proposed by Hulthen (Hulthen, 1944). Hulthen proposed a method, based on his variational principle, for the approximate calculation of the radial function and its phase, and verified his method on the simplest examples. In 1947, Schwinger (Schwinger, 1947) developed a variational method, different from Hulthen's method, which was based on an integral equation for the wave function. In 1948, Kohn (Kohn, 1948) geenralized Hulthen's formulation, extending it to the general case of scattering. Following on his work, a series of papers appeared (see for example, Newton, 1966) in which new variational methods were proposed. However, all these methods do not differ essentially from the two basic methods: the Hulthen-Kohn method based on Schrödinger's differential equation and Schwinger's method based on an integral equation. These variational methods are similar to those used for bound state problems but they differ in certain important respects. In both cases the aim is to obtain expressions involving wave functions describing the state of systems which remain correct to the first order when a variation is imposed on one or more of these functions. The bound state energies are found to be true minima with respect to the variational calculations under the imposed conditions while this is not true for problems of unclosed states which described collision processes. This has the consequence that greater flexibility in the choice of trial functions may even lead to less satisfactory results, a situation which can never arise in bound state problems. Much greater care has to be taken therefore in applying variational methods under these circumstances and it is usually difficult to estimate the accuracy of the results. The application of variational methods of scattering problems has also been limited by the complexity of the integration required in the use of the Schwinger formulation even for the simplest trial functions, and the difficulty of finding adequate trial functions in the relatively simple Kohn formulation. In spite of the difficulty of applying variational methods to collision problems, extensive development of the theory of elastic (Massey and Moiseiwitsch, 1950; Moiseiwitsch, 1953) and inelastic scattering of electrons by atoms using the variational methods of Hulthen and of Kohn has been carried out. The distorted wave functions used in Erskine and Massey's (Erskine and Massey, 1952),

Massey and Moiseiwitsch's (Massey and Moiseiwitsch, 1953), Khashaba and Massey's (Khashaba and Massey, 1958), and Massey and Moiseiwitsch's calculations (Massey and Moiseiwitsch, 1960) are determined by Hulthen's and Kohn's variational methods. The results obtained have been encouraging. The generalization of Hulthen's variational method to the inelastic scattering of electrons by atoms was first derived by Moiseiwitsch (Moiseiwitsch, 1951) by using the integral $L = \Psi^*(H-E)\Psi d\tau$ where H is the hamiltonian of the system. The complex parameters a, d, c_1, ..., c_n in the trial function are determined through the conditions

$$L_t = 0 \qquad (5)$$

$$\frac{\partial L_t}{\partial a} + 2i\frac{k_1}{k} d^* \frac{\partial L_t}{\partial a} = 0 \qquad (6)$$

$$\frac{\partial L_t}{\partial C_i} = 0, \ (i=1, \ldots . n) \qquad (7)$$

$$\text{where } L_t = \int \Psi_t^*(H-E)\Psi_t d\tau \qquad (8)$$

and a and d are complex. In contrast to Hulthen's variational method applied to the elastic (Moiseiwitsch, 1953) scattering of electrons, the condition L=O does not imply that a=O. A correction to the parameter may be obtained by considering the integral

$$L' = \int \Psi(H-E)\Psi d\tau \qquad (9)$$

For it can be shown that

$$\delta L' = 4\pi k \delta a \qquad (10)$$

therefore the corrected value of the parameter a is given by

$$\lambda = a - L'/4\pi k \qquad (11)$$

This variational method can be extended to include any order partial wave, and excitation to any state of the atom. Massey and Moiseiwitsch (Massey and Moiseiwitsch, 1953) first applied this method to the calculation of the 1s–2s electron excitation cross-section of hydrogen. Their results are qualitatively more in accord with those of the accurate numerical method of Marriott (Marriott, 1958) than either of the other methods. The poor quantitative agreement is probably a consequence of the use of an over-simplified trial function in the variational calculation. It is apparent that this variational method does not require the coupling between the motion in the initial and final states to be weak and the results of this method are better than those of other approximations. However, the great difficulty in applying this variational procedure is the complexity of the calculations involved even when using the simplest trial functions. For this reason, in Massey and Moiseiwitsch's calculation of the 1s–2s electron excitation cross-section of hydrogen, detailed numerical work was confined to trial functions of the form

$$f_o = \text{Sin } kr + (A + be^{-r})(1 - e^{-r}) \cos kr \qquad (12)$$

$$f_1 = (1 - e^{r})d \exp(ik_1 r) \qquad (13)$$

b being the additional variable parameter. These wave functions suffer from the defect that they do not allow for mixing between the incident and scattered waves. Even for such over-simplified trial functions the analysis involved, is very extensive and the determination of "a" from the equations (5) to (7) was quite involved.

Electron-Molecule Collision Problems

Although the scattering of slow electrons by atoms has been extensively studied theoretically, comparatively little theoretical work has been done on the elastic and inelastic scattering of slow electrons by diatomic or polyatomic molecules. This is undoubtedly due to the mathematical complexity of the problem. We cannot apply Born's approximation, because it is least reliable in the low energy domain. Thus we have to solve the Schrödinger equation for the incident electron directly. This problem is fairly simple in the case of atoms on account of the sperical symmetry of the potential field, but in the case of molecules, the molecular potential (force) is not spherically symmetric, so the Schrödinger equation is not separable and the analysis becomes very complicated. Under certain conditions, however, it is possible to treat the individual atoms in the molecule as independent scattering centers so that the amplitude for scattering by the individual atoms in the molecule can be obtained by adding the amplitudes for scattering by the individual atoms with proper allowance for phase differences. The resulting cross sections must then be averaged over all molecular orientations to give observable data. This appoximation will only be valid if multiple scattering of an electron within the molecule is negligible and if the distortion of the atomic fields by the valence forces is also unimportant. Both these conditions are likely to be well satisfied for impacts with fast electrons for which Born's first approximation gives an adequate representation of the atomic scattering (Massey, 1956). It is not so obvious that when Born's first approximation is inadequate the assumption of independent scattering will always be satisfactory, but there is evidence that it does have a range of validity extending to scattering of electrons with velocities much too slow for Born's first approximation to be applicable. On the other hand, for very slow electrons, with wave-lengths comparable with the atomic separations in the molecule, there is no doubt that the independent scattering approximation is no longer valid and the calculation of the scattering presents much greater difficulties.

According to our discussions in the above sections, it seems more flexible to use the variational method for inelastic scattering of the electrons by diatomic or polyatomic molecules. The behavior of phases at small energies, the relation between the discrete and the continuous spectrum are directly or indirectly connected with variational principles. Besides, it is well known that the basic equations of stationary perturbation theory for bound state can be derived from a variational principle. Similar results can also be obtained in collision theory for the scattering amplitudes and the phases if one starts from the stationary property of appropriate functionals. As regards numerical calculations, it seems as if only variational methods allow one to take into account effectively and rigorously such phenomena as the polarization of an atom by an incident electron and obtain results of the same degree of accuracy as is attained in the evaluation of atomic and molecular energy levels. However, in order to generalize Moiseiwitsch's variational method for inelastic scattering of electrons by molecules, first we have to solve the difficulties of the variational meth-

od (such as the complexity of the calculations involved and the question of choosing trial functions). As to the trial functions, the Hulthen's, Kohn's and Moiseiwitsch's criteria for selection of the "best" values of the parameters are reasonable, but of course not essential or necessarily the best to choose. Hulthen's and Kohn's criteria merely make the trial function simulate one property of the exact wave-function. We shall examine various alternative procedures and look for the possibilities for finding the best criteria. The special feature of the method of integral equations as mentioned before shall be studied and the applicability to obtaining appropriate classes of variation function associated with calculation of electron inelastic scattering by the variational method shall be examined. For diatomic molecule calculations, the difficulty due to the distortion of a two-center field also should be overcome. For inelastic scattering of electrons by the hydrogen molecule, Huzinaga's (Huzinaga, 1957) and Hoyland's (Hoyland, 1966) one-center wave functions shall be used for both the initial and final states of the molecule. This will allow all the integrals to be evaluated exactly and with little labor. Calculations on this line are now in progress in our laboratory.

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NOTES

WHAT EFFECT DOES PROLONGED FLOODING HAVE ON ANT COLONIES?

C. A. DENNIS, D. HENRY, D. WALCH, G. JONES, AND L. C. SHELL
Department of Biology, Millikin University, Decatur, Illinois

Abstract. — This study shows that prolonged flooding eliminated all species of ants, in the area worked, with the exception of *Camponotus pennsylvanicus* and *Camponotus (Myrmentoma) nearticus*. It is suggested that in some manner these species are able to block off the water from their nests.

This question came to the mind of the senior author after observing for several years the prolonged and frequent flooding of a tract of woodland along the Sangamon River. The area is flooded to a depth of several feet for from several days to several weeks during the spring and summer, and it seems reasonable to think that all the ants in this area except those living in trees would be drowned. If any colonies were found below flood level during summer or fall they would be the result of fertile queens moving in and establishing colonies after the water receded.

Collecting in August of 1967 showed six species. All the colonies with the exception of *Camponotus pennsylvanicus* and *Camponotus (Myrmentoma)* nearcticus were small, but the colonies of the two species of *Camponotus* were large and vigorous. In early May of 1968, after severe flooding the area was again searched. Only colonies of the species *Camponotus* were found. These were located in very large and soggy logs within a few feet of the rivers edge. This discovery led us to believe that in some manner these two species had survived after being submerged for a long period of time.

In the fall of 1968 the area was again covered, with practically the same results as August of 1967. Then in March of 1969 after the area had been flooded the results were the same as 1968.

There seems no doubt that these species are able to withstand being submerged for a long time. The area of these logs mentioned is some yards away from any standing timber and as rapid as the current is at this place it seems impossible for a large colony to be able to swim to safety of standing trees. Another factor was noticed which led us to believe the colonies did not move out. The logs were sopping wet everywhere with the exception of the region of the colonies.

We propose to continue the investigation to find, if possible, the method used by these two species to avoid drowning when flooded.

Acknowledgement

We are indebted to Dr. William S. Creighton for checking the identification of the species.

Manuscript received July 7, 1970

TRIBUTE TO DR. JAMES W. NECKERS

RICHARD T. ARNOLD
Department of Chemistry, Southern Illinois University, Carbondale 62901

It is rare enough for a young scientist to spend four decades in continuous service as a teacher at one institution before retirement, and even of this small group, few can look back on such a remarkable degree of change—much of which they promoted—as can Dr. James W. Neckers. On Saturday, October 10, 1970, Southern Illinois University, in grateful recognition of his superb contributions, dedicated its new six-million dollar Physical Science Building and officially named it the James W. Neckers Building.

Dr. Neckers, a native of New York, was graduated from Hope College; he completed his Ph.D. at the University of Illinois in 1927, at the age of 25, and joined the faculty at S.I.U. Two years later, he took over the chairmanship of the Department of Chemistry. Dr. Neckers played a leading role in the design of the new Parkinson Laboratory. But most important to those of us who were chemistry majors was the fact that we were exposed to enthusiastic, well-trained and dedicated teachers who devoted an enormous amount of time encouraging and counseling each of us.

At that time, the Chemistry faculty consisted of only four members (known affectionately as the "Four Horsemen"); namely, Drs. Neckers (Analytical-Inorganic), T. W. Abbott (Organic), R. A. Scott (Biochemistry) and K. A. Van Lente (Phyical). Although each man taught the advanced courses in his respective specialty, all cooperated in the teaching of Freshman Chemistry. It was not until 1945 that a fifth man was added to the staff, and shortly afterwards, an astounding rate of growth ensued.

When Dr. Neckers came to S.I.U., the institution conferred only the B.Ed. degree and consisted of a total student body numbering less than 2,000. At the time of his retirement (1967), S.I.U. had become a full-fledged university with over 20,000 students, a large graduate school with doctoral programs in many disciplines and a chemistry faculty of 24 members. Under his guidance, an ACS accredited program for Chemistry majors was approved, and, subsequently, M.S. and Ph.D. programs were started in 1957 and 1963, respectively.

Dr. Neckers served as a President of the Illinois Academy of Science, and was long active as a member of the Illinois Teachers Association, National Teachers Association, the American Chemical Society and other professional organizations.

His role, however, was not limited to things chemical and scientific. As a true scholar, his concern for the welfare of the entire University was great. He was active in A.A.U.P. and a leader in faculty participation in University affairs which led to the formation of the Faculty Council.

In 1966, former students elected him as recipient of the Alumni Association's Great Teacher Award. Many of these alumni do not know that he was active in the planning of the new building which now bears his name. They remember best his quick wit, his dry humor, his absolute dedication to high standards of scholarship and personal behavior and his untiring effort to impress these upon every student who was privileged to be associated with him. This is why hundreds of former students admire and respect him both as teacher and friend.

A CONVENIENT METHOD FOR THE PREPARATION OF AROMATIC α-DIKETONES

JERRY HIGGINS AND JOE F. JONES
Illinois State University, Normal, Illinois

ABSTRACT. — A convenient method has been developed for the preparation of aromatic α-diketones from α-halo aromatic ketones and pyridine-N-oxide. Desyl chloride (**1**) and phenylglyoxalybenzil (**4**) were prepared in 50% and 70% yields, respectively.

A new synthesis of glyoxals and α-diketones has recently been described (Kornblum, et al. 1966). This synthesis involves the reaction of *a*-halo ketones with nitrate to produce the organo nitrate esters followed by the elimination of nitrite with a weak base such as sodium acetate. Benzil was obtained in 95% yield from desyl bromide and yields of various glyoxals ranged from 82-90%. A similar method which has been used to form aromatic aldehydes from the reaction of benzyl halides and pyridine-N-oxide has been reported in the literature (Feely et al. 1957). In this particular reaction pyridine-N-oxide is used as the nucleophile for producing benzyloxypyridinium bromide from benzyl bromide. This salt is then treated with dilute sodium hydroxide to produce benzaldehyde by the abstraction of a proton from the benzyl carbon and the elimination of pyridine. Yields of benzaldehyde and aromatic dialdehydes ranged from 80-95%. The chemical requirements for reactions of this type are that a nucleophile capable of displacing reactive halides is used and that this nucleophile possesses a good leaving group with the elimination of a proton in the formation of the carbonyl group.

The method we would like to report in this communication is a combination of the procedures used by Feely and Kornblum. Our modified method uses aromatic α-halo ketones as in Kornblum's method and pyridine-N-oxide as in Feely's method.

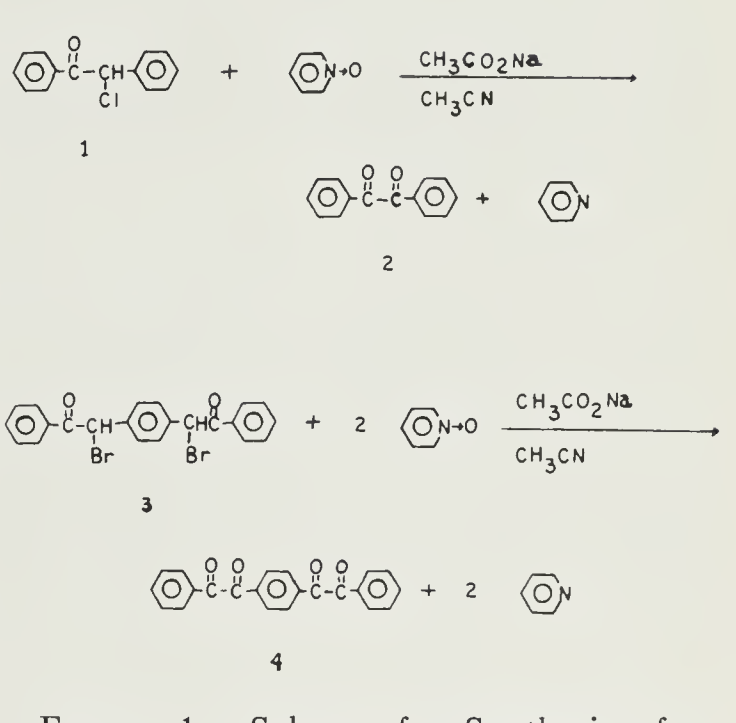

FIGURE 1. Scheme for Synthesis of α-diketones.

EXPERIMENTAL

Preparation of Benzil(2). — Equal molar quantities of desyl chloride and pyridine-N-oxide were refluxed for three hours in acetonitrile. An equal molar quantity of sodium acetate was then added, and the reaction mixture was refluxed for an additional three hours. After removal of two-thirds of the acetonitrile, the reaction mixture was poured into water and the product collected by filtration. Recrystallization from methanol gave 50% yield of benzil melting at 91-94°C (reported m.p. 95°).

Preparation of α α′-dibenzoyl-α, α′- dibromo-*p*-xylene (3).—to 150 ml of glacial acetic acid was added 11.6 g (0.037 moles) of α, α′-dibenzoyl-*p*-xylene (Wrasidlo and Augl, 1969). After dissolving the compound, 12.5 g (0.078 moles) of bromine was added dropwise to the warm reaction solution. The product was collected by filtration and washed three times with 100 ml portions of water. The yield of crude product was 15 g (86%). After recrystallization from carbon tetrachloride, 10.9 g (62%) of pure product, m.p. 179-180°C, was obtained. Calcd. for $C_{22}H_{18}Br_2O_2$: C, 55.94%; H, 3.42%; Br, 33.85%. Found: C, 55.83%; H, 3.21%; Br, 34.00%.

Preparation of phenylglyoxalylbenzil(*4*).

—To 200 ml acetonitrile were added 25.0 g (0.053 mole) of α, α'-dibenzoyl-α, α'-dibromo-*p*-xylene and 12.0 g (0.126 mole) pyridine-N-oxide. The reaction solution was refluxed for three hours and then 12.0 g of anhydrous sodium acetate was added to the hot solution. After refluxing for an additional three hours, about two-thirds of the acetonitrile was removed and the remaining residue added to 300 ml of water. The aqueous mixture was stirred for 30 minutes. The product was filtered off and then dried *in vacuo*. Recrystallization from methanol gave 12.3 g (0.036 mole) (70%) of product melting at 124-126°C. Reported m. p. 126°C (Oliarinso, et al. 1963).

Results and Discussion

Benzil and phenylglyoxalylbenzil can be prepared easily and conveniently by the reaction of pyridine-N-oxide with aromatic α-halo ketones in acetontirile solvent (Scheme 1). Other solvents such as alcohols, cyclic ethers, diols, N,N-dimethylacetamide were also used as solvents in the present procedure, and the yields were similar to those in acetonitrile solvent. Since this appears to be a general reaction, we are presently expanding this procedure to the preparation of glyoxals and other α-diketones.

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Manuscript received October 1, 1970

RE: HAWKINS AND KLIMSTRA, "DEER TRAPPING CORRELATED WITH WEATHER FACTORS" (TRANS., 63:198-201)

H. W. NORTON
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The use of factor analysis, probably always dubious, is exemplified in its uncertainties by application separately to data for two different years. After the independent variables were "loaded on 12 facors", selection of "the independent variable under each factor that contributed most" led to two sets of 12 variables having only a single variable (number 17) in common. This is significantly ($P = 0.017$) less agreement than should occur by chance (though there is at least one misprint affecting variables "deleted from 1964-65 trap period" without explanation). Can the authors still believe that the factor analysis serves "to eliminate redundant factors"? If so, must they not conclude that all factors were probably "redundant"? Furthermore, this common variable failed to exhibit a significant correlation with trap success in the first year (no similar statement was made about data for the second year).

Of the four independent variables which exhibited "significant t-test values" in the first year, it is said that only two "had significant r values". If this means partial correlation, the test of correlation coefficients should yield results identical to those from the t test; if total correlation is meant, the analysis is regressing from a more detailed and specific result to a relatively crude result.

The authors remark that, although 6 corral and 3 box traps were available, they were not always all in use and that data for nights with only one or two traps set were ignored. However wise that may be, surely the analysis should have taken account of the individuality of the traps, not to mention the possible difference between the two types. It may be that permanent differences between traps contribute so much to the variance relegated to "error" in the reported analysis as to obscure completely real effects of some of the weather variables studied.

The data should be reanalyzed taking explicit account of trap individuality, and involving data for the two years so as to test whether years are in reasonable agreement. Those of your readers who are interested in factor analysis may gain further understanding from J. Scott Armstrong, "Derivation of theory by means of factor analysis or Tom Swift and his electric factor analysis machine", American Statistician 21:17-21, 1967.

Letter received October 23, 1970

DR. LESTER WICKS

Dr. Lester Wicks, a member of the Illinois State Academy of Science and a former faculty member of McKendree College passed away in the summer of 1970 after a brain disorder illness of two and one-half years.

Dr. Wicks was born in St. Louis. He received his B.S. and M.S. degrees from St. Louis University and his Ph. D. degree from Washington University in Biochemistry. Before joining the chemistry faculty of McKendree College in 1959, he taught at Parks College from 1955 to 1959. As a research chemist he published extensively in scientific journals. He was a member of Sigma Xi and the Illinois Chemistry Teachers Association. *Dr. Boris Musulin, Chemistry, Southern Illinois University, Carbondale, Illinois 62901.*

Manuscript received Nov. 29, 1970

PROFESSOR JOSEPH TYKOCINSKI TYKOCINER

The Illinois State Academy of Science lost a senior member, Professor Emeritus Joseph Tykocinski Tykociner, at the age of 91. Professor Tykociner was referred to as the "father of sound movies". His first public demonstration of sound on film took place at the University of Illinois in 1922. A permanent display exhibit of his demonstration is in the Ford museum, Greenfield, Michigan. He was born in Vlaclavek, Poland and received his E.E. degree from the Hoheres Tech. Inst. Cothen. He later studied at the Tech. Inst. Berlin and Göttingen and received an hon. Dr. Eng. from Illinois in 1965. Professor Tykociner was a pioneer in wireless transmission, microwaves, and zetetics. He served on the staff of the Marconi Company (England) when the first wireless transmission was made across the Atlantic ocean. In 1905 he went to Russia to help the Imperial Navy equip itself with radio. He was a research engineer for Westinghouse Electric and Manufacturing in 1920 and joined the University of Illinois, department of electrical engineering, in 1921. He officially retired in 1948 but at the age of 84 came out of retirement to teach zetetics. Professor Tykociner received the award of merit from the National Electronics Conference in 1964, one of only three scientists to receive the prize which was instituted twenty years earlier. He was a fellow of the Physical Society. *Dr. Boris Musulin, Chemistry, Southern Illinois University, Carbondale, Illinois, 62901.*

Manuscript received October 15, 1970

DR. BYRON RIEGEL— PRESIDENT OF AMERICAN CHEMICAL SOCIETY

Dr. Byron Riegel, a member of the Illinois State Academy of Science since 1942, served as the president of the American Chemical Society in 1970. His administration of this society of over 100,-000 members is another demonstration of the versatility of this outstanding chemist. Dr. Riegel's academic career was principally at Northwestern University from 1937 to 1951 where he attained the rank of professor. His industrial career has been with G. D. Searle & Company as director of chemical research and development since 1951. He also served as a lecturer with Northwestern from 1951 to 1959. He was born in Palmyra, Mo. and received the A.B. degrée from Central Methodist College. His graduate degrees were taken at Princeton and Illinois. He was conferred the hon. D. Sc. by Central Methodist College in 1963. Dr. Riegel's research has been in Medicinal Chemistry specializing in steroids, structure and biological activity, vitamin K, cancer chemotherapy, anti-malarials and other drugs. *Dr. Boris Musulin, Chemistry, Southern Illinois University, Carbondale, Illinois 62901.*

BIOLOGICAL NOTES ON *PHLOEOTRIBUS SCABRICOLLIS* (HOPKINS) (COLEOPTERA: SCOLYTIDAE)

MILTON W. SANDERSON AND JAMES E. APPLEBY
Illinois Natural History Survey, Urbana

ABSTRACT. — *Phloeotribus scabricollis* (Hopkins) is recorded for Illinois and Ohio, the first records since it was originally described from Hessville, Indiana in 1916. Its occurrence on wafer ash, *Ptelea trifoliata* — a new host record — is discussed.

On July 5, 1970, one of the authors observed insect damage to wafer ash (*Ptelea trifoliata*) at the Morton Arboretum, Lisle, Illinois, DuPage County, in northeastern Illinois. Small tunnels and adult scolytid beetles were noted in stems at the bases of the petioles (fig. 1). Further damage and beetles were noted on August 31 and September 19, but no beetles were in evidence on October 22, 1970, when the plants were last examined.

FIGURE 1. Base of petiole of wafer ash, *Ptelea trifoliata,* showing beetles and injury caused by *Phloeotribus scabricollis.* (Photo by W. Zehr, Illinois Natural History Survey).

The beetle causing the damage was tentatively identified by Sanderson as *Phloeotribus scabricollis* (Hopkins), and confirmed by Dr. Stephen L. Wood, Brigham Young University, Provo, Utah, and by Dr. Donald M. Anderson, U.S. National Museum. The species was described by Hopkins (1916, p. 656) in the genus *Phloeophthorus* from one unassociated specimen collected by W. S. Blatchley at Hessville, Indiana, on July 14; the year of collection not indicated. Hessville now lies within Hammond, Indiana, in extreme northwestern Lake County approximately thirty miles east of Lisle, Illinois. A search of the literature disclosed no further records of this species, but Dr. Wood generously gave us an unpublished record of four specimens from Georgesville, Ohio, Franklin County, collected September 18, 1898. The specimens are labeled as having been collected on bladdernut, *Staphylea trifolia.* Georgesville, located in the central part of the state, is approximately 250 miles southeast of Hessville, Indiana.

Wafer ash and bladdernut are widely distributed in the eastern half of the United States, and both occur in the Morton Arboretum. However, there were no signs of the beetle on bladdernut located about 200 yards from the infestation on wafer ash, and no infestation was found on wafer ash in other areas of the arboretum. Wafer ash and bladdernut examined near Mahomet in Champaign County in east-central Illinois were uninfested. Because of the similarity of *Ptelea* and *Staphylea* when not in fruit, it is suggested that *Staphylea* as a host for *Phloeotribus* should be confirmed. Hutchinson (1969) shows a close relationship between the families, Rutaceae and Staphylaceae, to which these genera belong.

Samples of infested *Ptelea* twigs measured from 3 mm to 12 mm in diameter. In one 475-mm long twig, 33 live and 2

dead adults were found. Usually there was only one beetle in a tunnel in the twig beneath the petiole base, but occasionally two beetles were noted, and in one instance three betles. Most tunnel entrances were at the base of the petiole, and the tunnel penetrated diagonally to a maximum depth of about 7 mm. However, two entrances were about 3 mm from the petiole base, entering the stem at a right angle and nearly perforating it. Frass usually was present at the entrance of the tunnel, and some tunnels were filled with exudate, completely covering live beetles. These are believed to be feeding tunnels for no larvae were found in them.

The sex ratios of beetles differed markedly in the three collections as follows: July 5 (9♂, 24♀); August 31 (9♂, 8♀); September 19 (2♂, 5♀).

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PREPARATION OF MANUSCRIPTS FOR THE TRANSACTIONS

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Doe, J. H., and S. H. Jones. 1951. Mineralogy of Lower Tertiary deposits. McGraw-Hill Book Co., New York. iv + 396 pp.

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CONTENTS

EXCESS MOLAR VOLUMES OF MIXING OF SOLUTIONS OF NITROMETHANE AND CARBON TETRACHLORIDE

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ABSTRACT.—The direct measurement, by means of weight and density measurements, of the excess molar volume of mixing in solutions of nitromethane and carbon tetrachloride at 35°C. is described. The data confirm the quadratic nature of deviations from ideality for these solutions.

Gunter *et al.* (1967) have reported values of excess molar volumes of mixing of binary solutions of CH_3NO_2 and CCl_4 which were derived from measured densities assuming that the molar volumes of mixing of ideal solutions were additive. Although the reported values are consistent in functional form and magnitude with those reported by Brown and Smith (1955), they appear to be of lesser quality. The purpose of this investigation is to ascertain if directly measured values, from a simple experiment, are of better quality than derived values.

EXPERIMENTAL

Fisher Spectrograde nitromethane and carbon tetrachloride were used without further purification. Temperature, specific gravity, and weight measurements were made as described in Gunter *et al.* (1967). A temperature of 35°C. was used in this work. Two sets of pipets, one for CH_3NO_2 and one for CCl_4, each containing a 25-ml, a 10-ml, and a 5-ml pipet, were calibrated by weight techniques. Of the seven physically independent permutations that can be made by mixing a pipetful from each set, four were used to make the sample solutions whose weights were determined directly. The solution densities were measured directly and the solution volumes calculated from these densities and weights. The densities of the pure components used in calibration were taken from Gunter *et al.* (1967). The excess molar volumes of mixing were obtained by differences of the measured volumes.

RESULTS AND DISCUSSION

All measured and calculated values are given in Table 1. The mole fraction values for each solution are at least one magnitude more reliable than those given by Gunter *et al.* (1967). In calculations, the mole fraction was used, as reported, with four significant figures.

The measured solution densities are compared to values calculated from the density-mole fraction function derived by Musulin (1971).

TABLE 1.—Densities and Molar Volumes of Mixing of Nitromethane-Carbon Tetrachloride Solutions

Mole Fraction (CH_3NO_2)	Density (g/ml)	Calculated Density[a] (g/ml)	Molar Volume of Mixing (ml/mole)	Calculated Molar Volume of Mixing[a] (ml/mole)	Additive Molar Volume of Mixing (ml/mole)
0.4174	1.43137	1.43479	0.2064	0.1990	0.2404
0.6414	1.33347	1.33201	0.2088	0.1925	0.2465
0.8178	1.23861	1.23410	0.1078	0.1189	0.1652
0.8989	1.18568	1.18408	0.06250	0.07192	0.04940

a. Musulin (1971)

The maximum difference is 0.0045 g/ml, i.e. 0.36%. Excluding pure experimental error, two reasons may be given for the variation. First, Musulin derived the function using lesser quality mole fraction data, e. g. the 0.8989 mole fraction CH_3NO_2 solution of the present work would have been reported as 0.900 mole fraction CH_3NO_2 in the earlier work. Second, the temperature variation (inherent in the Musulin function) which was allowable with lesser quality mole fraction data becomes a controlling factor in the present investigation. Nevertheless, it is clear that the present densities are in good agreement with the earlier work.

The measured excess molar volumes of mixing are compared to values calculated from the excess molar volume of mixing-mole fraction function derived by Musulin (1971) and to values calculated assuming additivity of molar volumes for ideal solutions as was done by Gunter *et al.* (1967). The measured values are in good agreement with those calculated by the Musulin function. The deviations were about 0.01 ml. As before, these variations could be attributed to mole fraction and temperature measurements. The increased magnitude of the variation could be attributed to the fact that excess molar volume of mixing is a difference quantity. Comparison to the values obtained from the additivity assumption indicates that values obtained by that assumption are overstated in the middle of the mole fraction range. The existence of this error introduces another error in the function derived by Musulin and establishes the final reason for the greater deviations from calculated values with excess molar volumes of mixing compared to the deviations with densities.

These new, accurate measurements confirm the quadratic form and the magnitude of the deviation from ideality of solutions of CH_3NO_2 and CCl_4. The weight techniques presented in this work provide a meaningful way to obtain better quality data than that obtained by Gunter *et al.* (1967) and is an appropriate compromise if the vapor density equipment is not available. Finally, the present results empha-

size the necessity of utilizing an excess type equation for the excess molar volumes of mixing for CH_3-NO_2-CCl_4 solutions as opposed to utilization of a simple additive assumption.

Acknowledgment

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THE EVOLUTION OF GROWTH HABIT IN CYNODON L. C. RICH. (GRAMINEAE)

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ABSTRACT.—Vegetative characters studied included presence or absence of rhizomes, kind of rhizome, modification of stolon tips, branching patterns and winter hardiness. Growth habit, together with geographic distribution, ecological adaptation, and affinity based on cytogenetic studies suggests a sequence of evolutionary events in *Cynodon*.

A biosystematic study of the genus *Cynodon* was conducted at Stillwater, Oklahoma from 1963 to 1967 resulting in a revision of the genus (Clayton and Harlan 1970, Harlan and de Wet 1969, Harlan *et al.* 1969). The species and varieties as now recognized are presented in Table 1.

TABLE 1.—The Species and Varieties of *Cynodon*.

Epithet	2n Chromosome Number	Distribution
C. aethiopicus Clayton et Harlan	18, 36	East Africa; Ethiopia to Transvaal
C. arcuatus J. S. Presl ex. C. B. Presl	36	Malagasy, Ceylon, India, Southeast Asia, Philippines, Taiwan, Indonesia to Australia
C. barberi Rang. et Tad.	18	South India
C. dactylon (L.) Pers		
var. *dactylon*	36	Cosmopolitan
var. *afghanicus* Harlan et de Wet	18, 36	Afghanistan
var. *aridus* Harlan et de Wet	18	South Africa to Palestine to South India; intro. in Hawaii, Arizona
var. *coursii* (A. Camus) Harlan et de Wet	36	Madagascar
var. *elegans* Rendle	36	Southern Africa; Mozambique, Zambia and Angola southward
var. *polevansii* (Stent) Harlan et de Wet	36	Baberspan, South Africa
C. incompletus Nees		
var. *incompletus*	18	South Africa
var. *hirsutus* (Stent) Harlan et de Wet	18, rarely 36	South Africa
C. nlemfuensis Vanderyst		
var. *nlemfuensis*	18, rarely 36	Tropical Africa; Ethiopia to Zambia, west to Angola
var. *robustus* Clayton et Harlan	18, 36	East Africa; Ethiopia to Rhodesia
C. plectostachyus (K. Schum.) Pilger	18	Ethiopia, Uganda, Kenya, Tanzania
C. transvaalensis Burtt-Davy	18	Transvaal and Orange Free State

Specimens were examined at Kew, British Museum, Paris, Brussels, Berlin, Florence, Geneva, Honolulu, Bangkok, Manila, Los Banos, and Washington. The revised classification, however, was based primarily on a living collection of some 700 accessions grown in uniform nurseries at Stillwater. In the living material, it was noted that most of the taxa could be distinguished by characteristic growth habits. Vegetative characters are difficult to describe and are highly subject to environmental modification, yet they are useful for field identification and have evolutionary implications. They tend to go unnoticed in the herbarium because few specimens are sufficiently complete to reveal growth characteristics. The main types of growth habits are described in this paper as an aid to identification and to shed some light on evolution in the genus.

Description

RHIZOMES: The genus is essentially caespitose; only *C. transvaalensis* and four of the six varieties of *C. dactylon* have rhizomes. There are, however, two kinds of rhizomes, Fig. 1. One tends to be relatively slender, straight, with long internodes and the tip always stays below the surface. Lateral buds grow upward and emerge to form culms, but the rhizome itself remains underground, Fig. 1A. The other kind of rhizome is relatively large in diameter, fleshy, usually crooked with short internodes and the tip may grow to the surface where the rhizome is converted into a stolon, Fig. 1B. The first type grows faster and deeper than the second. The distribution of rhizome types is given in Table 2.

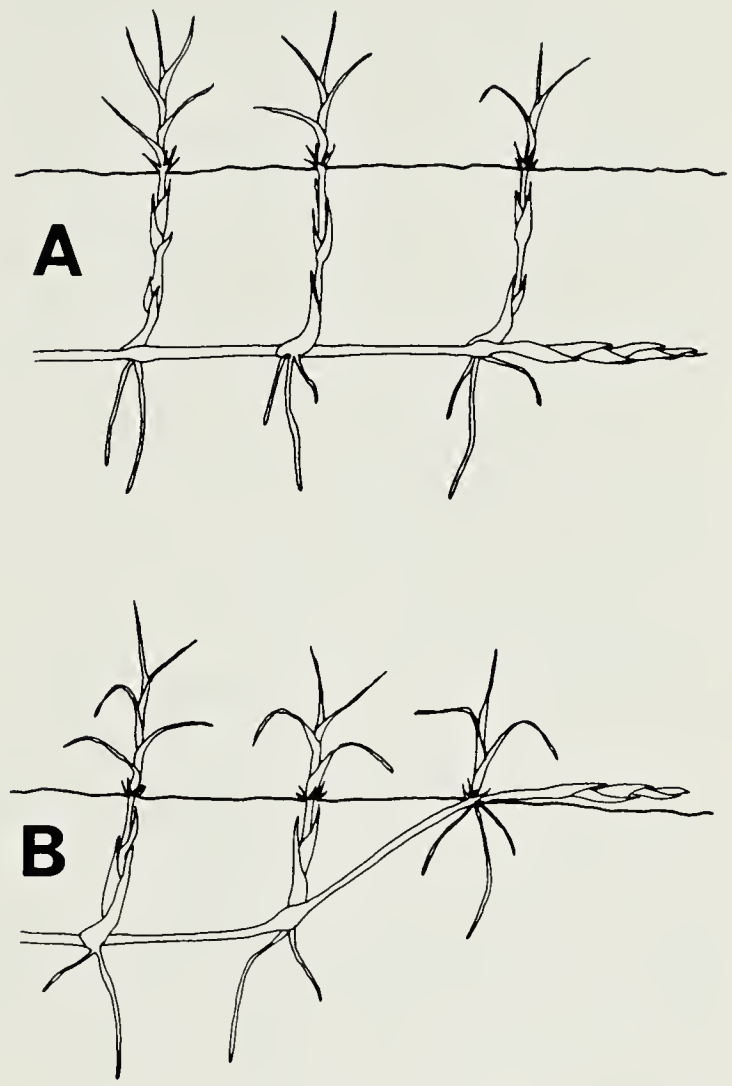

Figure 1. Two types of rhizomes in *Cynodon*.
A. A true rhizome in which the tip always stays below the soil surface.
B. Rhizome that emerges and is converted to a stolon.

The inheritance of rhizome formation was studied in a limited number of the hybrids produced in the course of the biosystematic study, Table 3. In hybrids between nonrhizomatous and rhizomatous species, rhizome formation was suppressed, but the nonrhizomatous varieties of *C. dactylon* were not able to suppress rhizome formation. The tetraploid race of *C. nlemfuensis* var. *robustus* does not suppress rhizomes completely, and, indeed, rhizomes were produced in hybrids between it and

TABLE 2.—Some Characteristics of Growth Habit in *Cynodon*.

Taxon	Rhizome Type (Fig. 1)	Stolon Tip (Fig. 2)	Stolon Branching (Fig. 4)	Turf Formation	Size*	Tissue Hardiness
C. aethiopicus	None	A	A	Very Open	Large	None
C. arcuatus	None	A	A	Open	Small	None
C. barberi	None	A	A	Open	Small	None
C. dactylon						
var. *dactylon*	A & B	A, R, B	B, C	Dense	Small-Medium	Variable
var. *afghanicus*	None	A	A	Very Open	Medium	Yes
var. *aridus*	A	A	A	Open	Small-Medium	None
var. *coursii*	None	R	B	Dense	Large	None
var. *elegans*	B	B	A	Lax	Medium	None
var. *polevansii*	A	B	C	Dense	Small	Yes
C. incompletus						
var. *incompletus*	None	R	B	Dense	Small	Yes
var. *hirsutus*	None	R	B	Dense	Small	Yes
C. nlemfuensis						
var. *nlemfuensis*	None	A	A	Open	Medium-Large	None
var. *robustus* (2x)	None	A	A	Very Open	Large	None
var. *robustus* (4x)	None	A	C	Open	Large	None
C. plectostachyus	None	A	B	Open	Large	None
C. transvaalensis	B	B	B	Dense	Small	Yes

* Small plants are usually less than 15 cm tall; large plants are usually over 40 cm tall under nursery conditions.

TABLE 3.—Rhizome Formation in Hybrids Between Nonrhizomatous and Rhizomatous Taxa

Nonrhizomatous Parent	Rhizomatous Parent	Number Hybrids Examined	Rhizome Formation
C. aethiopucus (4x)	*C. dactylon* (4*x*)	4	—
C. incompletus (2x)	*C. dactylon* (2*x*)	3	—
C. incompletus (2x)	*C. dactylon* (4*x*)	65	—
C. nlemfuensis var. *nlemfuensis* (2x)	*C. dactylon* (2*x*)	35	—
C. nlemfuensis var. *nlemfuensis* (2x)	*C. dactylon* (4*x*)	22	—
C. nlemfuensis var. *robustus* (2x)	*C. dactylon* (2*x*)	4	—
C. nlemfuensis var. *robustus* (4x)	*C. dactylon* (4*x*)	15 + 4*	—
C. dactylon var. *afghanicus* (2x)	*C. dactylon* (2*x*)	35	+
C. dactylon var. *afghanicus* (2x, 4x)	*C. dactylon* (4*x*)	47	+
C. dactylon var. *coursii* (4x)	*C. dactylon* (2*x*)	41	+
C. dactylon var. *coursii* (4x)	*C. dactylon* (4*x*)	43	+
C. dactylon var. *coursii* (4x)	*C. transvaalensis* (2x)	6	+

* Four F_1 plants of this combination had short, poorly developed rhizome-like structures.

tetraploid *C. dactylon* var. *afghanicus*. The fact that nonrhizomatous parents could produce rhizomatous offspring suggests that both parents carried recessive genes for rhizome production. Our collection also contained populations of *C. dactylon* var. *coursii* that apparently had already crossed with var. *dactylon* in Madagascar and segregated for rhizome production in our nurseries. The character gives every evidence of being rather simply inherited.

STOLON TIPS: There are striking differences among taxa in the morphology and behavior of stolon tips. Two extremes are shown in Fig. 2. In one kind, the tips are soft and leafy and the blades, when fully developed, are little different from those of the culms, Fig. 2A. In the other type, the blades are reduced to small flaps and the encasing sheaths are hardened, producing a sharp organ capable of penetrating soil, Fig. 2B. The organ resembles the penetrating tip of a rhizome, and, in fact, is readily converted into a rhizome when buried in the soil. Stolons with leafy tips simply stop growing when buried.

In some races of *C. dactylon* var. *dactylon,* the stolons regularly bury themselves, pushing the sharp tips down into the soil where the stem is converted to a rhizome which may emerge again after a short distance to be reconverted into a stolon. The horizontal stems of these plants seem to "gallop" alternately plunging into the soil and emerging again, producing rhizomes and stolons alternately along the length of the stem.

The extremes, as figured, are easily recognized, but there are intermediate forms that are very difficult to classify, especially with herbarium specimens. In *C. incompletus,* for example, the blades of the stolon leaves near the tip are much reduced and they never develop as fully as the leaves on the culm. The stolon tip may resemble the sharp-pointed organ just described, but it is softer and cannot be converted into a rhizome by burial in the soil. In Table 2, we have listed such intermediates as "R" for the reduced blades of stolon leaves. *C. dactylon* var. *dactylon* is especially variable and all

FIGURE 2. Stolon tips.
A. Leafy type relatively unmodified.
B. Extreme of modified type in which the sheaths are hardened and the blades much reduced.

classes of stolon tips are found in it.

BRANCHING HABIT: Branching patterns depend on the number and position of buds differentiated and on the timing of their development. In most grasses, leaves are arranged alternately in two ranks on the culm, one leaf to the node and each subtending a lateral bud in the axil. In *Cynodon* and some other grasses the culms have two leaves at each node, but only the lower subtends a lateral bud (Bogdan 1952). If the nodes were truly compound, one would expect each leaf to subtend a bud. The culm nodes could be interpreted as simple with the upper leaf subtending the terminal bud even though the terminal bud becomes far removed by subsequent growth of the culm, Fig. 3A. The lateral bud is usually suppressed, although those at the base of the culm may develop into a branch if the culm is allowed to develop fully.

The stolon nodes of *Cynodon* are compound and have three leaves, the lower two subtending lateral buds on opposite sides of the stolon, Fig. 3B. In some taxa, notably races of *C. dactylon* var. *dactylon,* the stolon nodes appear to have more than three leaves, but careful examination of young nodes near the tip shows that the basic ground plan is essentially constant. The additional leaves are derived from precocious growth of the lateral buds.

Of the two buds, the lower one is the first to grow and develop into either a stolon branch or a culm. Growth of the upper bud may be suppressed for a long time resulting in a stolon with conspicuously alternate branching, Fig. 4A. In such

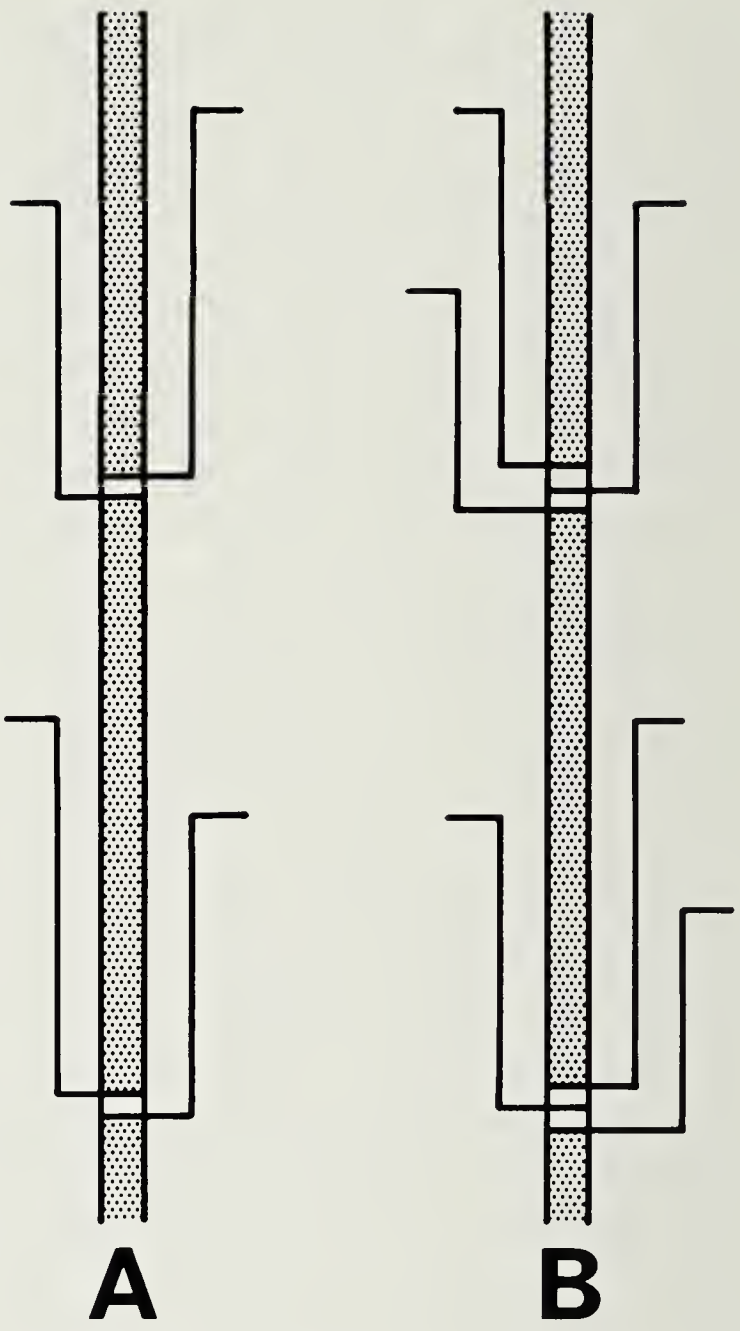

FIGURE 3. Diagram of culm leaves, *A* and stolon leaves, *B*.

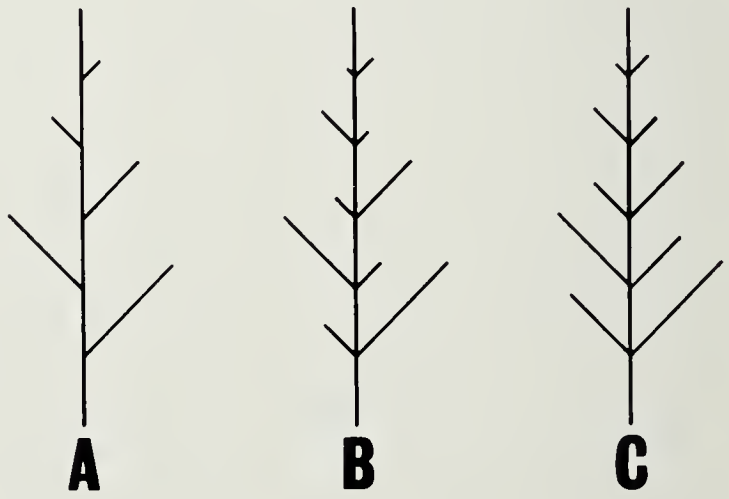

FIGURE 4. Branching patterns.
A. Alternate, one bud suppressed.
B. Intermediate, one bud delayed.
C. Opposite subequal.

stolons, rooting is usually also suppressed so that in the larger forms, one may lift up a stolon one or two meters long unattached to the soil, and with regular alternate branches down its length. Under natural conditions, stolons of this type may festoon shrubs and even small trees three meters or more in height. They tend to have long internodes ($>$ 10 cm and sometimes $>$ 20 cm) and are very fast growing. Under nursery conditions they may grow 10 meters or more in a single season. Plants of this type produce a loose, open mat of growth rather than a dense turf.

At the other extreme, are taxa in which the upper bud at a node develops almost immediately after the lower one, producing a branching pattern that appears to be opposite and subequal, Fig. 4C. These tend to have short internodes, root freely, and form a dense turf covering the soil almost completely. Furthermore, the branches themselves branch quickly and nodes only a short distance back from the tip may show a knot of growth consisting of several culms, short stolon branches, and many leaves. In the absence of competition, the turf creeps in a closed, dense front across the soil surface, rooting immediately behind the stolon tips.

Again, the two extremes are conspicuously different, but some taxa show intermediate behavior as in Fig. 4B. An individual plant may also be rather variable. During periods of maximum growth, internode elongation is sufficiently rapid that the branching appears alternate, but as growth slows down the development of the two buds at a node become more nearly synchronous and the branching approaches opposite. The upper lateral bud usually grows eventually even in forms that have a distinctly alternate branching pattern. Despite the variability, branching habits of some taxa are conspicuously different from others. *Cynodon aethiopicus, C. nlemfuensis* var. *nlemfuensis* and 2x var. *robustus, C. arcuatus, C. barberi* and *C. dactylon* var. *aridus, afghanicus* and *elegans* have consistently alternate branching, at least along the distal portions of the stolons. *Cynodon plectostachyus, C. nlemfuensis* var. *robustus* (4x), *C. incompletus, C. transvaalensis,* and *C. dactylon* vars. *dactylon, coursii,* and *polevansii* have essentially opposite branching patterns.

SIZE: There are striking differences among taxa with respect to plant size. This, again, is a variable character and readily influenced by environment. Under conditions of a uniform nursery in full sunlight and with competition with other vegetation removed, the differences are so consistent that plant size is one of the most conspicuous of all characters. Under these conditions, small plants are usually 15 cm or less in height and large plants are generally 40 cm or more. Some of the most robust forms may exceed a meter in height. A rough classification of plant size is presented in Table 2.

HARDINESS: *Cynodon* is basically a tropical genus and plants of most species are very sensitive to freezing. Plants of *C. arcuatus, C. barberi, C. aethiopicus, C. plectostachyus, C. nlemfuensis, C. dactylon* var. *coursii* and some races of

var. *dactylon* are completely destroyed by a killing frost under Oklahoma conditions. *C. dactylon* var. *aridus* apparently has no tissue hardiness, but can usually overwinter at Stillwater by virtue of the deep rhizomes that escape killing temperatures. *C. dactylon* var. *elegans* has the same faculty, but the rhizomes do not go as deep and mortality is very high. Plants of the tropical race of var. *dactylon* may survive especially mild winters, but always with very severe injury. Deep rhizomes can act as a survival mechanism for plants without tissue hardiness.

Cynodon incompletus and *C. dactylon* var. *afghanicus,* on the other hand, have good tissue hardiness but no rhizomes. Both overwinter well in Oklahoma. *Cynodon transvaalensis, C. dactylon* var. *polevansii* and many accessions of var. *dactylon* have both tissue hardiness and rhizomes.

LEAF SHAPE: Three species can be easily recognized by leaf shape. In *C. barberi,* the leaves are broadly ovate-lanceolate, conspicuously different from all other taxa in the genus. In *C. arcuatus,* the leaves are broadly linear-lanceolate, rather intermediate between *C. barberi* and most of the other taxa. There is no overlap, however, and the leaves of *C. arcuatus* are readily recognizable. *Cynodon transvaalensis* represents the other extreme, with slender linear leaves finer than in any other species in the genus. Plants of all other taxa have linear-lanceolate leaves more or less alike in form. There is a conspicuous range in size, but only the three species mentioned can be consistently distinguished by leaf shape.

Interpretation

Cynodon barberi and *C. arcuatus* are well separated from the rest of the genus not only by leaf shape and growth habit, but by inflorescence and spikelet characters and by genetic barriers (Harlan and de Wet 1969, Harlan *et al.* 1969). The distribution of *C. arcuatus* across the islands of the Indian and South Pacific Oceans from the Comoros and Seychelles to Australia suggests an ancient distribution of ancestral forms, clearly distinct from the geographic patterns of the rest of the genus. *Cynodon barberi* shows some morphological affinity to the nearest genus, *Brachyachne,* which is represented by a number of species in both tropical Africa and Australia that were at one time assigned to *Cynodon. Cynodon barberi* and *C. arcuatus* appear, therefore, to represent a very early differentiation from the ancestral *Cynodon* stock and are no longer closely related to the remaining taxa.

A second clearly separable group includes the large East African species *C. aethiopicus, C. nlemfuensis,* and *C. plectostachyus.* They share a number of growth habit characteristics such as lack of rhizomes, leafy stolon tips, lack of hardiness, large size, open growth and mostly alternate branching patterns. All three have distributions closely associated with the Great Rift Valley. In crossability studies reported by Harlan *et al.* (1969), it was shown that *C. plectostachyus* is completely

isolated genetically from other taxa and that *C. aethiopicus* is isolated by very strong genetic barriers.

A third natural group includes the South African endemics, *C. incompletus* and *C. transvaalensis*. They are small, turf-forming diploids with far more winter hardiness than is required by their present habitats in South Africa. Although sympatric in part, they do not seem to cross in nature, but can be hybridized artifically (Harlan *et al.* 1970).

In the complex species *C. dactylon,* there is nothing that appears directly related to the first group. *C. dactylon* var. *coursii,* however, is a large, tropical, nonhardy and nonrhizomatous form with evident connections to *C. nlemfuensis*. Harlan *et al.* (1969) report that it crosses rather easily with other varieties of *C. dactylon* and that some hybrids with *C. nlemfuensis* were produced. The variety *polevansii* is a small, turf-forming, winterhardy endemic of South Africa evidently associated with the third group. The other African variety, *elegans* has growth habits that suggest it is a tetraploid form derived, in part at least, from the diploid var. *aridus*.

Finally, the Asian forms of *C. dactylon* seem to form a fourth group. Harlan and de Wet (1969) have shown that var. *aridus,* var. *afghanicus,* and the winterhardy temperate races of var. *dactylon* interact genetically in Asia. Introgression between hardy races of var. *dactylon* and hardy var. *afghanicus* is especially evident in Afghanistan. There is no apparent genetic connection between the winterhardy forms of Asia and those of South Africa. Hardiness has evolved independently in two widely separated regions. Furthermore, it is the Asian group that has produced the cosmopolitan weed var. *dactylon* (Harlan and de Wet 1969).

We, therefore, postulate the following sequence of evolutionary events:

1). *C. barberi* and *C. arcuatus* separated early from the remainder of the *Cynodon* stock. They are different morphologically, completely isolated genetically, and have distributions and affinities that suggest an early differentiation. Croizat (1968) assigns distributions of this type to precretaceous events.

2). The large, robust forms of East Africa (*C. aethiopicus, C. nlemfuensis, C. plectostachyus*) evolved, adapted to rather high rainfall and warm temperatures. Their distributions are closely associated with the Great Rift Valley and adjacent highlands which began to assume their present conformation in mid-Tertiary.

3). A diploid form evolved rhizomes and invaded the more arid regions of Africa, the Near East, and India. There were both small and large races and *C. dactylon* var. *aridus* represents the modern survivor of the progenitor rhizomatous *C. dactylon.*

4). The South African endemics evolved in isolation from the rhizomatous forms of India and the Near East. Winterhardy forms, with and without rhizomes, emerged more or less simultaneously in South Africa (*C. incompletus, C. transvaalensis*) and in Asia (*C. dactylon*

var. *afghanicus* and var. *dactylon*). The high degree of winterhardiness in the South African species implies natural selection and survival through the Pleistocene.

5). *C. dactylon* var. *dactylon,* in part through genetic interaction with var. *afghanicus* and var. *aridus* became a cosmopolitan weed (Harlan and de Wet 1969). The high degree of winterhardiness of the temperate races of this variety also implies a Pleistocene evolution, but the cosmopolitan distribution, especially in the Pacific Islands and the New World is a recent historical phenomenon.

Summary

Although highly subject to environmental modification, the vegetative characters in the genus *Cynodon* are useful for field identification and have evolutionary implications. Detailed observations of growth habit and characteristic features of rhizomes and stolons are described.

The genus is essentially nonrhizomatous; only *Cynodon transvaalensis* and four of the six varieties of *C. dactylon* have them. In some taxa, the rhizomes and stolons are interconvertible; others have true rhizomes and true stolons that are not convertible.

The compound nodes of stolons have three leaves, each subtending a bud, one terminal and two lateral. Of the two lateral buds, the lower one grows first and develops into either a stolon or a branch. The second bud grows after a delay of varying duration. The length of the delay accounts for the branching patterns of stolons.

A sequence of evolutionary events in *Cynodon* has been postulated after taking into account the information available from geographic distribution and cytogenetic studies.

Acknowledgment

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DERIVATION OF 1/Z EXPANSION OF HARTREE-FOCK EQUATIONS

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Abstract.—A simple method of deriving the 1/Z expansion of the Hartree-Fock equation by using the properties of determinants and the orthonormalities of spin and space is described. The method consists of an elementary derivation of the energy expression and subsequent variation of it using Lagrange multipliers. It can also be used to derive the regular (restricted or unrestricted) Hartree-Fock equations.

In recent years, the usefulness of the 1/Z expansion perturbation method for calculating atomic wave functions and energy states, and for predicting atomic properties has been widely explored (Hirschfelder, Brown and Epstein, 1964; Dalgarno, 1962). The method involves use of the perturbation parameter, which is the inverse nuclear charge Z. It has the advantage that all members of an iso-electronic sequence may be treated simultaneously. Furthermore, it also provides a convenient basis for comparison with similar expansions corresponding to the non-relativistic many-electron Schrödinger equation. The zeroth-order equations of this method are satisfied by hydrogenic wave-functions and energies; the first-order perturbed equations using this method have been solved for a number of systems either variationally (Dalgarno, 1960) or in terms of infinite sums of Laguerre functions) Linderberg, 1961; Sharma and Coulson, 1962). For predicting many atomic properties, the calculations are very simple using this method, convergence is rapid and results are comparable in accuracy with those of the Hartree-Fock approximation. The 1/Z expansion method may be developed further to yield results superior in accuracy to those of the Hartree-Fock approximation. It is well known that the Hartree-Fock approximation has played an important role in the development of theories of atomic structure. Its quantitative application leads to a set of coupled integro differential equations, specific to the particular atomic system under consideration, which can be solved only by lengthy numerical techniques. If we apply the 1/Z expansion method to the Hartree-Fock equations, it leads to sets of uncoupled ordinary differential equations, specific to the particular electron shell under consideration, which can be solved exactly in analytical form. For this reason, many calculations have been done by this method in the last few years (e.g. Cohen and Dalgarno, 1961, 1963a, 1963b, 1964, 1965a, 1965b, 1967; Schwartz, 1962; Coulson and Hibbert, 1967; Stewart, 1964; Sharma, 1962). However, no detailed derivation of the 1/Z expansion of the Hartree-Fock

equations has been shown in any of those papers or in any standard quantum mechanics textbooks except perhaps for a very brief description of the standard procedure of the derivation. The purpose of the present note is to show that a simple method of derivation can be obtained by using the properties of determinants, spin orthonormality and space orthonormality.

Notation

In order to illustrate the procedure, let us consider the case of the restricted Hartree-Fock wave function for a $1s^2 2p 2^2 p$ state of excited lithium atom by using the following notations:

$\overset{+}{S}(i)$: 1s orbital with α spin for the ith electron.

$\overset{-}{S}(i)$: 1s orbital with β spin for the ith electron.

$\overset{+}{P}_o(i)$: $2p_o$ orbital with α spin for the ith electron.

$\overset{-}{P}_o(i)$: $2p_o$ orbital with β spin for the ith electron.

$U(r_i)$: the radial part of the 1s orbital for the ith electron.

$Y_o^o(\theta_i,\phi_i)$: the angular part of 1s orbital for the ith electron which is equal to $\frac{1}{\sqrt{4\pi}}$

$V(r_i)$: the radial part of the $2p_o$ orbital for the ith electron.

$Y_1^o(\theta_i\phi_i)$: the angular part of the $2p_o$ orbital for ith electron which is equal to $\sqrt{\frac{3}{4\pi}}\cos\theta_i$.

so the total wavefunction for the 1s orbital with α spin for the ith electron can be expressed as $\overset{+}{S} = Y_o^o(\theta_i,\phi_i)U(r_i)\alpha_i = \frac{1}{\sqrt{4\pi}}U(r_i)\alpha_i$, and the space part of the wavefunction is $S(i) = \frac{1}{\sqrt{4\pi}}U(r_i)$. Similarly, $\overset{+}{P}_o(i) = Y_1^o(\theta_i,\alpha_i)V(r_i)\alpha_i = \sqrt{\frac{3}{4\pi}}\cos\theta_i V(r_i)\alpha_i$ and $P_o(i) = \sqrt{\frac{3}{4\pi}}\cos\theta_i V(r_i)$. The inverse interelectron distance, $\frac{1}{r_{ij}}$, can be expressed as follows (Eyring, Walter and Kimble, 1947):

$$\frac{1}{r_{ij}} = \sum_{k=o}^{\infty}\sum_{m=-k}^{+k}\frac{4\pi}{2k+1}\frac{r_<^k}{r_>^{k+1}}Y_k^m(\theta_i,\phi_i)Y_k^{m*}(\theta_j,\phi_m).$$

We define the following integrals:

$$|S(1)P_o(1)) = \int_{v_1}\frac{1}{r_{12}}U(r_1)V(r_1)Y_o^o(\theta_1,\phi_1)Y_1^o(\theta_1,\phi_1)\sin\theta_1 d\theta_1 d\phi_1 r_1^2 dr_1$$

$$= \sum_{k=0}^{\infty} \sum_{m=-k}^{+k} \frac{4\pi}{2k+1} \int_0^{\infty} \frac{r_<^k}{r_>^{k+1}} U(r_1)V(r_1)r_1^2 dr_1$$

$$\times \int_0^{\pi}\int_0^{2\pi} \frac{1}{\sqrt{4\pi}} Y_0^0(\theta_1,\phi_1) Y_k^m(\theta_1,\phi_1) \sin\theta_1 d\theta_1 d\phi_1 Y_k^{m*}(\theta_2,\phi_2).$$

The above reduces to:

$$|S(1)P_0(1)) = \sqrt{\frac{4\pi}{3}}\, Y_1^0(\theta_2,\phi_2)\left\{ \frac{1}{r_2^2} \int_0^{r_2} r_1^3 U(r_1)V(r_1)dr_1 + r_2 \int_{r_2}^{\infty} U(r_1)V(r_1)dr_1 \right\}$$

since the spherical harmonics form an orthonormal set, i.e.

$$\int_0^{2\pi}\int_0^{\pi} Y_k^{m*}(\theta,\phi) Y_{k'}^{m'}(\theta,\phi) \sin\theta\, d\theta\, d\phi = \delta_{k,k'}\delta_{m,m'}$$

$$(S(2)S(2)|\, S(1)P_0(1)) = \langle S(2)S(2)| \frac{1}{r_{12}} |\, S(1)P_0(1)\rangle$$

$$= \frac{1}{\sqrt{4\pi}} \cdot \frac{1}{\sqrt{4\pi}} \cdot \frac{\sqrt{4\pi}}{3} \int_0^{\infty} U(r_2)\left\{ \frac{1}{r_2^2} \int_0^{r_2} r_1^3 U(r_1)V(r_1)dr_1 + r_2 \int_{r_2}^{\infty} U(r_1)V(r_1)dr_1 \right\} r_2^2 dr_2$$

$$\times \int_0^{\pi}\int_0^{2\pi} Y_1^0(\theta_2,\phi_2) \sin\theta_2 d\theta_2 d\phi_2 = 0,$$

because $\int_0^{\pi}\int_0^{2\pi} Y_1^0(\theta_2,\phi_2)\sin\theta_2 d\theta_2 d\phi_2 = 0$ therefore the integral vanishes.

In general,

$$|SP_0) = \sqrt{\frac{4\pi}{3}}\, Y_1^0(\theta,\phi)\left\{ \frac{1}{r^2} \int_0^{r} x^3 U(x)V(x)dx + r \int_r^{\infty} U(x)V(x)dx \right\},$$

$(SS|\, SP_0) = 0$ and $(P_0P_0|\, SP_0) = 0$ due to the orthonormal property of the spherical harmonics.

However, $(SP_0|\, SP_0) = \frac{1}{\sqrt{4\pi}} \sqrt{\frac{4\pi}{3}} \int_0^{\pi}\int_0^{2\pi} Y_1^0(\theta,\phi) Y_1^0(\theta,\phi) \sin\theta\, d\theta\, d\phi \int_0^{\infty} U(r)V(r)$

$$\times \left\{ \frac{1}{r^2} \int_0^{r} x^3 U(x)V(x)dx + r \int_0^{\infty} U(x)V(x)dx \right\} r^2 dr,$$

$$\text{or } (SP_o|SP_o) = \frac{1}{3}\int_o^\infty U(r)V(r)\{\frac{1}{r^2}\int_o^r x^3U(x)V(x)dx + r\int_r^\infty U(x)V(x)dx\}r^2dr,$$

$$\text{because } \int_o^\pi \int_o^{2\pi} Y_1^o(\theta,\phi)Y_1^o(\theta,\phi)\text{Sin}\theta\, d\theta\, d\phi = 1$$

Similarly, we define

$$|S(1)S(1)) = \int_{V_1} \frac{1}{r_{12}} U^2(r_1)Y_o^o(\theta_1,\phi_1)Y_o^o(\theta_1,\phi_1)\text{Sin}\theta_1 d\theta_1 d\phi_1 r_1^2 dr_1$$

$$= \sum_{k=o}^{\infty} \sum_{m=-k}^{+k} \frac{4\pi}{2k+1} \int_o^\infty \frac{r_<^k}{r_>^{k+1}} U^2(r_1)r_1^2dr_1 \int_o^\pi \int_o^{2\pi} Y_o^o(\theta_1,\phi_1)Y_o^o(\theta_1,\phi_1)Y_k^m(\theta_1,\phi_1)$$

$$x\ \text{Sin}\theta_1 d\theta_1 d\phi_1 Y_k^{m*}(\theta_2\phi_2) = \frac{1}{r_2}\int_o^{r_2} U^2(r_1)r_1^2dr_1 + \int_{r_2}^\infty U^2(r_1)r_1dr_1$$

$$\text{So, } (SS|P_oP_o) = (P_oP_o|SS) = \int_o^\pi \int_o^{2\pi} Y_1^o(\theta,\phi)Y_1^o(\theta,\phi)\text{Sin}\theta d\theta d\phi \int_o^\infty v^2(r)$$

$$\mathbf{X}[\frac{1}{r}\int_o U^2(x)x^2dx + \int_r^\infty v^2(x)xdx]r^2dr$$

$$\text{In general, } (P_oP_o|SS) = \int_o^\infty v^2(r)[\frac{1}{r}\int_o^r U^2(x)x^2dx + \int_r^\infty U^2(x)xdx]r^2dr,$$

$$\text{and } (SS|SS) = \int_o^\infty U^2(r)[\frac{1}{r}\int_o^r U^2(x)x^2dx + \int_r^\infty U^2(x)xdx]r^2dr.$$

1/Z Expansion of the Angular and Radial Hartree-Fock Equations

We use a unit of length equal to 1/Z A.U., a unit of energy equal to Z^2 A.U. and the notations of the preceding section. The Hamiltonian of this system can be written as

$$\hat{H} = \sum_i^3 \hat{h}(i) + \frac{1}{Z} \sum_{i<j} g(i,j)$$

where $\hat{h}(i) = -\frac{1}{2}\nabla_i^2 - \frac{1}{r_i}$ and

$$\sum_{i<j} g(i,j) = \frac{1}{r_{12}} + \frac{1}{r_{23}} + \frac{1}{r_{13}}$$

In order to obtain the total energy of this atomic state, we have to evaluate the diagonal matrix elements of the Hamiltonian of this system over the restricted Hartree-Fock wavefunctions. Let us first consider the matrix elements of $\sum_i^3 \hat{h}(i)$. Let $\hat{\mathcal{P}}_i$ be the permutation operator and $\mathcal{P}_i$ be the number of permutation.

Then, after multiplying by inverse permutations and their corresponding signs to eliminate the normalization factor (Eyring, Walter and Kimble, 1947) we have,

$$\langle |\overset{+}{S}\bar{S}\overset{+}{P}_o| \sum_i^3 \hat{h}(i) |\overset{+}{S}\bar{S}\overset{+}{P}_o| \rangle = \frac{1}{\sqrt{3!}}\frac{1}{\sqrt{3!}} \sum_i (-1)^{\mathcal{P}_i} \hat{\mathcal{P}}_i \int \overset{+}{S}(1)\bar{S}(2)\overset{+}{P}_o(3) \sum_i \hat{h}(i) \begin{vmatrix} \overset{+}{S}(1) & \bar{S}(1) & \overset{+}{P}_o(1) \\ \overset{+}{S}(2) & \bar{S}(2) & \overset{+}{P}_o(2) \\ \overset{+}{S}(3) & \bar{S}(3) & \overset{+}{P}_o(3) \end{vmatrix}$$

$$= \langle \overset{+}{S}(1)|\hat{h}(1)|\bar{S}(1)\rangle + \langle \bar{S}(2)|\hat{h}(2)|\bar{S}(2)\rangle + \langle \overset{+}{P}_o(3)|\hat{h}(3)|\overset{+}{P}_o(3)\rangle$$

$$= 2\langle S|\hat{h}|S\rangle + \langle P_o|\hat{h}|P_o\rangle ;$$

where $\langle \overset{+}{S}(i)|\bar{S}(i)\rangle = \langle \bar{S}(i)|\overset{+}{S}(i)\rangle = 0$ and $\langle \overset{+}{S}(i)|\hat{h}(i)|\bar{S}(i)\rangle = \langle \bar{S}(i)|\hat{h}(i)|\overset{+}{S}(i)\rangle = 0$, because of spin orthogonality; $\langle \overset{+}{P}_o(i)|\overset{+}{S}(i)\rangle = \langle \overset{+}{S}(i)|\overset{+}{P}(i)\rangle = 0$ and $\langle \overset{+}{P}_o(i)|\hat{h}(i)|\overset{+}{S}(i)\rangle = \langle \overset{+}{S}(i)|\hat{h}(i)|\overset{+}{P}_o(i)\rangle = 0$, because of space orthogonality; $\langle \overset{+}{P}_o(i)|\hat{h}(i)|\bar{S}(i)\rangle = \langle \bar{S}(i)|\hat{h}(i)|\overset{+}{P}_o(i)\rangle = 0$, because of spin and space orthogonality;

$\langle \overset{+}{S}(i)|\hat{h}(i)|\overset{+}{S}(i)\rangle = \langle S(i)|\hat{h}(i)|S(i)\rangle\langle\alpha(i)|\alpha(i)\rangle =$
$\langle S(i)|\hat{h}(i)|S(i)\rangle \times 1 = \langle S|\hat{h}|S\rangle$ and similarly $\langle \overset{+}{P}_o(i)|\hat{h}(i)|\overset{+}{P}_o(i)\rangle =$
$\langle P_o|\hat{h}|P_o\rangle$. For matrix elements of $1/Z \sum_{i<j} g_{ij}$, we can use similar arguments to get the following results.

$$\langle|\overset{+}{S}\bar{S}\overset{+}{P}_o|\frac{1}{Z}\sum_{i<j} g_{ij}|\overset{+}{S}\bar{S}\overset{+}{P}_o|\rangle = \frac{1}{Z}\int \overset{+}{S}(1)\bar{S}(2)\overset{+}{P}_o(3)g_{12}\begin{vmatrix}\overset{+}{S}(1) & \bar{S}(1) & \overset{+}{P}_o(1)\\ \overset{+}{S}(2) & \bar{S}(2) & \overset{+}{P}_o(2)\\ \overset{+}{S}(3) & \bar{S}(3) & \overset{+}{S}(3)\end{vmatrix} dV_{123}^{+}$$

$$\frac{1}{Z}\int S^{+}(1)S(2)P_o(3)g_{13}\begin{vmatrix}\overset{+}{S}(1) & \bar{S}(1) & \overset{+}{P}_o(1)\\ \overset{+}{S}(2) & \bar{S}(2) & \overset{+}{P}_o(2)\\ \overset{+}{S}(3) & \bar{S}(3) & \overset{+}{P}_o(3)\end{vmatrix} dV_{123}$$

$$+\frac{1}{Z}\int \overset{+}{S}(1)\bar{S}(2)\overset{+}{P}_o(3)g_{23}\begin{vmatrix}\overset{+}{S}(1) & \bar{S}(1) & \overset{+}{P}_o(1)\\ \overset{+}{S}(2) & \bar{S}(2) & \overset{+}{P}_o(2)\\ \overset{+}{S}(3) & \bar{S}(3) & \overset{+}{P}_o(3)\end{vmatrix} dV_{123}$$

The above equation can be simplified by multiplying the third orbital ($\overset{+}{P}_o$) into the third row in the first term, the second orbital ($\bar{S}$) into the second row in the second term and the first orbital ($\overset{+}{S}$) into the first row in the third term, as shown by the arrows. After using the orthonormality argument, we have the following results:

$$\langle|\overset{+}{S}\overset{-}{S}\overset{+}{P}_o|\frac{1}{Z}\sum_{k<j} g_{ij}|\overset{+}{S}\overset{-}{S}\overset{-}{P}_o|\rangle = \frac{1}{Z}\{\int \overset{+}{S}(1)\overset{-}{S}(2)g_{12}\begin{vmatrix}\overset{+}{S}(1) & \overset{-}{S}(1)\\ \overset{+}{S}(2) & \overset{-}{S}(2)\end{vmatrix} dV_{12}$$

$$+ \int \overset{+}{S}(1)\overset{+}{P}_o(3)g_{13}\begin{vmatrix}\overset{+}{S}(1) & \overset{+}{P}_o(1)\\ \overset{+}{S}(3) & \overset{+}{P}_o(3)\end{vmatrix} dV_{13}$$

$$+ \int \overset{-}{S}(2)\overset{+}{P}_o(3)g_{23}\begin{vmatrix}\overset{-}{S}(2) & \overset{+}{P}_o(2)\\ \overset{-}{S}(3) & \overset{+}{P}_o(3)\end{vmatrix} dV_{23}\ \}$$

$$= \frac{1}{Z}(SS+\overline{SS}) + \frac{1}{Z}(SS|P_oP_o) - \frac{1}{Z}(SP_o|P_oS) + \frac{1}{Z}(\overline{SS}|P_oP_o)$$

$$= \frac{1}{Z}\ (SS|SS) + 2(SS|P_oP_o) - (SP_o|P_oS)\}$$

So, the total energy of $(1s^2 2p\ 2^2\mathbf{p})$ is

$$E(1s^2 2p, 2^2 p) = 2(S|\hat{h}|S) + (P_o|\hat{h}|P_o) + \frac{1}{Z}(SS|SS) + \frac{2}{Z}(SS|P_oP_o) - \frac{1}{Z}(SP_o|P_oS)$$

(1)

The orthonormality conditions are

$$\int S^2 dV = 1, \qquad \int P_o^2 dV = 1 \text{ and } \qquad \int SP_o dV = 0.$$

Applying the variational principle to obtain the Hartree-Fock equations, we use a star * to label the quantities being varied in Eq. (1). We have then

$$E = 2(S^*|\hat{h}|S) + (P_o^*|\hat{h}|P_o) + \frac{1}{Z}(S^*S|S^*S) + \frac{2}{Z}(S^*S|P_o^*P) - \frac{1}{Z}(S^*P_o|P_o^*S)$$

(2)

and we note that

$\delta\{E-2\lambda_s(S^*|S)-\lambda_p(P_o^*|P_o)-\lambda_{sp}(S^*|P_o)-\lambda_{ps}(P_o^*|S)\} = 0$ is the same as $\delta\{E-2\lambda_s(S^*|S)-\lambda_p(P_o^*|P)-\lambda^*_{sp}(S|P_o^*)-\lambda^*_{ps}(P_o|S^*)\} = 0$, since λ_{sp} is real, $\lambda_{sp} = \lambda^*_{ps}$ where λ's are Lagrange multipliers and $(S|S)$, $(P_o|P_o)$ and $(S|P_o)$ are the constraints.

Carrying out the variation with respect to δs^*, δP_o^*, (and to δs, αP_o separately); we have,

$$2(\delta S^*|\hat{h}|S) + (\delta P_o^*|\hat{h}|P_o) + \frac{2}{Z}(\delta S^*S|S^*S) + \frac{2}{Z}(\delta S^*S|P_o^*P_o)$$

$$+ \frac{2}{Z}(S^*S|\delta P_o^*P_o) - \frac{1}{Z}(\delta S^*P_o|P_o^*S) - \frac{1}{Z}(S^*P_o|\delta P_o^*S)$$

$$- \frac{2}{Z}\lambda_s(\delta S^*|S) - \lambda_p(\delta P_o^*|P_o) - \lambda_{sp}(\delta S^*|P_o) - \lambda_{ps}(\delta P_o^*|S) = 0$$

where $\lambda_{sp} = \lambda_{ps}$. Equating to zero the coefficients of δs^* and δP_o^*, we obtain the Hartree-Fock equations:

$$\hat{h}S + \frac{1}{Z}S|SS) + \frac{1}{Z}S|P_oP_o) - \frac{1}{2Z}P_o|P_oS) = \lambda_s S + \frac{1}{2}\lambda_{sp}P_o \quad \text{(3)}$$

$$\hat{h}P_o + \frac{2}{Z}P_o|SS) - \frac{1}{Z}S|SP_o) = \lambda_p P_o + \lambda_{ps}S \quad \text{(4)}$$

Since S and P_o are orthonormal, we do not need to introduce the Lagrangian multipliers λ_{sp} and λ_{ps}; therefore, we set $\lambda_{sp} = \lambda_{ps} = 0$, and Eqs. (3) and (4) become

$$\hat{h}S + \frac{1}{Z}S|SS) + \frac{1}{Z}S|P_oP_o) - \frac{1}{2Z}P_o|P_oS) = \lambda_s S \quad \text{(5)}$$

$$\hat{h}P_o + \frac{2}{Z}P_o|SS) - \frac{1}{Z}S|SP_o) = \lambda_p P_o \quad \text{(6)}$$

These equations depend on the parameter Z^{-1} as well as the λ's. S and P_o can be obtained as functions of that parameter by solving these equations. The present procedure also can be used to derive regular Hartree-Fock (restricted or unrestricted) equations. The derivation of the regular Hartree-Fock equations can be considered as a special case of the present method. All procedures are the same, except that we omit the 1/Z factor in the equations.

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DEATH OF CELLS IN PITH TISSUE OF SOYBEAN SEEDLINGS

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ABSTRACT.—Parenchyma cell death in the hypocotyl pith tissue of soybean seedlings was discovered in each of the 24 varieties studied representing seven maturity classes. Dead cells generally were observed on the fifth day after planting; and chlorenchyma tissue formed between the dead pith parenchyma cells and xylem in the above-ground stem. Dead parenchyma cells were observed in the below-ground cortex and in the pith of the elongating internode above the cotyledons. No dead cells were observed in the cotyledonary node during the 20-day study period.

The death of cells in pith parenchyma of the hypocotyl and the first and second internodes of seedlings of 18 soybean varieties was studied in relation to two or three planting depths (sub-surface, 2.5 cm, and 5.0 cm). Differences were noted in the rate of cell death in hypocotyls but not in epicotyl internodes as a result of planting depth differences. In the hypocotyls, most pith parenchyma cells died during the first week of growth, and the greater depth of planting resulted in the fastest rate of cell death in each variety. Cell death in pith parenchyma of internodes occurred as the internodes elongated.

Patterns of parenchyma cell death have been reported for normal and injured sorghum (Katsanos and Pappelis, 1969), sugarcane (Pappelis and Katsanos, 1965), and corn (Pappelis and Katsanos, 1969). It was considered desirable to seek similar parenchyma death in a plant more suited for study in growth chambers. This paper reports the discovery of parenchyma cell death in soybean seedlings and describes variations in cell death patterns associated with variations in planting depth.

MATERIALS AND METHODS

Twenty-four soybean varieties (Class 00, Acme, Flambeau, Portage; Class 0, Grant, Merit, Norcheif; Class I, A-100, Chippewa, Chippewa 64, Ontario; Class II, Hawkeye, Lindarin; Class III, Adams, Ford, Harosoy, Shelby, Wayne; Class IV, Clark, Kent, Midwest, P.I. 84.946-2, Clark 63; Class V, Dorman, Hill) were grown under greenhouse conditions at various times from December 29, 1964, to March 31, 1965. Seeds of each variety were planted in separate wood flats 2.5 cm apart at a depth of 5 cm in a soil mixture of 50% sand and 50% peat moss. Five plants from each variety were selected for study each day beginning at the second day after planting and continued until the first internodes had elongated. The maximum study period was 20 days.

Twelve soybean varieties were planted (April 29, 1965) in separate wood flats, 2.5 cm apart at depths of 2.5 or 5.0 cm. Watering was accomplished with a mist sprayer to reduce soil packing or washing. Five seedlings of each variety were selected from each planting depth on the seventh, tenth, and twentieth days after planting. Also, seedlings of six varieties were studied after planting (May 20, 1965) at three depths: 5.0 cm, 2.5 cm, and just below the soil surface. For the latter, seeds were placed on the soil surface and covered with a thin layer of soil to permit germination below the soil. Watering and sampling procedures were as those described

above but with two samples studied seven and fourteen days after planting.

Dead cells in hypocotyl and epicotyl pith tissue were discovered in cross section and longitudinal section using the plasmolyzing neutral red stain solution described by Tribe (1955).

The length of white hypocotyl tissue of the below-ground part was used as the final estimate of planting depth. The lengths of hypocotyls and internodes were measured before the stem was cut longitudinally. Lengths of pith composed of dead cells were measured and the per cent of total length with dead cells calculated.

Results

Dead and living pith cells were easily distinguished microscopically in cross and longitudinal sections of the hypocotyls using the plasmolyzing-neutral red stain. In stained sections, living cells contained dark-red, plasmolyzed protoplasts, while dead cells were light in color and contained no plasmolyzed protoplasts. In every case, dead cells contained a gas bubble. No remains of the protoplast or cell organelles were observed. When masses of dead cells occurred in the pith tissue, macroscopically the pith appeared white in color and was spongy. Tissue composed of living cells was well hydrated and appeared light green in color in the above-ground part, and cream in color in the below-ground part.

In all varieties dead cells were first observed in the central area of the below-ground hypocotyl pith five days after planting (Figure 1). Sev-

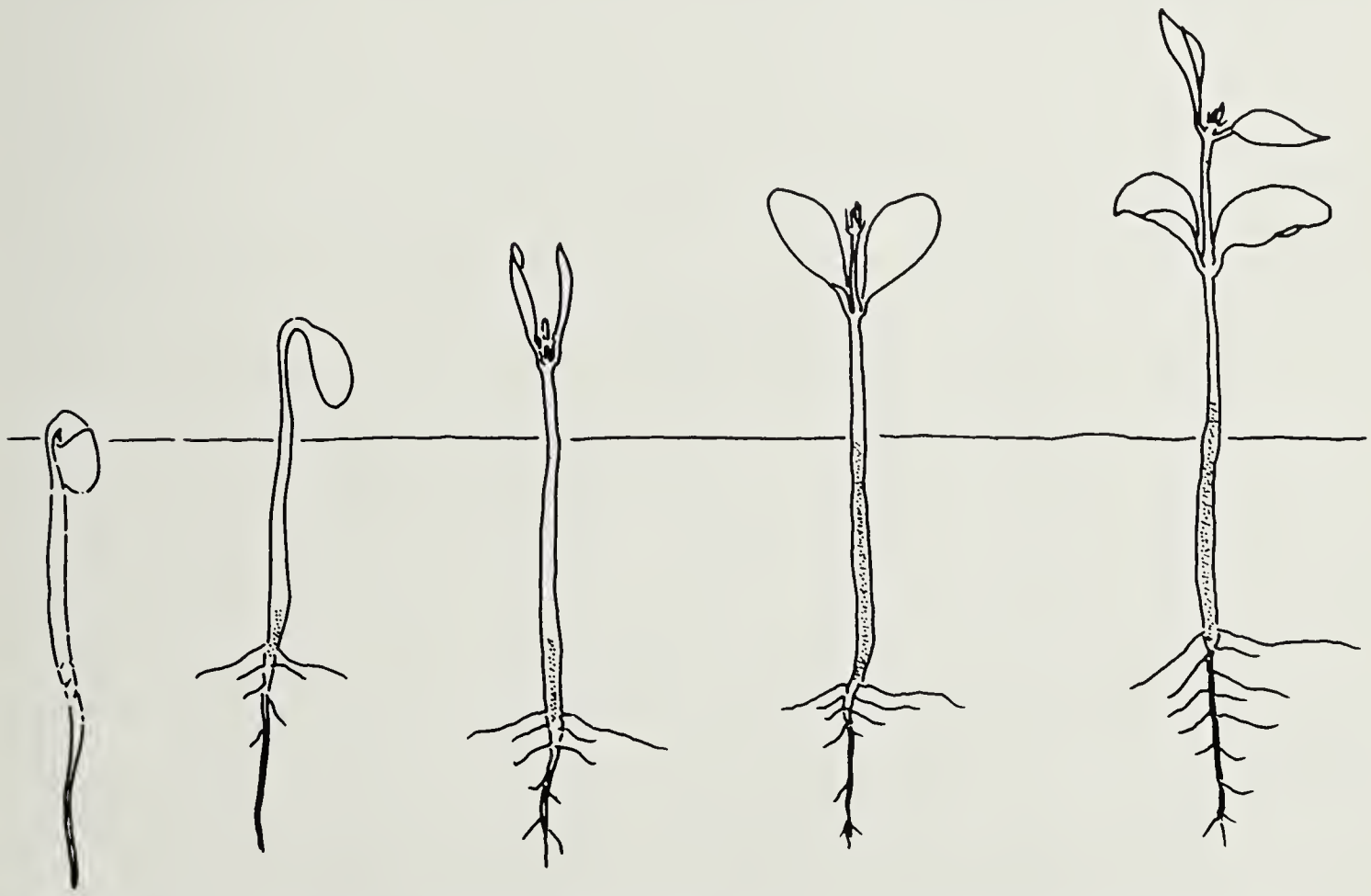

Figure 1. Dead pith parenchyma cells occur in hypocotyls five days after planting. From left to right, appearance of seedlings and areas of dead cells at 4, 5, 6, 8, and 10 days after planting; dotted areas representing areas of dead parenchyma cells in the pith.

en to eight days after planting, almost all of the cells in pith tissue of the below-ground hypocotyl were dead. Ten days after planting dead cells were observed in the central region of the pith of the above-ground hypocotyl and a hollow area formed in the below-ground hypocotyl pith. This was associated with an increase in below-ground diameter of the hypocotyl; a result of cortical expansion. At this stage, dead cells and hollow areas were also observed in the cortex. In the above-ground hypocotyl, pith cells adjacent to the xylem developed into a layer of chlorenchyma, four to five cells in thickness.

In the first internodes of all varieties studied, dead cells appeared in the pith as the internodes elongated. A chlorenchyma layer developed in the internode pith adjacent to the xylem tissue. No dead cells appeared in the cotyledonary node or in nodes above this point.

In the two experiments designed to determine the effects of planting depth on cell death in stem tissue, little differences were noted in the cell death patterns in the epicotyl internodes. For this reason, only the results from two varieties, one from each experimental group, will be presented (Table 1). In the hypocotyls, pith cells died in different patterns due to planting depth. The data for 12 varieties, planted at 2.5

TABLE 1.—Cell death percentages in the hypocotyl, first internode, and second internode pith tissue at different stages of seedling development from seeds of Harosoy planted at two depths and Kent planted at three depths. (% DC = per cent of the area of pith tissue with dead cells.)

Depth of Planting (cm)	Days After Planting	Seedling Part					
		Hypocotyl		First Internode		Second Internode	
		Length	% DC	Length	% DC	Length	% DC
Harosoy (Planted April 29)							
2.5	7	6.6	45	1.7	0		
	10	7.0	85	5.3	5		
	20	7.7	87	8.5	96	4.0	0
5.0	7	8.1	56	1.9	0		
	10	8.2	92	6.2	8		
	20	8.0	96	9.2	96	4.4	27
Kent (Planted May 20)							
Sub-surface	7	4.5	0	2.5	0		
	14	4.4	52	11.3	91	7.2	54
2.5	7	6.0	50	4.8	0		
	14	5.9	76	12.4	84	7.8	57
5.0	7	8.2	88	5.0	4		
	14	8.7	95	11.7	99	7.5	49

and 5.0 cm, and sampled 7, 10, and 20 days after planting are given in Table 2. The data for 6 varieties, planted at sub-surface, 2.5 cm, and 5.0 cm, and sampled 7 and 14 days after planting are given in Table 3.

In the study of cell death in 12 varieties at two planting depths, seven days after planting, the lengths of hypocotyls of seedlings planted at 2.5 cm depths ranged from 6 to 9 cm. During the next 13 days, additional growth was generally less than 1 cm; Acme being most extreme with an additional 1.9 cm of growth. Hypocotyls of seedlings at 5.0 cm depth ranged in length from 7 to 9 cm 7 days after planting and increased about 1 cm in length during the next 13 days, Hawkeye being most extreme with an additional 2.5 cm of growth. The length of hypocotyl with dead pith cells on the seventh day after planting ranged from 41 to 65% for seeds planted at 2.5 cm depth and 53 to 88% for those planted at 5.0 cm depth. In all but one case, the seedlings from the deepest planting depth had the highest percentage of dead cells in each variety. By the twentieth day after planting, the amount of dead cells in the pith ranged from 88 to 100% in the plants from both planting depths

TABLE 2.—Per cent of the area of pith tissue of hypocotyls composed of dead cells in seedlings of 12 varieties of soybeans planted at two depths and sampled three times after planting (April 29).

Variety	Depth of Planting (cm)	Per cent of hypocotyl with dead cells; days after planting		
		7	10	20
A-100	2.5	53	96	96
	5.0	66	99	96
Acme	2.5	42	95	96
	5.0	53	96	98
Chippewa 64	2.5	64	61	97
	5.0	88	97	97
Chippewa	2.5	44	96	96
	5.0	68	98	98
Clark	2.5	46	84	95
	5.0	66	75	96
Dorman	2.5	57	94	96
	5.0	64	94	95
Flambeau	2.5	41	97	100
	5.0	66	97	96
Ford	2.5	57	96	97
	5.0	64	96	97
Grant	2.5	61	71	90
	5.0	57	95	95
Harosoy	2.5	45	85	87
	5.0	55	95	96
Hawkeye	2.5	50	40	97
	5.0	62	80	98
Hill	2.5	58	75	91
	5.0	66	68	88

with no apparent difference due to the depth of planting. Generally, most of the pith parenchyma cells had died before the tenth day after planting.

The above-ground growth in the 12 varieties showed some variations but within each variety, the growth of the first and second internodes and the per cent of pith tissue with dead cells were similar in the seedlings from both the 2.5 and 5.0 cm planting depths. The average growth of the first internodes seven days after planting ranged from none visible to 1 cm, 4 to 10 cm on the tenth day, and 6 to 14 cm on the twentieth day after planting. The second internodes began to elongate between the tenth and twentieth days after planting, ranging in size from about 3 to 7 cm on the twentieth day. Cell death in the first internodes occurred after the seventh day. On the tenth day, the amounts of dead cells in the pith ranged from 0 to 43% in seedlings from the 2.5 cm depth and 8 to 55% in those from the 5.0 cm depth. On the twentieth day after planting, the amounts of pith with dead cells in the first internodes ranged from 92 to 98% in seedlings from the 2.5 cm depth (except Grant, 45%) and 95 to 98% in those from the 5.0 cm depth. The amounts of dead cells in the pith tissue of the second internodes ranged from 0 to 82% in seedlings from the 2.5 cm depth and 25 to 82% in those from the 5.0 cm depth. Generally, death of pith cells began in the lower part of the pith tissue of the internodes after they had grown a few centimeters in length. The slight differences noted in lengths and percentages of pith length with dead cells for Harosoy grown at two planting depths (Table 1) are typical for the 12 varieties studied.

The effects of sub-surface, 2.5, and 5.0 cm planting depths on cell death in stem tissue were most noted for hypocotyl pith. The results with Kent (Table 1) were typical both for hypocotyl and epicotyl pith cell death observed in all six varieties.

For all six varieties, hypocotyl lengths from the sub-soil plantings ranged from 4 to 6 cm on the seventh day and had little additional growth. The percentages of dead cells ranged from 0 to 84% on the seventh day and from 5 to 92% on the fourteenth day (Table 3). When these six varieties were planted at a depth of 2.5 cm, hypocotyls at 7 days after planting ranged in length from 5 to 7 cm with little or no additional growth during the next week. The amount of dead pith parenchyma ranged from 38 to 92% 7 days after planting and 14 to 93% 14 days after planting. The hypocotyls of the seedlings of the six varieties planted at 5.0 cm depths ranged in length from 7 to 12 cm 7 days after planting with little additional growth occurring during the next week. The ranges of cell death percentages for the hypocotyl pith tissue were 88 to 93% on the seventh day and 95 to 97% on the fourteenth day after planting. For all six varieties planted at sub-soil depth, the first internode lengths ranged from 3 to 11 cm 7 days after planting with 0 to 43% of the pith parenchyma cells dead, and 9 to 13 cm 14 days after planting with 80 to 100% of the cells dead. The second internodes began to elongate during the

TABLE 3.—Per cent of the area of pith tissue of hypocotyls composed of dead cells in seedlings of six varieties of soybeans planted at three depths and sampled two times after planting (May 20).

Variety	Depth of Planting (cm)	Per cent of hypocotyl with dead cells; days after planting	
		7	14
Kent	sub-surface	0	52
	2.5	50	76
	5.0	88	95
Lindarin	sub-surface	37	45
	2.5	66	82
	5.0	93	96
Merit	sub-surface	17	5
	2.5	38	14
	5.0	88	96
Norcheif	sub-surface	32	92
	2.5	62	93
	5.0	96	95
Portage	sub-surface	58	58
	2.5	66	46
	5.0	97	97
Wayne	sub-surface	84	58
	2.5	92	90
	5.0	89	97

second week and ranged from 5 to 8 cm in length 14 days after planting with 39 to 60% of the pith parenchyma cells dead. For the six varieties planted at a depth of 2.5 cm, first internodes lengths ranged from 5 to 13 cm 7 days after planting and 7 to 11 cm one week later. The dead cell percentages ranged from 0 to 38% and 84 to 99% on the seventh and fourteenth days, respectively. The second internodes began to elongate after the first sampling and ranged in length from 6 to 9 cm on the fourteenth day with cell death percentages ranging from 39 to 79%. For the six varieties planted at a depth of 5.0 cm, the first internode lengths for the six varieties ranged from 4 to 8 cm on the seventh day with 3 to 29% of the length containing dead cells and 8 to 17 cm on the fourteenth day with 68 to 99% of the lengths containing dead pith cells. The second internodes began to elongate during the second week after planting and, on the fourteenth day, the lengths ranged from 7 to 9 cm with cell death averages ranging from 49 to 74%.

No death of pith cells was observed in nodal areas in any of the experimental groups.

DISCUSSION

The death of parenchyma cells in the pith tissue of soybean occurred in all varieties studied, regardless of maturity class. Dead cells first

appeared in the central rows of pith cells in the below-ground part of the hypocotyl, usually within 5 days after planting. In all three planting groups, dead cells were observed in the pith of the above-ground and below-ground parts of the hypocotyl within two weeks. Hollow areas sometimes formed in the pith of the hypocotyl, especially in areas where increased cortical diameter had occurred. Whether expansion caused cortical cells to die is not known.

Death of pith parenchyma in the first and second internodes appeared after internode elongation began. Death of cells was first observed in the lower part of the internode.

In both planting depth experiments, the patterns for cell death were like those observed for all varieties at one planting depth, death of cells first occurring in the lower part of the internode or hypocotyl (Fig. 2). The death patterns were similar in all varieties studied but the rate of cell death differed between varieties.

In the study of the six varieties at 3 planting depths, it was very clear that the differences in growth and cell death in the first and second internode of any variety was very small. When compared as a group, some differences were noted in growth or cell death trends between varieties, but these too were small. The obvious differences in each variety were the percentages of cell death in pith cells of the hypocotyls of seedlings planted at the three depths, the greatest amount of cell death occurring at the deepest planting depth and the least amount occurring when the seeds were planted just below the soil surface. Varietal differences were noted (Table 3), with cell death percentages in Norcheif showing little difference due to planting depth while great differences occurred in Merit. Since cell death patterns in parenchyma tissue in stalks of corn, sorghum, and sugarcane is correlated with the areas susceptible to stalk rot in those crop plants (Katsanos and Pappelis, 1969; Pappelis and Katsanos, 1965 and 1969), it may be that the patterns of cell death discovered in the stem of soybeans will provide a new approach to the study of the nature of resistance to spread of *Cephalosporium gregatum* Allington and Chamberlain, the causal organism for the brown stem rot disease in soybean.

Acknowledgment

We wish to thank Dick Bernard, U.S. Regional Soybean Laboratory, Urbana, Illinois, for providing the seed used in this study.

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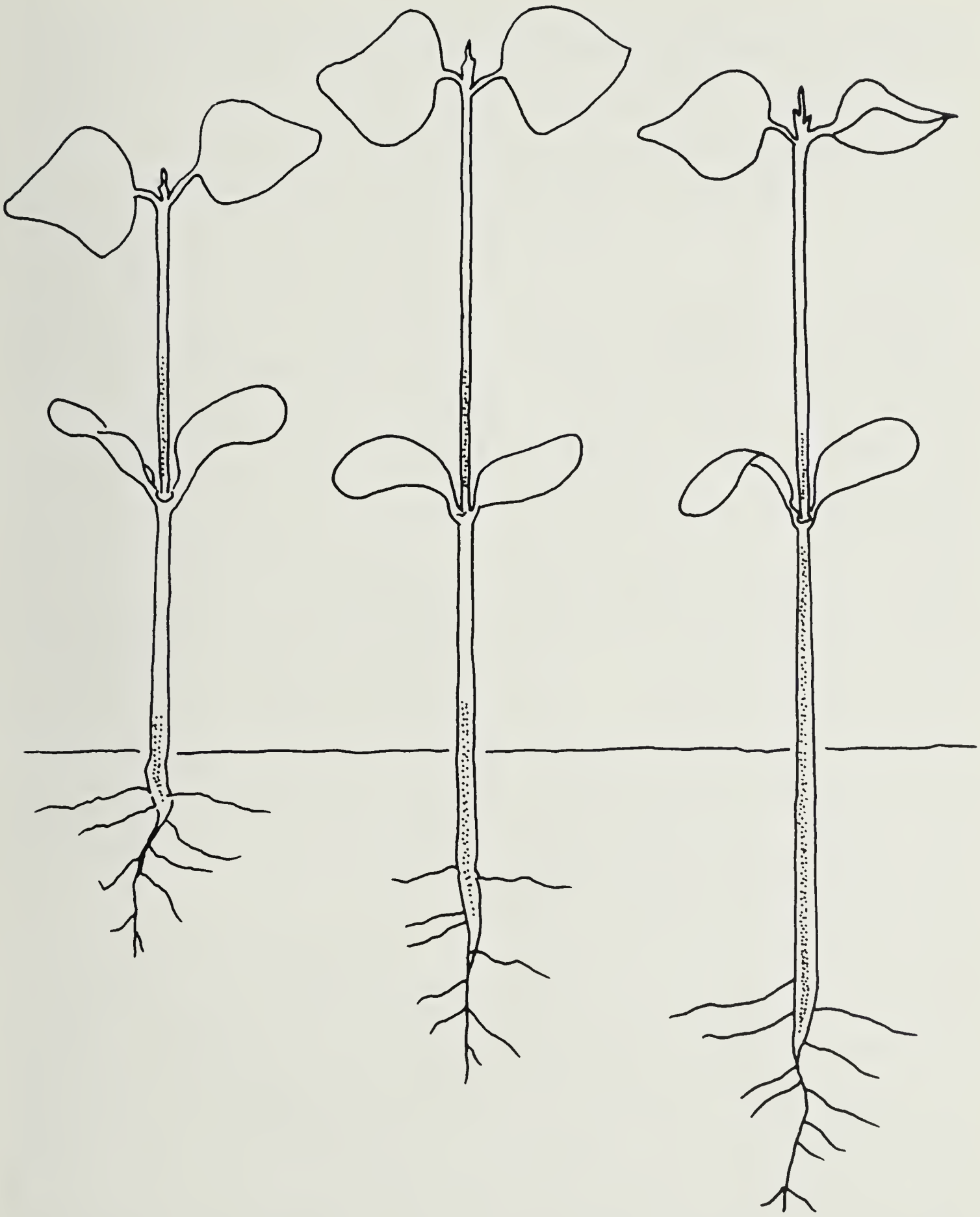

FIGURE 2. Effect of planting depth on the death of cells in pith tissue of soybean seedlings. From left to right, seedlings from sub-surface, 2.5 cm, and 5.0 cm depths, respectively. Dotted areas in the stem represent the areas (averages of six varieties) where dead cells occurred one week after planting (May 20; greenhouse conditions; equal parts sand and peat).

MICROBODIES OF SOYBEAN COTYLEDON MESOPHYLL

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ABSTRACT.—Microbodies were found in the upper mesophyll cells of soybean [*Glycine max* (L.) Merr., var. Wayne] cotyledon from the germinating stage (24 hr after planting) to cotyledon abscission (12 hr day, 12 hr night, 25°C, and 700 ft-c). These organelles were associated with endoplasmic reticulum, mitochondria, and chloroplasts. The shape of these organelles changed from oval, circular, or elongate in the earlier stage of seedling development to circular forms when cotyledons became yellow. The microbodies in senescent cells often lacked a continuous bounding membrane.

The occurrence, structure and the enzymatic function of plant microbodies is a subject of increasing interest to many investigators. Some of the characteristics of plant microbodies can be summarized as follows: they are bounded by a single membrane; they have a diameter from 0.5 to 1.5 μ; a dense granular stroma; and they are often associated with endoplasmic reticulum. Some are reported to contain crystals. These characteristics apply equally to two types of cell particulates isolated from homogenates. One of these, obtained from leaves, is involved in photo-respiration and is referred to as a peroxisome. The second, obtained from endosperm, is involved in the formation of succinate from fatty acids and is referred to as a glyoxysome. In cotyledons, although glyoxysomes appear early and are replaced by peroxisomes, the cytological events associated with these changes are unclear. Much of the literature describing plant microbodies and their associated enzymes has recently been reviewed (Beevers, 1969; Breidenbach, 1969; Gruber, *et al.*, 1970; Tolbert and Yamazaki, 1969; and Vigil, 1970).

No description of soybean microbodies has yet been published. In our recent study of cellular senescence in soybean cotyledons, we found organelles similar to published electronmicrographs and descriptions of glyoxysomes and peroxisomes. This paper presents some of our observations.

MATERIALS AND METHODS

Soybean [*Glycine max* (L.) Merr., var. Wayne] seeds were planted at a depth of 5 cm in sand and peat mixture (1:1 by volume) and grown in a growth chamber (25° C both for 12 hr of 700-ft-c of light and 12 hr of dark period). Sampling was at 24 hr intervals after planting for six days (at which time the hypocotyls were about 5 cm above the soil surface). On the sixth day, seedlings were standardized for uniform height and for uninjured cotyledons. All other seedlings were re-

moved. After the sixth day, sampling was obtained at three-day intervals until cotyledon abscission.

For each sample, tissue blocks of 1 x 1 x 4 mm were cut from the upper central area of the cotyledon. The blocks were fixed with 3% glutaraldehyde in 0.066 M phosphate buffer at pH 7.4 for 4 hours. After rinsing three times with the same buffer, the tissues were post-fixed in a 1:1 mixture of 2% osmium tetroxide and the above buffer for two hours. Both fixations were at room temperature. The tissues were dehydrated through an ethanol series, treated with propylene oxide and embedded in Epon 812 (Luft, 1961).

Sections were obtained with a diamond knife on a Reichert Om-U2 ultramicrotome, mounted on 200 mesh copper grids, stained in uranyl acetate (Watson, 1958), and counter-stained with lead citrate (Reynolds, 1963), and examined with a Hitachi HU-11A electron microscope at 50 KV.

Results

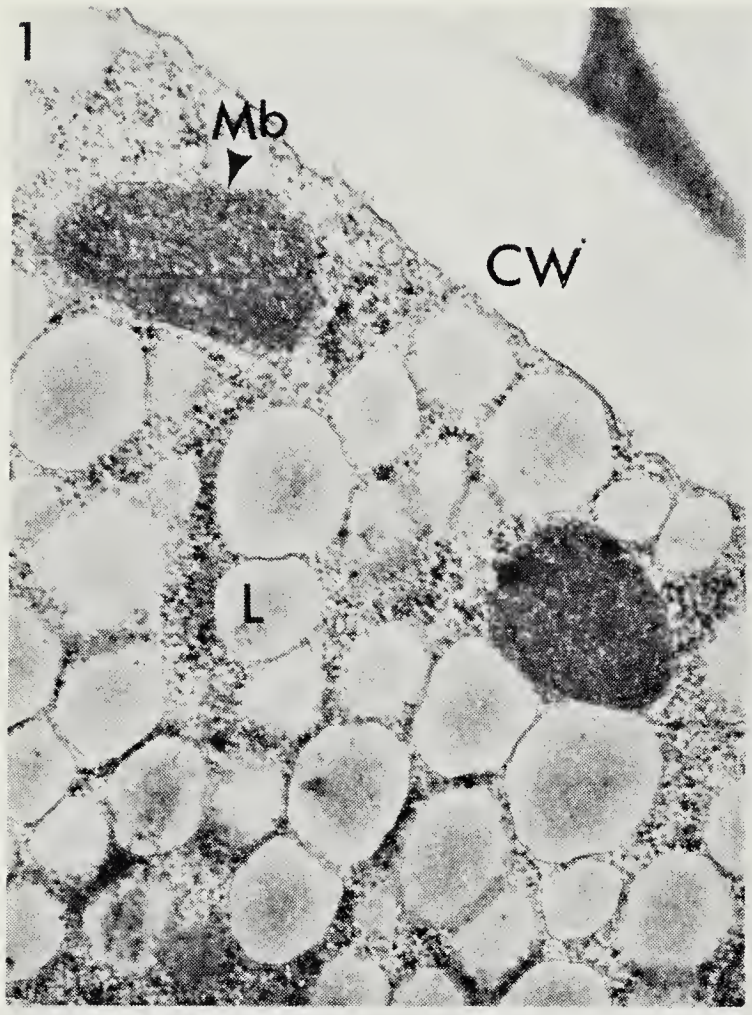
We did not find microbodies in cotyledons sampled 24 hrs after planting. Single membrane bounded electron dense, granulate organelles (Figs. 1-3) were observed in samples obtained at 48 hr after planting and in all other subsequent samples. Endoplasmic reticulum was associated with or in the vicinity of those organelles. The diameters of the dense organelles ranged from 0.5 to 1.3 μ, which is in the range of plant microbodies. We concluded that these were microbodies.

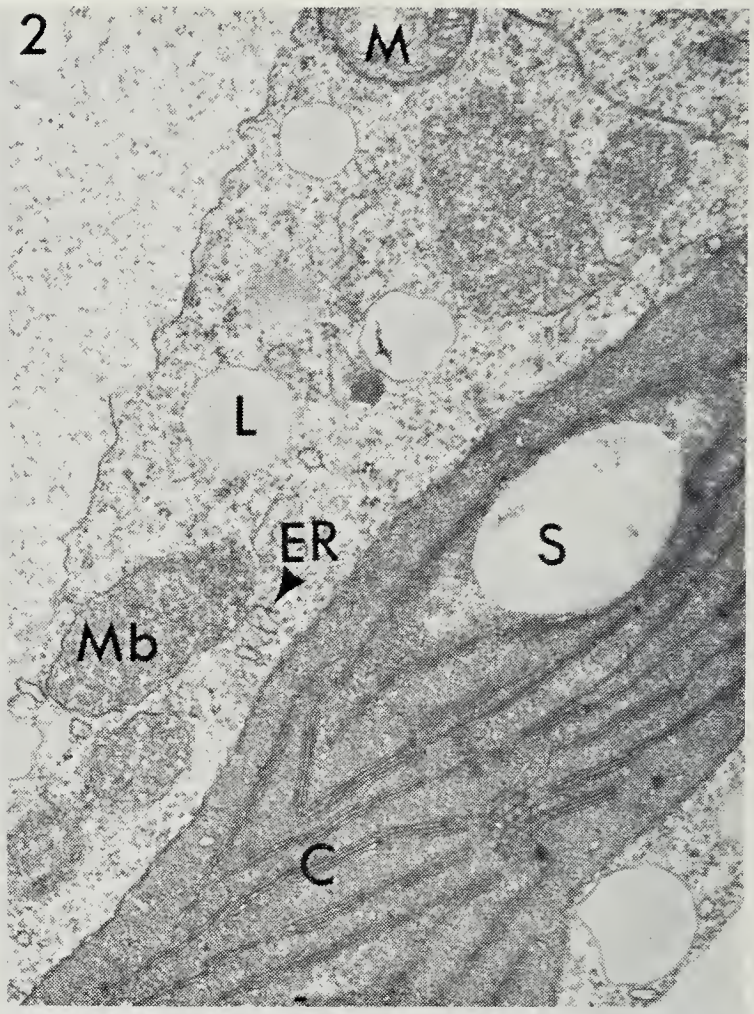
The shapes of the microbodies were spherical, elongated, irregular, or dumbbell (Figs. 2-6). Elongated microbodies had diameters of 0.3 μ at the narrowest region to 1.3 μ at the widest region. The stroma appeared as electron dense granules with transparent areas scattered within it. A single membrane was clearly identified as surrounding the microbodies of the earlier samples (Figs. 1-4). The bounding membrane of the microbodies from the sample of yellow cotyledon (21 days after planting) could not be seen clearly, but the endoplasmic reticulum associated with the organelles persisted (Figs. 5-6). The diameters of the microbodies in senescent cells were about 0.5 to 1.0 μ.

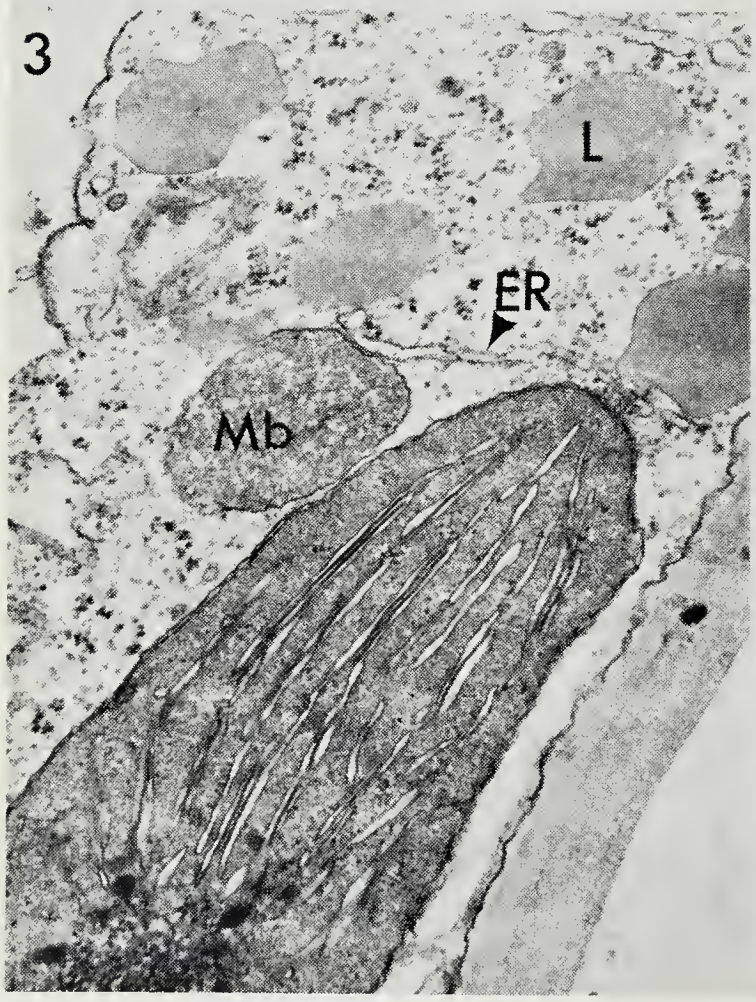
In addition to the close association between the microbodies and endoplasmic reticulum, in most of the cells the microbodies and chloroplasts were appressed (Figs. 3-4), changing the shape of the microbody at the place where the close spatial association occurred. Similar close associations between microbodies and mitochondria, and between mitochondria and chloroplasts were also observed.

Discussion

In fatty cotyledons, stored lipids are converted to carbohydrates in the early post-germinative stages. The microbodies observed in the soybean cotyledons at this stage were assumed to be glyoxysomes. As the cotyledons expand and emerge from the soil, they become photosynthetic organs. The microbodies observed in soybean cotyledons associated with the chloroplasts at this stage of growth are assumed to be peroxisomes. A time must exist when both







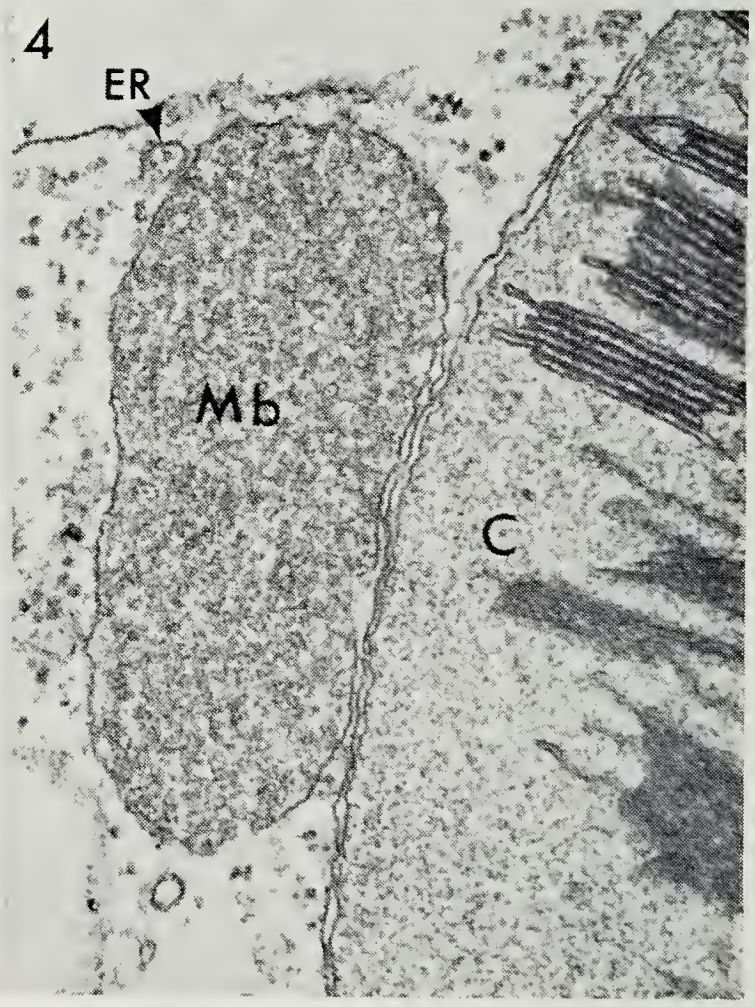


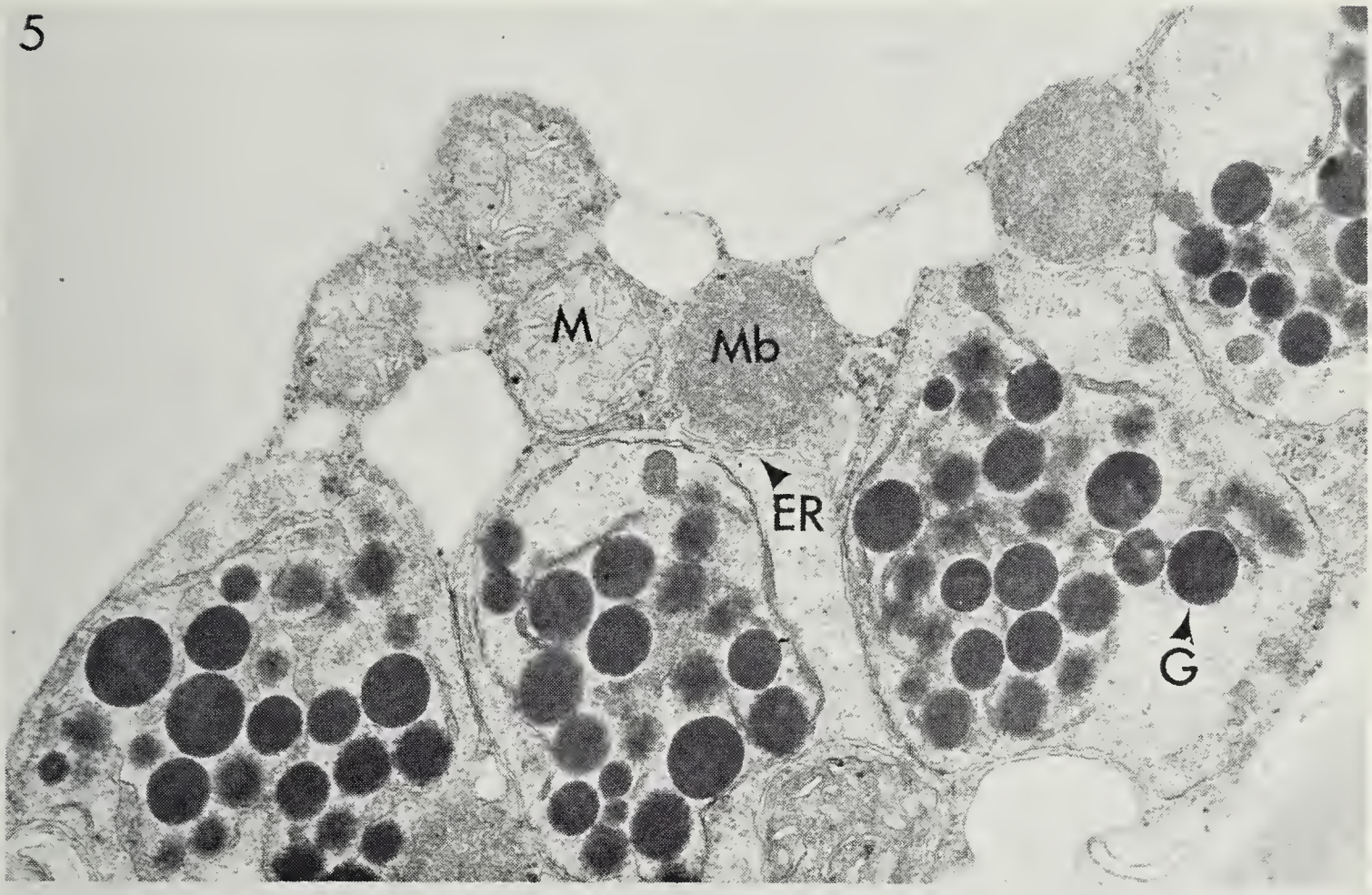
PLATE I

FIGURE 1. Two microbodies (Mb) among lipid bodies (L) in a cotyledon from three day old seedling; Cw, cell wall, x26,000.

FIGURE 2. Microbody (Mb) with endoplasmic reticulum (ER) in a cotyledon from four day old seedling. Mitochondrion (M), chloroplast (C), and starch grain (S) also can be identified, x26,000.

FIGURE 3. Microbody (Mb) associated with endoplasmic reticulum (ER) and developing chloroplast (C) in a cotyledon from five day old seedling, x33,000.

FIGURE 4. Microbody (Mb) with endoplasmic reticulum (ER), and chloroplast (C) in a cotyledon from a ten day old seedling, x52,000.



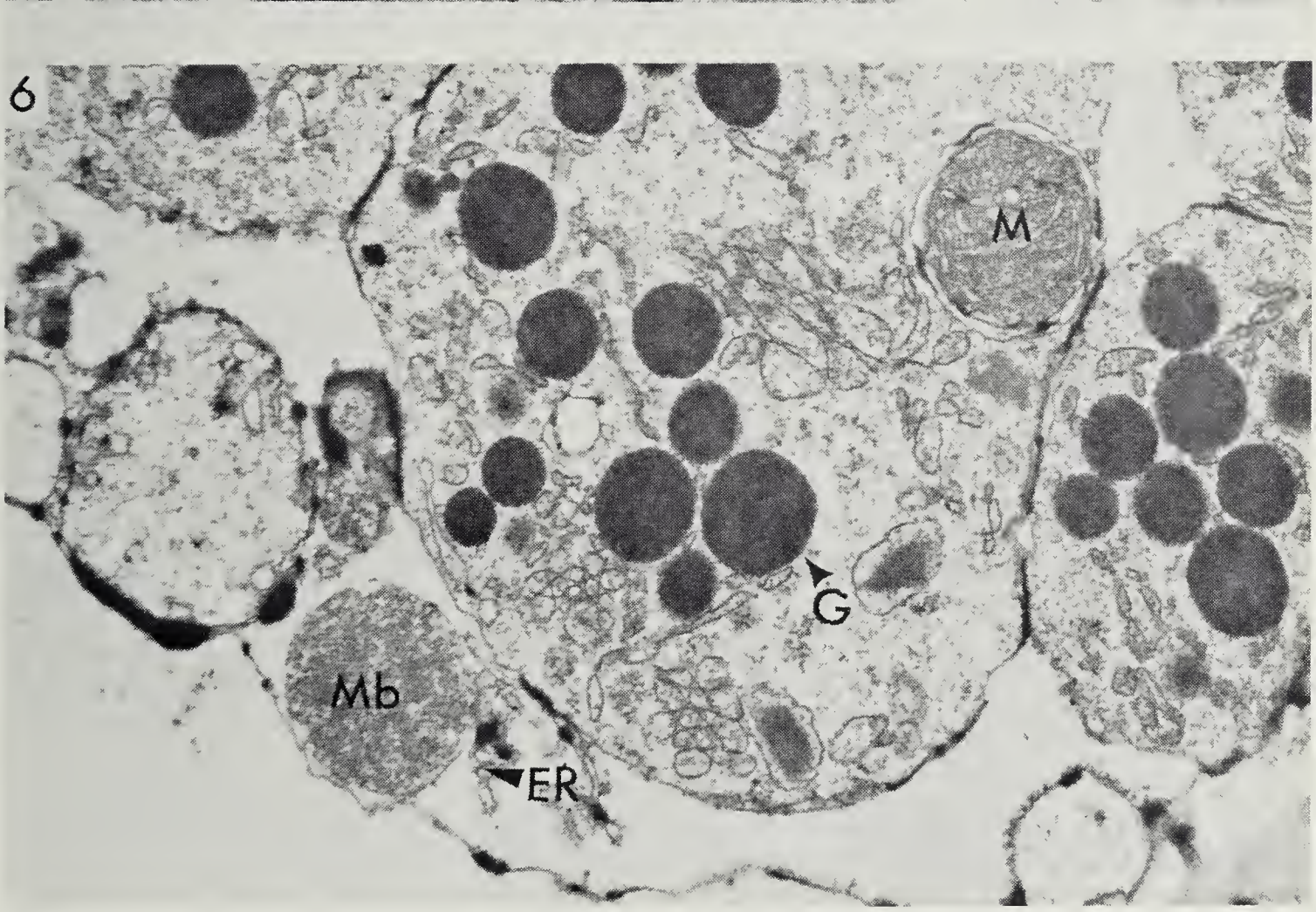


PLATE II

FIGURE 5. Microbodies (Mb) associate with endoplasmic reticulum (ER) from yellow cotyledon of 21 day old seedling. Degenerated mitochondrion (M) and degenerated chloroplasts with electron dense globules (G) also can be seen, x35,000.

FIGURE 6. As Figure 5, x44,000.

types of microbodies exist within the same cell of a cotyledon. Gruber *et al.* (1970) showed that microbodies are present at three distinct stages of cotyledon development of sunflower, cucumber, and tomato. The microbodies progress from catalase-containing particles located among lipid and protein bodies, to glyoxysomes closely associated with lipid bodies, to peroxisomes frequently appressed to chloroplasts. Although a transitional period occurred involving decline of glyoxysomes and a rapid rise of peroxisomes, the origin of the particles and their mode of destruction were not described. They did not determine whether any of the particle types were derived from preexisting microbodies or whether each arise as a separate population.

Vigil (1970) demonstrated that castor bean cotyledon microbodies showed membrane continuity with rough endoplasmic reticulum, suggesting a mode of formation similar to that in animal cells. As cotyledon development progresses, some microbodies disappear *in toto* by sequestration into autophagic vacuoles. The loss of enzymes did not appear to occur prior to digestion of the sequestered microbodies.

The rapid loss of lipids during germination can be seen in Figs. 1-4, the latter being sampled five days after planting. Starch was observed in developing proplastids at the stage of germination when seedlings were still underground. The glucose for this starch synthesis was believed to be derived from the conversion of lipids to hexose in a process involving glyoxysomes. The close association of microbodies and chloroplasts at a later stage is interpreted to imply that peroxisomal photorespiration occurs in soybean cotyledons.

The change in shape of plant microbodies from oval, elongate, and irregular in young tissue to almost perfectly circular (Figs. 5-6) in old tissue, and the inability to visualize a distinct bounding membrane, are the only noticeable changes during senescence. The chloroplast changes during aging (osmiophilic bodies, disruption and loss of grana stacks, and the appearance of circular fragments of lamellae) are similar to those reported by Barr and Arntzen (1969).

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THIN FILMS OF INTERPHASE CHROMATIN PREPARED FOR ELECTRON MICROSCOPY BY OSMOTIC SHOCK TECHNIQUE

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Abstract. — Interphase chromatin of chicken erythrocytes does not spread in a langmuir trough. By a new technique of osmotic disruption it spreads into a mass of knobby 70-625 Å wide fibers which aggregate to form dense chromocenters. The new method is simpler and faster than the conventional one. Enzymatic digestions indicate that DNA is necessary for the linear continuity of nucleoprotein fibers and histone seems to be necessary for fiber aggregation and the condensation of chromatin.

With the advent of the electron microscope, it was anticipated that the internal organization of hereditary material in higher organisms could be resolved. But except for the synaptinemal complexes in paired meiotic chromosomes (Moses, 1960), examination of thin sections has offered very little information. Thus, the recent discovery by Gall (1963) of a method for spreading nuclear content into monolayer films in a "Langmuir trough" represents a technical breakthrough. Gall's technique has been used successfully by several investigators to study the fine structure of interphase chromatin, and mitotic and meiotic chromosomes in quite a few different organisms (Ris and Chandler, 1963; Gall, 1966; Wolfe, 1965a, Wolfe, 1965b; Wolfe and John, 1965; DuPraw, 1965). However, nuclei of certain cell types do not spread readily in a Langmuir trough. For example, chicken nucleated erythrocytes and human sperm heads spread very little or not at all while those of amphibian erythrocytes and grasshopper sperms spread well (Fig. 1a, b). Apparently, the former possess a rather rigid membrane system. Removing the membranes of human or bull sperm heads by alkaline thioglycolate exposes their nuclei and gives adequate chromatin spreading (Lung, 1968).

Chicken erythrocyte nuclei can be spread by osmotic shock (Fig. 1b, c). This method is a modification of the protein monolayer technique devised recently by Freifelder and Kleinschmidt (1965) to spread isolated viral DNA.

Materials and Methods

One ml of rooster's venous blood was collected in a heparinized syringe and washed 3 times in 0.01 M Tris buffer containing 0.003 M $MgCl_2$, pH 7.5. Red blood cells were sedimented at 200 g for 5 min. They were then resuspended in hypotonic solution or distilled water and centrifuged at 2000 g for 10 min. This step was repeated 2 or 3 times until an upper layer of intact free nuclei was obtained.

The free nuclei were suspended to a final concentration of about 10^5/ml in a hyperphase solution of

1.0 M ammonium acetate, 0.5% neutralized formaldehyde and 0.01% cytochrome c (Nutritional Biochem. Corp.). A volume of 0.01 ml of well mixed hyperphase was poured down an inclined wet glass slide into a petri dish containing chilled hypophase solution of 0.3 M ammonium acetate and 0.5% neutralized formaldehyde. Excellent results in delivering such a small volume was obtained by using a 10 lambda Eppendorf micropipette (Brinkmann Instruments).

The surface film was picked up by touching it with a Formvar-carbon coated copper grid. Immediately after picking up the surface film, uranyl staining was achieved by dipping the grids in freshly pre-

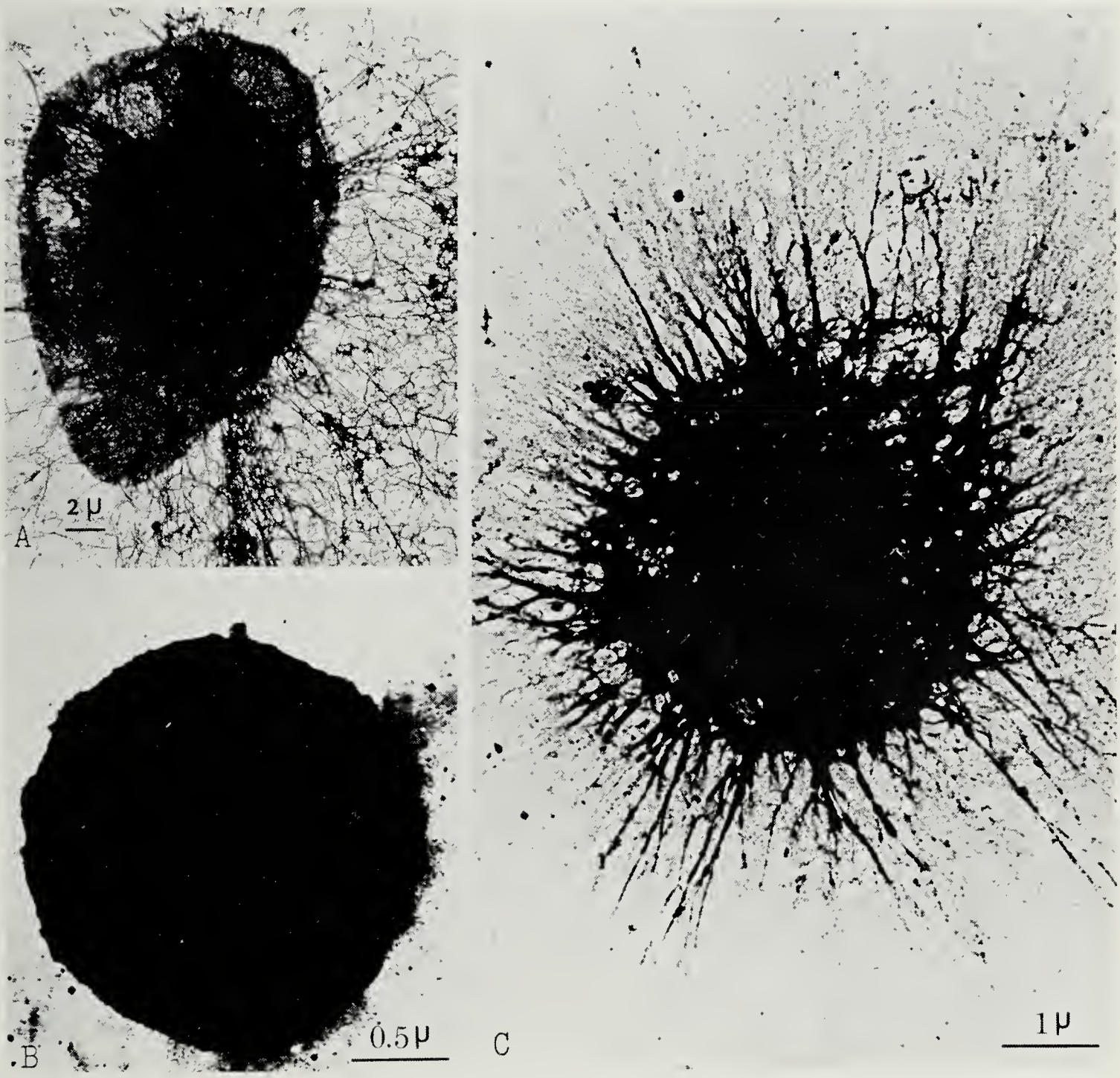

Figure 1. (a) A single erythrocyte nucleus of the Mexican salamander *Amby-stoma mexicanum* spread in a Langmuir trough into essentially a monolayer preparation. It shows the dense fibrillar components of interphase chromatin in these cells. X 2,090; (b) The erythrocyte nucleus of a chicken remained intact after being spread in a "Langmuir trough". X 2,010; (C)A single erythrocyte nucleus of chicken spread by osmotic shock technique into a mass of knobby chromatin fibers. X 10,400.

pared Wetmur's ethanol solution of the stain (Wetmur et al., 1966) for 30 sec. and then dehydrated for 15 sec. in chilled isopentane followed by air drying.

In DNase, RNase or trypsin treatments, digestion was carried on prior to staining. Trypsin (Sigma, 2X crystallized) and RNase (Nutritional Biochem. Corp. Crystalline) solutions were prepared as 1.0 mg/ml and 0.1 mg/ml of glass distilled water respectively. But DNase (Worthington deoxyribonuclease I, electrophor. purified) was prepared as 0.1 mg/ml of .003 M $MgCl_2$ and .01 M Tris buffer, pH 7.5. Stock solutions of the hyperphase, hypophase, Wetmur's stain, and Tris buffer were all millipore filtered at least once. The grids were examined in a JEM-T7 at 60 KV and an EMU-3 RCA at 50 KV.

Results

Interphase chromatin of chicken erythrocytes spreads into an interconnected mass of 70-625 Å wide nucleoprotein fibers. Except for the 70 Å fibers which seem to be the basic structural unit of chicken chromosomes, two parallel or relationally coiled sub-units were often observed within the wider fibers. At first glance the 625 Å fibers seemed to consist of two 300 Å units, the 300 Å fiber of two 150 Å units and the latter of two 70 Å units. However, fibers show definite polarity in their diameter tapering off from their base outward. They also exhibit a random pattern of branching into, or association between, fibers of different widths. Thus, observed differences in fiber diameter could not be explained by simple association in twos of equal size subunits. Chromatin fibers are spaced irregularly with chromomere like knobs and aggregate into a thick mass of chromatin similar to chromocenters.

Digestion with 0.1 mg/ml DNase for 15 min. disrupted drastically the linear continuity of chromatin fibers leaving a heterogeneous mixture of granules and ghost fibers (Fig. 2a). Aggregates of electron dense granules represent the remnants of condensed heterochromatin. Treatment with 0.1 mg/ml RNase for 15 min. showed no marked change in chromatin fiber diameter or continuity (Fig. 2b). Both knobby appearance and aggregation of fibers into a chromocenter were still evident. Digestion with 1.0 mg/ml trypsin for 30 min. resulted in dissociation of thick fibers into thinner ones and a reduction in their diameters to 40-100 Å with no loss in fiber continuity (Fig. 2c). Here, however, the fibers were smooth, largely free of knobs and the chromocenter appeared less dense.

Discussion

A number of observations demonstrate that these interphase chromatin fibers represent the DNA — histone complex of chromosomes, and trypsin digestion removes the histone component of this complex (Ris, 1966; Bernhard and Granboulan, 1963; Bastia and Swaminthan, 1967). Therefore, it is justifiable to define the electron dense granules and aggregates left after DNase treatment of chicken erythrocyte chromatin to be primarily histone residue. Furthermore, the dissociation of thick chromatin fibers

witnessed in trypsin treatment indicates that histones act as an adhesive promoting the aggregation of fibers and may play a definitive role in the condensation of heterochromatin.

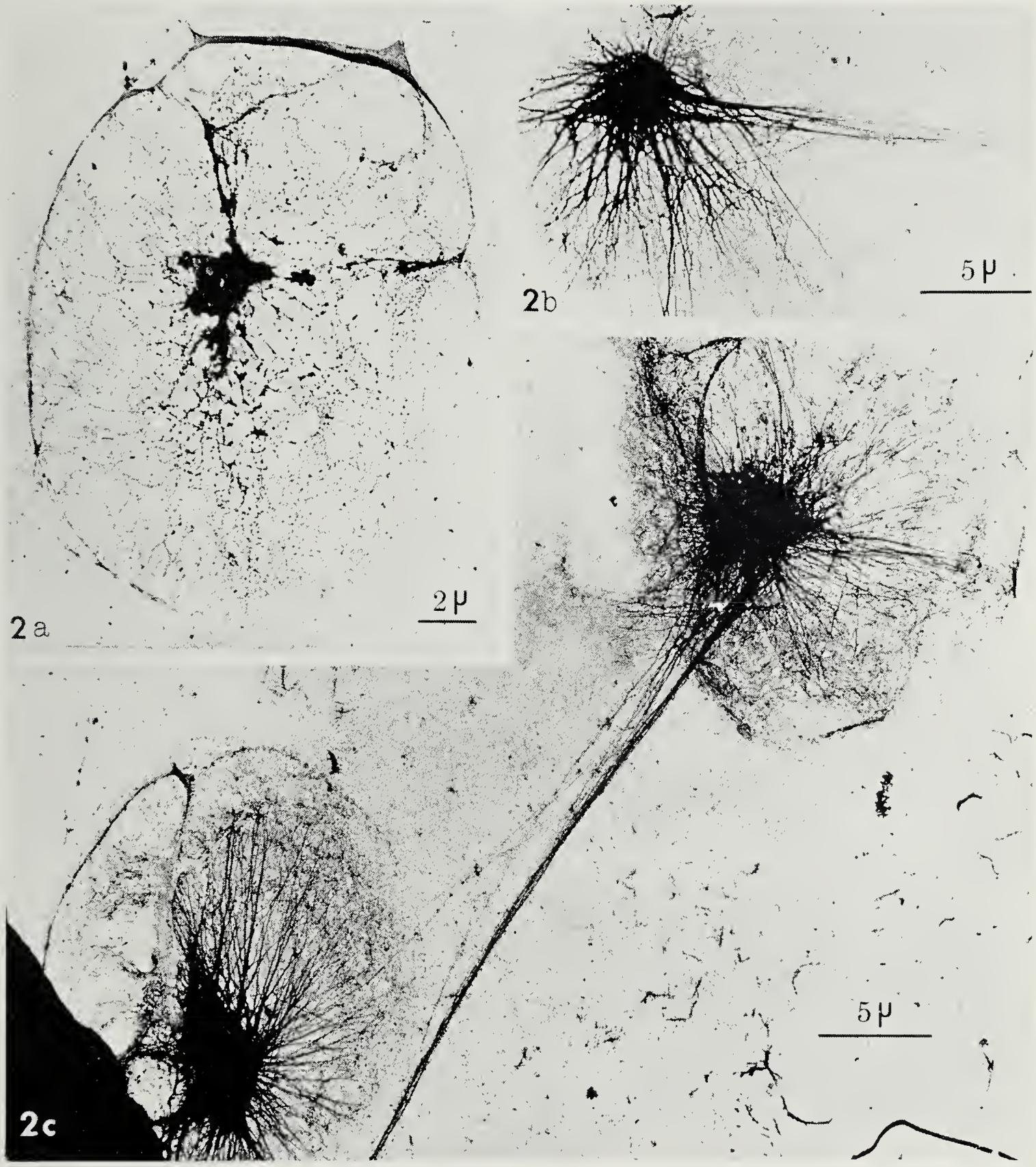

FIGURE 2. (a) A single chicken erythrocyte nucleus after DNase treatment showing ghost fibers and granules and their aggregation into one large central mass representing the remnant of interphase chromocenter. Ghost fibers extent to what is left of the nuclear membrane on the periphry. X 3,460; (b) A single erythrocyte nucleus of chicken treated with RNase. Knobby fibers remain intact with no change in diameter. X 2,590; (c) Two erythrocyte nuclei of chicken digested with trypsin. Fibers lose their knobby appearance and dissociate into a multitude of thinner fibers. X 2.670.

The above described osmotic shock technique used here to release chromatin of intact nuclei into monolayer protein films is much simpler and less time consuming than Gall's Langmuir trough method, especially in the spreading and dehydration steps. It gives reproducible results and should be generally applicable to nuclear fractions of various eukaryotes. Success has already been obtained with macronuclear fraction from the amicronucleate (GL) strain of the ciliate *Tetrahymena pyriformis* and the results are summarized elsewhere (Abdel-Hameed, 1969). Interestingly, both Gall's technique and the one described here are modifications of the water-spreading technique developed originally by Kleinschmidt and his coworkers (Kleinschmidt and Zahn, 1959; Kleinschmidt et al., 1962) to study the fine structure of bacterial and viral genophores.

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PATHOGENESIS OF *CLOSTRIDIUM BOTULINUM: IN VIVO* FATE OF *C. BOTULINUM* TYPE A SPORES

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ABSTRACT.—C. botulinum Type A spores contain sufficient toxin to produce fatal botulism in experimental animals. After 4 hours, i.p. injected spores undergo conversion from a heat-resistant spore to a heat-sensitive cell, probably a germinated spore or vegetative cell. This conversion appears to be prerequisite for liberation of spore bound toxin, since no botulinal toxin was noted before loss of heat-resistance. Within 0.5 minutes after i.p. injection, spores enter the bloodstream, liver, spleen and kidneys. The process of spore clearance and germination appear to be retarded by Type A antitoxin binding to spores rendering them non-phagocytizable. A significant number of viable heat-sensitive cells of *C. botulinum* are found in peritoneal exudates at later stages of survival in both antitoxin-protected and non-protected animals, indicating the capability of *in vivo* spore germination in the peritoneal cavity. No significant numbers of vegetative cells are found in liver, kidneys, or spleen of antitoxin protected mice. This implies that *C. botulinum* spores are trapped by the splanchnic mechanism, and may not be able to germinate in these organs.

Early works (Orr, 1922) on botulism pathology report *C. botulinum* spore dissemination into various tissues and production of toxin after oral challenge. Coleman and Meyer (1922) extended this work in demonstrating invasion of all organs of the body regardless of the mode of administration of spores. They were first to implicate toxin production *in vivo* from toxin-free spores.

Following intramuscular (i.m.) challenge, Keppie (1951) found clusters of heterophil leukocytes gathered at the site of injection which resulted in engulfment of spores and transportation away from the original site of injection. *In vivo* spore germination was rejected on the theorectical basis that spores could not germinate in the presence of oxygen. Keppie (1951) concluded that the toxin was released from spores by phagocytic digestion.

Delayed infections by latent spores of *C. botulinum* has been observed in clinical cases. Assuming no recurrent exposure to the toxin, these cases may have resulted from the ingestion of foods heated sufficiently to destroy toxin, but not heat-resistant spores. Therefore, ingestion of *C. botulinum* spores as an etiological agent in botulism food poisoning must be considered.

Two possibilities have been offered (Grecz and Lin, 1967) to explain spore toxicity: 1. *in vivo* spore germination and subsequent release of toxin from vegetative cells, and 2. degradation of the spore releasing its bound toxin.

The purpose of this paper is to demonstrate that *C. botulinum* spore do, in fact, germinate *in vivo,* and to define *in vivo* loci of mice which are conducive to this germination.

Materials and Methods

Culture methods: Clostridium botulinum Type A strain 33A was obtained from Dr. W. E. Perkins, National Canners Association, Berkeley, California. The culture was grown at 30 C in 5% Trypticase (BBL), 0.5% peptone (Difco) and 0.1% sodium thioglycolate. Within 6 days, abundant sporulation had occurred, at which time, the spores were harvested in a refrigerated Sorvall RC-2 continuous centrifugation system and cleaned with trypsin and lysozyme by method of Grecz, *et al.* (1962). The spores were washed twice, resuspended in 0.87% NaCl and stored at 4 C until used.

Heat-shocking procedure: The spore inoculum was heated in a screw-cap tube for 15 minutes at 80 C denaturing any free botulinal toxin in the medium. *C. botulinum* spores are able to survive this treatment.

Colony counts: The number of viable organisms of *C. botulinum* were determined by subculture in Wynne's broth (Wynne, *et al.*, 1955) plus 0.75% agar. One ml portions of serial dilutions were transferred to oval flat tubes and melted sterile Wynne's agar was added. To achieve anaerobiosis, an additional layer (2 cm.) of sterile Wynne's agar was poured. The tubes were plugged with foam rubber stoppers and incubated at 30 C. Colonies were counted after 96 hours.

Mice: White Swiss mice raised for 10 generations in a closed colony at the Illinois Institute of Technology were utilized in all experiments. They were fed and watered *ad libitum,* and attained a weight of 25 grams before experimentation.

Injections: Intraperitoneal (i.p.) injections into mice were made with 26 gauge, 2.5 ml disposable syringes.

Preparation of Exudates for Viability Analysis: Mice were injected i.p. with 2 x 10^8 spores. At selected time intervals, the peritoneal cavity was washed with 1 ml of sterile 0.87% saline, and exudates withdraw using a 26 gauge syringe. The peritoneal exudate was diluted 1:10 with sterile distilled water. Serial dilutions in distilled water for viable cell count in Wynne's agar were performed as described above.

Preparation of Tissues for Viability Analysis: Following removal of peritoneal exudates from mice, the animals were etherized and the peritoneal cavity surgically opened. The body cavity was rinsed with sterile saline, and the liver, kidneys and spleen removed.

The organs were placed in sterile plastic centrifuge tubes and washed with sterile saline by agitation on a vortex mixer. Washing was continued until the saline supernates became clear. Each organ, regardless of volume, was placed in 20 ml of sterile saline in a second sterile plastic centrifuge tube, and sonicated (Branson Sonifier, Model S-125, 8 amps) for 2-4 minutes to rupture the cells and release all spores.

The tubes were centrifuged (300 x g, 1 hour), and the supernatant discarded. Appropriate decimal dilutions of the pellet were analyzed for the number of viable spores in Wynne's agar.

Examination of Blood for Viable Spores and Vegetative Cells: Blood was removed from the mouse by two methods: (i) the animal was etherized and the abdominal and pleural

cavities were surgically opened using sterile techniques. A 26 gauge, 2.5 ml syringe was inserted into the heart and the blood was withdrawn; (ii) a direct cardiac puncture was made into a mouse slightly stunned by ether. Decimal dilutions of the blood were made and examined for the number of viable organisms in Wynne's agar.

Results

Peritoneal Cavity: After i.p. injection of 2 x 10^8 heat-shocked spores of *C. botulinum* Type A, only 6.6 x 10^5 heat-resistant spores could be recovered from the peritoneal cavity at the start of the experiment (Table 1). This apparent 300 fold reduction in number of spores was due to: (i) dilution of spores by body fluids; or (ii) rapid distribution of the spores throughout the animal body.

The first line of Table 1 shows that the intraperitoneal cavity of control mice receiving no spore inoculum contained some spores (6.0 x 10^2) as well as some heat-sensitive cells (4.4 x 10^3). This relatively low level of contamination was considered as "background" flora. Examination of Table 1 showed that the number of spores recovered from the animal body after initial i.p. injection gradually but consistently declined so that by 48 hours the number of spores recovered was approximately 100 fold lower than the initial level. Furthermore, at 48 hours, the number of spores recovered was only slightly higher than "background" spore load in control mice.

Incubation beyond 3 hours rapidly increased the number of heat-

Table 1.—Number of viable spores recovered from the peritoneal cavity of mice after intraperitoneal injection of 2 x 10^8 heat-shocked spores of *Clostridium botulinum* 33A.

Time After Intraperitoneal Injection Hours	Number of Animals Challenged	Number of Viable Organisms Recovered	
		Heat-Shocked (Spores)	Heat Sensitive Cells (Total Minus Spores)
Control[a]	3	6.0 x 10^2	4.4 x 10^3
0	3	6.6 x 10^5	4 x 10^3
1	2	5.0 x 10^5	4 x 10^4
2	3	1.0 x 10^5	1.0 x 10^3
3	3	2.0 x 10^5	5 x 10^4
4	3	1.0 x 10^5	2 x 10^5
8	3	2.0 x 10^4	1.5 x 10^5
24	3	2.0 x 10^4	1.8 x 10^5
		4.0 x 10^4	5.0 x 10^4
36	1	2.0 x 10^4	4.3 x 10^5
	1	1.0 x 10^4	1.0 x 10^4
	1	5.0 x 10^2	2.5 x 10^3
	1	1.0 x 10^3	3.0 x 10^3
48[b]	2	7.0 x 10^3	5.0 x 10^5
	1	1.0 x 10^3	2.0 x 10^5
	1	3.0 x 10^3	1.0 x 10^5
	1	2.0 x 10^3	5.0 x 10^3

[a]/Control mice received 1 ml sterile saline but no spores.
[b]/Recovered from surviving animals; only 10% of the injected animals survived 48 hrs.

sensitive cells and reached a plateau of approximately 2 x 10^5 cells which seemed to be relatively stable between 8 to 48 hours (the upper limit of this experiment). Within the precision of these experiments, the number of heat-sensitive (germinated) cells was essentially equivalent to the initial number of spores which could be recovered from the peritoneal cavity of the mouse during the first 8 hours. Thus, these results suggest that after injection into the mouse, heat-resistant spores were essentially all converted into heat-sensitive (probably germinated) forms within 4-8 hours. The heat sensitive forms appeared not to be destroyed to a detectable degree in the mouse body for up to 48 hours.

The cells recovered from the peritoneal cavity of injected mice were shown to produce specific Type A toxin as determined by mouse toxicity tests of subculture. Control mice in which the animals were challenged with 1 ml of sterile saline without spores contained low levels of viable cells. The organisms recovered from the controls were nontoxic.

It is important to note that mice

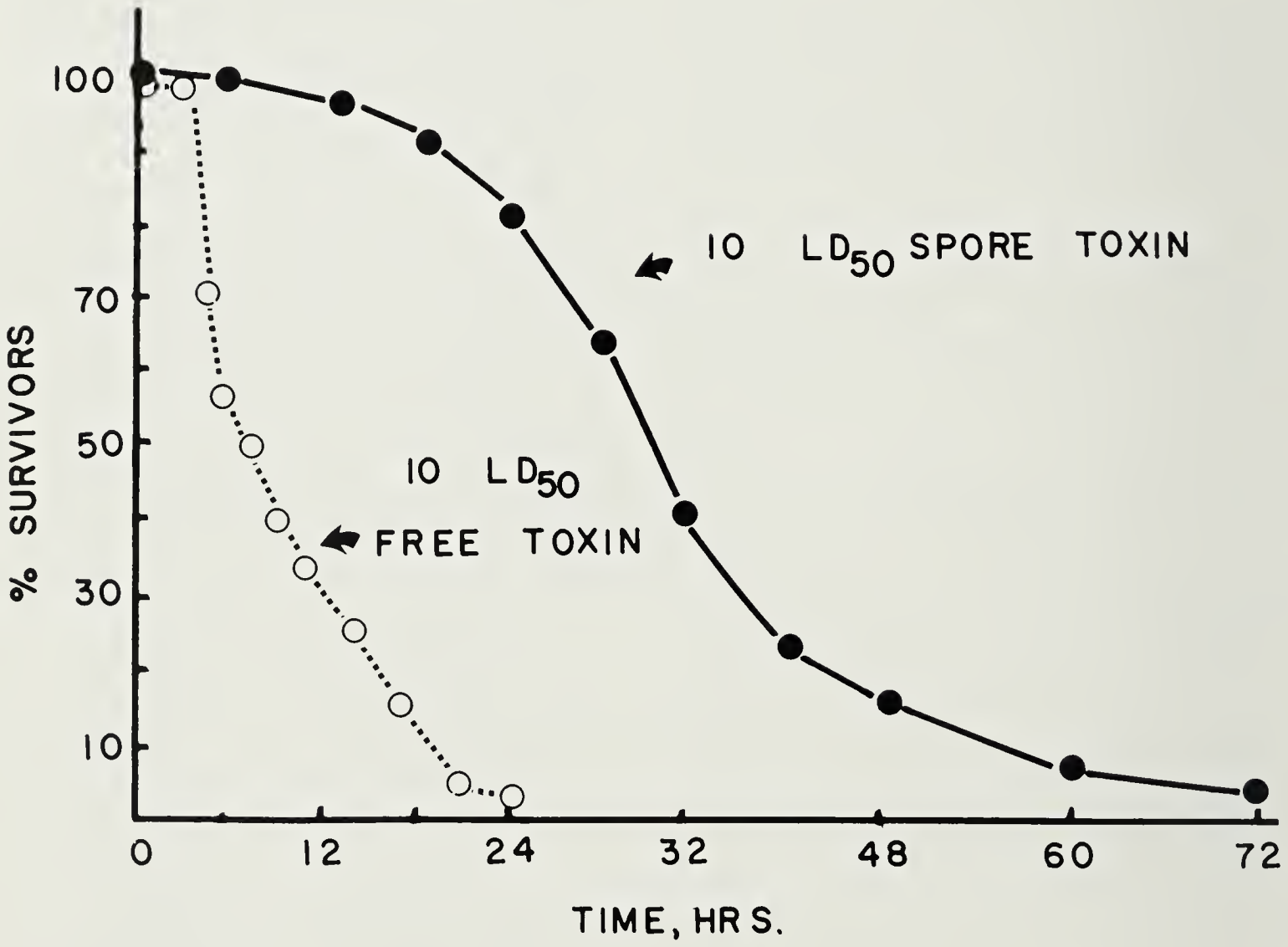

FIGURE 1. Rate of death of mice injected with spores of *C. botulinum* as compared with mice injected with free type A botulinum toxin.

●—● Mice injected i.p. with 2 x 10^8 heat-shocked spores containing 10 LD_{50} of type A toxin.

○·····○ Mice injected i.p. with a dilution of pure type A botulinum toxin.

injected with 2 x 10^8 spores (containing the equivalent of 10 LD_{50} botulinal toxin by mice assay) did not show any typical symptoms of botulism for the first 8 hours. Fifty-percent of the animals died 32 hours after injection (Figure 1). Normally, mice injected with 10 LD_{50} of botulinum toxin expire within 8-18 hours, with 50% expiring within 10 hours. Presumably, the increased expiration time with spores is needed for *in vivo* spore germination. Millipore Filtrate (0.22 μ) of the spore suspension were not lethal to any mice injected.

These observations suggest that germination of *C. botulinum* spores occurs previous to toxin release *in vivo*.

Spores in the Blood: Table 2 demonstrates that heat-resistant spores penetrate into the blood stream within 0.5 minutes after i.p. injection. It can be seen that the cells remain heat-resistant for at least 70 minutes, a fact which is in accordance with lack of spore germination or loss of heat-resistance during the first 3 hours as pointed out in Table 1. The number of spores entering the blood appears to be somewhat lower than the number of spores recovered from peritoneal exudate during comparable time intervals. However, the data definitely establish the fact that spores penetrate extremely rapidly into the bloodstream. Background flora bacteria, upon penetration into the bloodstream are effectively destroyed by phagocytes in the bloodstream.

TABLE 2.—Number of Viable Spores in the Blood of Mice Injected Intraperitoneally with 2 x 10^8 Spores.

Time of Withdrawal (min.)	Method of Withdrawal*	Number of Heat-Shocked Spores (per ml.)	Number of Non-Heat-Shocked Spores (per ml.)
0	A	0	0
.5	A	1 x 10^4	1 x 10^4
15	B	3 x 10^5	5 x 10^4
20	A	6 x 10^3	6 x 10^3
	A	3 x 10^2	3 x 10^4
30	B	7 x 10^4	1 x 10^3
	B	2.5 x 10^4	5 x 10^3
40	A	1 x 10^3	1 x 10^3
	A	2 x 10^3	2 x 10^3
60	A	2.1 x 10^3	2 x 10^3
70	A	7 x 10^2	5 x 10^2

* Method A—direct cardiac puncture while animal etherized.
Method B—animal sacrificed, body opened, cardiac puncture.

Spores in Liver, Kidney and Spleen: Table 3 shows that spores of *C. botulinum* 33A injected i.p. are rapidly disseminated to the liver, kidneys and spleen between 0.5 and 4 hours. The spores persist in the liver and kidneys at a relatively constant level up to 36 hours at which time a rapid decrease in the number of viable organisms occurred. In the spleen, spore clearance initiated at 8 hours and continued for 24-48 hours. The number of heat-sensitive (germinated) spores or vegetative cells was usually approximately 5 fold higher than the number of heat-resistant spores.

As with the peritoneal exudate, control colonies from organ preparations were not toxic to mice. The Colonies in 10 experimental plates proved to be toxic as determined by mice i.p. assay (antitoxin-protected mice survived). Moreover, organs from healthy animals, ground and injected into mice, did not cause ill effects. These controls substantiate the presence of *C. botulinum* spores in the organs.

RES Clearance of Spores in Mice Protected by Antitoxin: In order to obviate the effect of spore toxin, a series of samples similar to those in the preceding experiment were analyzed, with the exception that 0.1 ml of antitoxin sufficient to protect the animal from death was administered with each spore inoculum. In this way, it was possible to evaluate the survival of spores *in vivo* for extended periods of time.

In the peritoneal exudate of passively immunized mice, appearance of a heat-sensitive element could not

TABLE 3.—Number of viable cells recovered from organs of mice after intraperitoneal injection of 2 x 10^8 heat-shocked spores of *Clostridium botulinum* 33A. (Mice received no antitoxin).

Time After Intraperitoneal Injection (hrs.)	Viable Cells Recovered From Organs[a]					
	Livers		Kidneys		Spleens	
	Spores	Heat Sensitive Cells	Spores	Heat Sensitive Cells	Spores	Heat Sensitive Cells
0 (controls)	1×10^2	2×10^4	3×10^1	1.7×10^2	3×10^1	1.7×10^2
1/2	2×10^3	8×10^4	1×10^3	1×10^4	3×10^3	2.7×10^4
1	1×10^4	1×10^5	3×10^4	2×10^3	8×10^3	7.2×10^4
2	5×10^4	3×10^4	2×10^3	3×10^4	3×10^4	1.7×10^5
3	7×10^4	1×10^5	2×10^3	2.8×10^4	2×10^4	1.8×10^5
4	7×10^4	2×10^5	2×10^3	2.8×10^4	7×10^4	1.3×10^5
8	5×10^4	1.6×10^5	1×10^4	2×10^4	1×10^5	1×10^5
18	7×10^4	2.3×10^5	.....	.....	3×10^4	7×10^4
24	1×10^5	3×10^5	3×10^4	7×10^4	3×10^3	2.7×10^4
36	1×10^5	2×10^5	3×10^4	2×10^4	1×10^3	8×10^3
48	2×10^3	5×10^4	1×10^3	2×10^3	2×10^3	1.5×10^3

[a] Control animals received 1 ml sterile saline without spores Averages of 4 animals.

be observed as in mice not passively immunized, (Figure 2). The number of spores decreased slightly during the first 5 days, but dropped approximately 100 fold between the fifth and seventh day. Between one

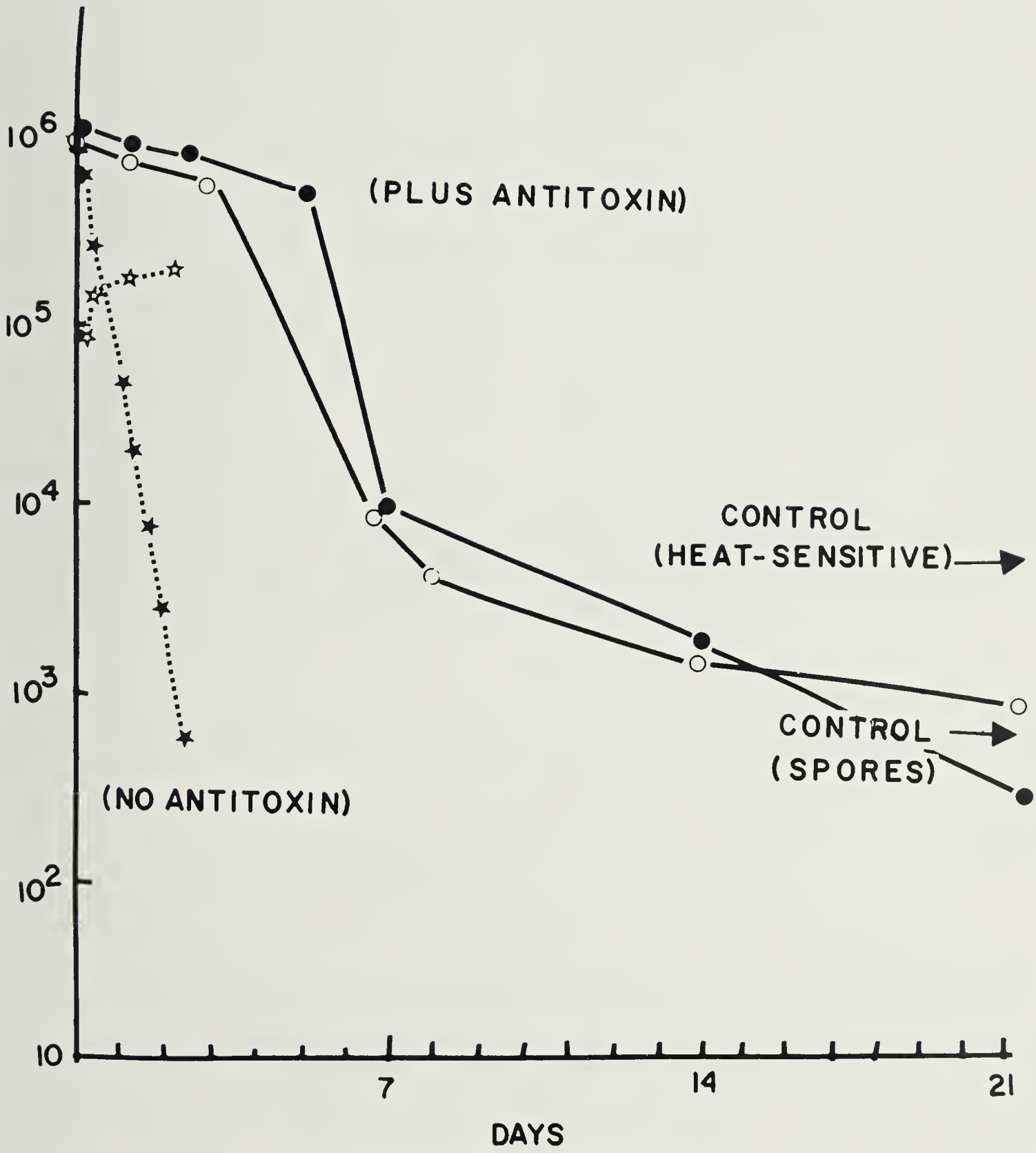

FIGURE 2. Number of Viable Spores in the Peritoneal Cavity of Mice Injected with 2×10^8 Heat-shocked Spores of *C. botulinum 33A*.

●—● Spores: 1 ml of cells surviving 80C for 10 min. in type A antitoxin protected mice.

○—○ Heat-sensitive cells: Cells killed by 80C for 10 min. in type A antitoxin protected mice.

★·····★ Spores: 1 ml of cells surviving 80C for 10 min. without antitoxin.

☆···☆ Heat-sensitive cells: Cells killed by 80C for 10 min. without antitoxin.

week and 3 weeks the numbers of spores continued to decrease further. In the presence of antitoxin, heat-sensitive *C. botulinum* cells did not appear in numbers greater than normal background contamination, i.e. approximately 4 x 10^3, indicating that destruction of spores *in vivo* after germination must have been extremely rapid.

In the liver, spleen and kidneys, no situation was found which could be attributed to the presence of vegetative cells in presence of antitoxin, since heat-resistant and heat-sensitive counts were always essentially equal (Table 4). There was gradual disappearance of spores from all these organs during the three weeks. Controls injected with antitoxin alone, contained 50 to 1000 spores, and 1000 to 10,000 heat-sensitive cells, all of which did not produce toxin in subculture.

Discussion

The significant finding emerging from these studies is the fact that spores injected i.p. — with or without antitoxin — are disseminated within seconds or minutes into the blood, liver, spleen and kidneys of the experimental animal. Subsequent clearance of spores from the RES is very gradual.

The mechanism of intoxication of the animals from spore toxin appears to depend on conversion of the heat-resistant spores to heat-sensitive forms; perhaps germinated spores or vegetative cells. The heat-sensitive cells appear at 4 hours and may persist for over 48 hours after i.p. injec-

Table 4.—Number of viable cells recovered from organs of antitoxin protected mice after intraperitoneal injection of 2 x 10^8 heat-shock spores of *Clostridium botulinum* 33A.

Time After Intraperitoneal Injection	Viable Cells Recovered From Organs					
	Livers		Kidneys		Spleens	
	Spores[a]	Total	Spores	Total	Spores	Total
0	1 x 10^2	2 x 10^3	3 x 10^1	3 x 10^2	3 x 10^1	2 x 10^2
20 hours	2 x 10^6	1 x 10^6	7 x 10^4	5 x 10^4	6 x 10^5	5 x 10^5
42 hours	2 x 10^6	2 x 10^6	7 x 10^5	2 x 10^5	1 x 10^6	6 x 10^5
48 hours	1 x 10^6	3 x 10^6	2 x 10^4	1 x 10^4	4 x 10^5	2 x 10^5
67 hours	9 x 10^5	5 x 10^5	7 x 10^4	3 x 10^4	3 x 10^5	1 x 10^5
120 hours	1 x 10^6	7 x 10^5	2 x 10^4	3 x 10^4	2 x 10^5	1 x 10^5
1 week	9 x 10^5	1 x 10^6	1 x 10^5	1 x 10^5	2 x 10^5	1 x 10^5
2 weeks	1 x 10^5	1 x 10^5	1 x 10^4	9 x 10^3	2 x 10^4	2 x 10^4
3 weeks	2 x 10^4	2 x 10^4	1 x 10^4	1 x 10^4	5 x 10^3	5 x 10^3
	5 x 10^5	5 x 10^5	1 x 10^4	2 x 10^4	5 x 10^4	5 x 10^4

[a]/Spore = cells surviving heating at 80 C for 10 minutes.
No germination, no appearance of heat-sensitive forms.
Control mice inoculated with 0.1 ml antitoxin but no spores contained 50 to 1000 spores and 1000 to 10,000 heat-sensitive cells.

tion. Symptoms of botulism intoxication are observed after time intervals necessary for loss of heat-resistance by injected spores.

The presence of botulinal antitoxin A appears to depress normal spore clearance levels *in vivo* by a factor of 5-10x. By a technique developed by this laboratory (Suzuki, *et al.*, 1971a), botulinal toxin was found not to affect phagocytic clearance (Suzuki, *et al.*, 1971b) which is in agreement with other investigators (Freeman, 1961). We speculate that delayed disappearance of spores *in vivo* when antitoxin is administered may be due to antitoxin binding to spores rendering them dormant. This seems plausible as a host defense mechanism against botulism pathogenesis since Suzuki, *et al.* (1970, 1971c) reported the PMN leukocyte as requisite for *C. botulinum* spore germination and toxin release. Staining techniques of phagocytized *C. botulinum* spores have verified this relationship (Booth, *et al* 1971).

Acknowledgement

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THREE IRON SULFATE MINERALS FROM COAL MINE REFUSE DUMPS IN PERRY COUNTY, ILLINOIS

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ABSTRACT.—Three iron sulfate minerals have been found on coal mine refuse dumps in Perry County, Illinois. Szomolnokite ($FeSO_4 \cdot H_2O$) usually occurs on oxidizing pyrite on the dump surface, rozenite ($FeSO_4 \cdot 4H_2O$) is found beneath the surface and along some watercourses, and melanterite ($FeSO_4 \cdot 7H_2O$) appears to be restricted to moist areas. The occurrences indicate the hydration state of the ferrous sulfate is at least partly dependent upon relative humidity of the microenvironment.

Pyrite is a common accessory mineral in the coal deposits of southern Illinois and elsewhere. During the benefication of the coal, pyrite, as well as clay, shale, and other impurities, is discarded and piled up in refuse dumps. Oxidation of pyrite in the upper portion of these dumps has resulted in a variety of products, three of which are described in this paper. This study was conducted on dumps located in Perry County, Illinois, but conditions similar to those found in this area are so common throughout coal mining regions of midcontinent United States and elsewhere that the minerals described herein are probably of widespread occurrence.

METHODS

The samples used in this study were collected from two coal mine refuse dumps located two miles southwest of DuQuoin and five miles south of Pinckneyville, Perry County, Illinois. Collection of samples was accomplished during the months of September and October, 1969, in fairly dry conditions. Approximately 100 specimens were collected from dry runoff ditches, along flowing streams and seeps, and from fresh bulldozer cuts in the refuse dumps. Samples were grouped in the laboratory on the basis of observable properties. They were then hand cleaned under a microscope to remove impurities in order to insure relatively pure specimens for X-ray identification. The purified minerals were finely ground and identified by X-ray powder diffraction techniques.

RESULTS

X-ray diffraction study reveals the occurence of three hydrated iron sulfate minerals: szomolnokite ($FeO_4 \cdot H_2O$); rozenite ($FeSO_4 \cdot 4H_2O$); and melanterite ($FeSO_4 \cdot 7H_2O$). About 15% of the fifty samples identified by X-ray diffraction were melanterite, 30% rozenite, and 55% szomolnokite (percentages do not necessarily reflect relative abundance on the refuse dumps, but rather proportion of X-ray test samples; however, the abundance of the various minerals in the test runs are approximately proportional to their abundance

in the total samples collected.) These three minerals have sufficiently different physical properties so that they can generally be identified in the field without the aid of sophisticated equipment. A brief description of each mineral follows.

Szomolnokite. Szomolnokite occurs as a white powdery coating on pyrite and occasionally over the entire surface of an area where oxidizing pyrite is abundant. Thus far we have found it only on material exposed directly at the surface.

It is distinguished by its occurrence as a white powder on pyrite, its hardness of 2.5 (determined only with considerable difficulty), and its slow rate of dissolution in water. It is reported that a brown residue is left when the mineral is dissolved in water (Palache, et. al., 1951), but we have not found this to be an important characteristic. It does not have a cleavage. The specific gravity is about 3.05 (Winchell and Winchell, 1964), but its mode of occurrence generally makes an estimation of specific gravity impossible.

Rozenite. Rozenite is rather common is Perry County. The best occurrences are a few inches beneath the surface of the spoil piles, where considerable masses of granular crystals can occasionally be found. It also occurs along the sides of some small streams that drain the spoil piles, but in such locations it usually turns to a crumbly white material if the water dries up.

Rozenate has a hardness of 2.5, which is the same as szomolnokite but clearly harder than melanterite. The specific gravity is about 2.3. Winchell and Winchell (1964) report artificial rozenite has one direction of good cleavage, but the well developed conchoidal fracture of the Perry County material often makes the cleavage difficult to discern. Most samples used in this study were blue to blue-green, but occasionally colorless or white material was found. Unaltered specimens are transparent to translucent and have a vitreous luster; partially dehydrated specimens are translucent to opaque and have a dull luster. The mineral is readily soluble in water and gives a metallic, astringent taste.

Melanterite. This mineral is generally found as a crust on rocks and mud along the sides of flowing drainage ditches, usually within a few inches of water. It occasionally is found as small clumps of white radiating crystals.

Melanterite is transparent to translucent and has a vitreous luster. The color ranges from colorless to green or blue-green. Both its hardness (2.0) and its specific gravity (1.90) are considerably less than those of rozenite, with which it can easily be confused. Like rozenite, it dissolves easily in water and has a metallic, astringent taste. Its three directions of cleavage are not easily seen in most specimens. The most useful field test to distinguish melanterite from rozenite is the hardness.

Discussion

Collection precautions. In collecting samples of these minerals for research or mineral collections, care must be taken to preserve them in their natural hydration states. It is suggested that they be placed in airtight containers and tightly sealed

immediately upon collection, because only a few hours exposure to dry air will cause drastic changes in appearances. Rozenite appears to be particularly susceptible to alteration, for it will change from a glassy, transparent blue or blue-green material to a dull, opaque, white, crumbly material overnight if exposed to dry air. On the other hand, we have successfully kept one sample for over a year with no sign of alteration by storing it in a tightly sealed polyethylene bottle.

Another problem in the handling and storage of these minerals is their decidedly acid reactions. Dissolving appreciable quantities of the minerals in water will lower the pH to the range of 2 to 4. Paper or aluminum foil left in contact with the minerals, particularly in a moist atmosphere, will rapidly decompose.

Stability relations. The occurrence of melanterite and rozenite in Perry County does not seem to entirely conform to the present state of knowledge of the system ferrous sulfate-water-sulphuric acid. Experimental work by Cameron (1930) indicates that melanterite should be the stable ferrous sulfate phase crystalizing from solutions with less than 28 percent sulphuric acid at 25° C. Ehlers and Stiles (1965) proved that melanterite can be reversibly dehydrated to rozenite under relative humidities of less than 70 to 80 percent at temperatures that would be found on the surfaces of the refuse dumps. Taken together, these two studies suggest that the ferrous sulfate phase crystallizing from coal mine drainage water ought to be melanterite and subsequent exposure to low humidity air could cause dehydration to rozenite. The close association of melanterite with water is in agreement with this conclusion. However, we have repeatedly obtained rozenite directly by evaporation of a saturated solution of rozenite in water under conditions that should have given, according to Cameron (1930), melanterite. Furthermore, the subsurface occurrence of rozenite in moist soil suggests that it can exist under conditions of high relative humidity. This is in disagreement with the earlier studies and suggests the natural system differs in some significant way from the artificial one. Work is being initiated in this laboratory in an attempt to discover the factors that stabilize rozenite in the coal mine refuse dumps of Perry County.

Acknowledgments

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THE BEHAVIOR OF IRON IN PEORIA LAKE

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ABSTRACT.—Iron in Peoria Lake can be differentiated into several fractions, of which the great majority was found to be particulate Fe(III). The particulate Fe (III) possess several characteristics. Its concentration is much higher in the upper reaches of the lake than in the downstream sector; it can be correlated with water turbidity; there is a significant correlation between particulate Fe(III), particulate Fe(II), particulate silica, and particulate phosphate. The dissolved Fe(III) concentration is in excess of its solubility, suggesting that it is not in a complete soluble form.

Aqueous solutions of iron have been intensively studied during the past decade. Many investigations have been concerned with the chemical behavior of iron as related to thermodynamic and kinetic models (Hem and Cropper, 1959; Hem, 1960; Stumm and Lee, 1960; Morgan and Stumm, 1964; Ghosh, O'Connor, et. al., 1966; Larson, 1967). Numerous as such studies have been, most of them have dealt with distilled water systems. The results obtained from such systems can not, without modification, be applied to a natural water system. For example most iron studies have been performed under abiotic laboratory conditions. Such studies though informative are not substantive; biological activity does affect the iron behavior in natural water, as suggested by Lee and Hoadley (1967). Furthermore, Morgan and Stumm (1964) have shown, in distilled water studies, that iron most likely plays a role in surface chemical reactions. As natural water contains suspended solid such as detritus, silt, and clay, it is conceivable that the surface phenomenon may be even more important in the natural water system. In an effort to gain some insight into the behavior of iron in a natural water environment the observations presented herein were made, as a part of a limnological study of Peoria Lake.

PROCEDURE

Peoria Lake, is fundamentally a wide basin of the Illinois River (Figure 1). It has been channelized for navigation and is located in one of a series of eight pools extending from the river's confluence with the Mississippi River. At normal pool stage the lake is about 13 miles long and has an average width of 1.5 miles; the maximum depth is 5 meters with a considerable portion of water depth less than 1 meter. During the study period, the residence time within the lake ranged from 2 to 6 days.

Five transects and nine stations were established on the lake (Figure 1). On four of the transects two stations were assigned; one was representative of the channel area and the other the shallower area of the lake. A single station was

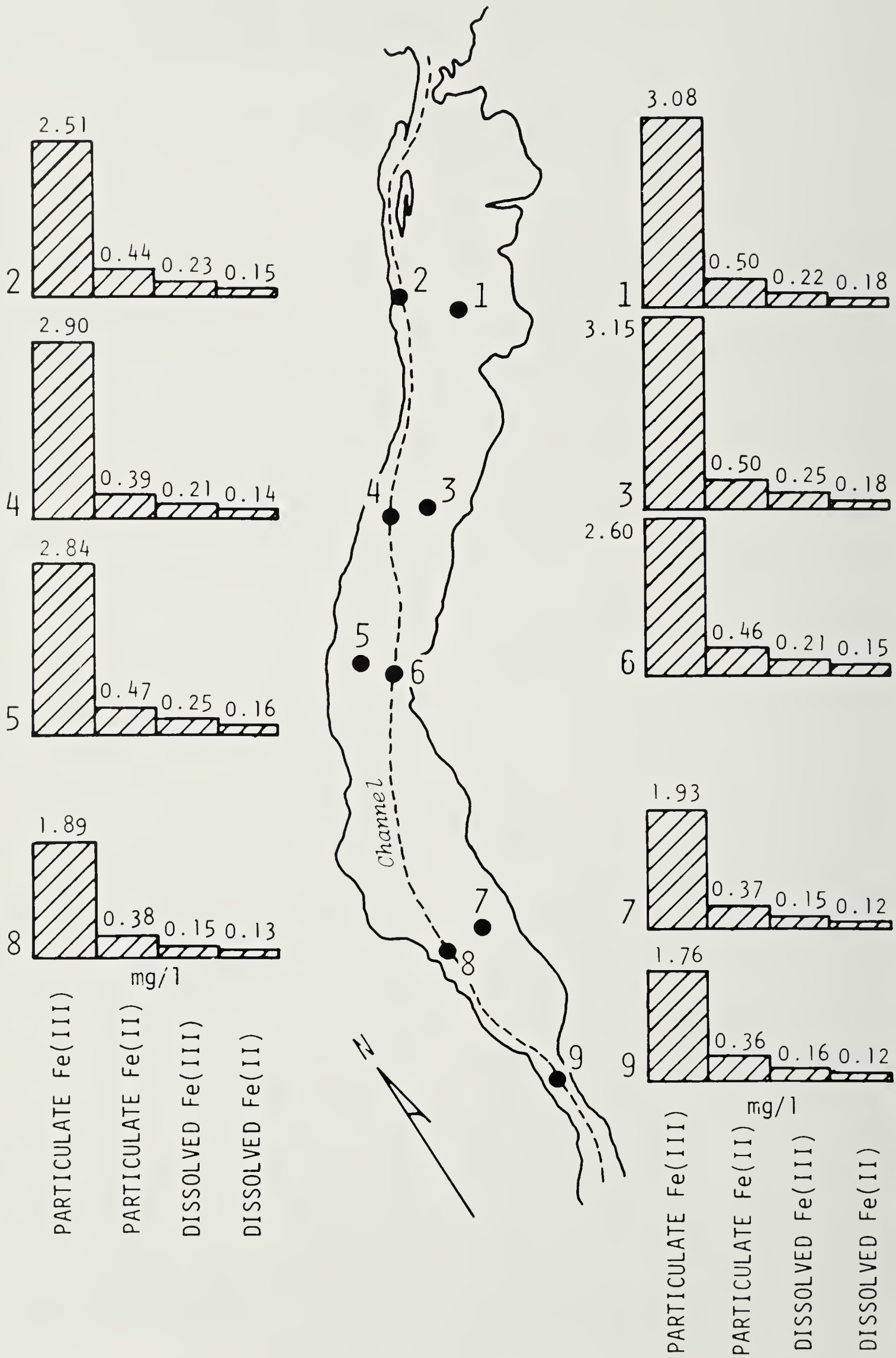

FIGURE 1. Various iron fractions in Peoria Lake.

selected in the "Narrows", the outlet of the lake.

Water samples were collected at the nine stations at a depth of 1 meter using a Kemmerer sampler. Collection was made at one to two weeks intervals. All water samples were transferred to glass bottles which were prewashed with acid.

Analytical Methods

The turbidities of all samples were determined immediately upon delivery to the laboratory. The method used closely parallel the Jackson candle method (American Public Health Association, 1965). Also upon delivery a portion of each sample was filtered through an 0.45 micron membrane filter.

The filtrate and unfiltered portions of each sample were separately analyzed for Fe(II) and total iron by using the phenanthroline method (American Public Health Association, 1965). In this manner the concentration of the various fractions of iron were determined, i.e., dissolved Fe(III) and Fe(II) and particulate Fe(III) and Fe(II). All analyses were performed within 48 hours after the collection of samples.

Dissolved oxygen, temperature, and pH were determined in the field. Dissolved oxygen and temperature were determined by an oxygen analyzer manufactured by Precision Sientific Company, and pH was determined by a Beckman model N pH meter.

Results and Discussion

Distribution

Some characteristics of Peoria Lake water are shown in Table 1. In general, the water is turbid and rich in the bicarbonates of calcium and magnesium. Nutrient levels

Table 1.—Chemical Characteristics of Peoria Lake

	Range	Mean**
Temperature, °C	5.0 — 27.3	19.5
Turbidity, JTU	28.0 — 296.0	115.0
pH	7.57 — 8.69	8.19
Dissolved Oxygen, mg/l	1.4 — 15.3	5.6
Alkalinity*, mg/l	136.0 — 213.0	165.0
Hardness*, mg/l	215.0 — 324.0	268.0
Iron (total), mg/l	0.69 — 13.01	3.21
Ferrous	0.16 — 1.89	0.58
Ferric	0.52 — 11.12	2.63
Fluoride, mg/l	0.17 — 2.06	1.08
Silica (total), mg/l	1.96 — 14.80	6.10
Nitrogen (total), mg/l	3.88 — 14.98	8.85
Nitrate (NO_3–N)	1.65 — 11.12	4.33
Ammonia (NO_3–N)	0 — 5.45	1.15
Organic–N	0.64 — 9.84	3.37
Phosphorus (total), mg/l	0.47 — 3.02	1.13
Orthophosphate–P	0.25 — 2.30	0.84
Polyphosphate–P	0 — 0.67	0.15
Organic Phosphate–P	0 — 0.58	0.14

* expressed as $CaCO_3$
** based on 225 samples

are quite high and upstream wastes discharges are reflected in the relatively low dissolved oxygen content.

The distribution of various iron fractions is shown in Figure 1. Of the four iron fractions, particulate Fe(III) was dominant and constituted over 70 percent of total iron in the lake waters. The next abundant fraction was particulate Fe(II), followed by dissolved Fe(III), and dissolved Fe(II), in that order.

An attempt was made to determine the fluctuation of particulate Fe(III) from station to station. A two-way analysis of variance was made. The result showed that particulate Fe(III) concentration was significantly different from station to station, i.e., it was not uniformly distributed in the whole lake. Further attempts were made to group the nine stations into three regions; stations 1 and 3, stations 2, 4, 5, and 6, and stations 7, 8, and 9. The analysis of variance was again made in three regions separately. The results showed that there was no longer significant variation of iron concentration within each region. Figure I depicts the results for the three regions.

Particulate Fe(III) was highest at station 1 and 3, ranging from 3.08 to 3.15 mg/l. The intermediate zone was at station 2, 4, 5, and 6, ranging from 2.51 to 2.90 mg/l. The lowest concentrations were at stations 7, 8, and 9, ranging from 1.76 to 1.93 mg/l. This distribution pattern is the same as that observed for turbidity in the lake (Wang and Brabec, 1969).

It should be noted that the percentage of particulate Fe(III), with regard to the total, ranged from 73 to 80. The highest level was at station 2 with a gradual decrease to the lowest level at station 9. This was apparently due to a greater loss of particulate Fe(III) through precipitation along in the water course compared with that of other iron fractions.

If station 2 is considered representative of the inlet and station 9 the outlet of the lake the iron budget can be computed. Figure 2 depicts the results in terms of concentration and load. The positive iron balance indicates that the iron input is greater than the output with the consequence accumulation of iron within the lake.

The observed dissolved Fe(III) concentration averaged 0.20 mg/l which is ten times higher than the solubility of this fraction as cited in the literature (Stumm and Lee, 1960). This suggests that the preponderance of dissolved Fe(III) is not insoluble form but exist possibly as particles of less than 0.45 micron diameter. Shapiro (1964) reported the existence of $Fe(OH)_3$ in the form of a precipitate and peptized sol.

Iron and Turbidity

Since turbidity can be regarded as an index of particulate matter, an attempt was made to correlate particulate iron and turibidty. Figures 3 and 4 show the relationship between turbidity and Fe(III) and turbidity and Fe(II), respectively. Regardless of location within the lake, particulate Fe(III) and Fe(II) were significantly related with turbidity. A similar analysis with dissolved Fe(III) and Fe(II) did not reveal a similar relationship.

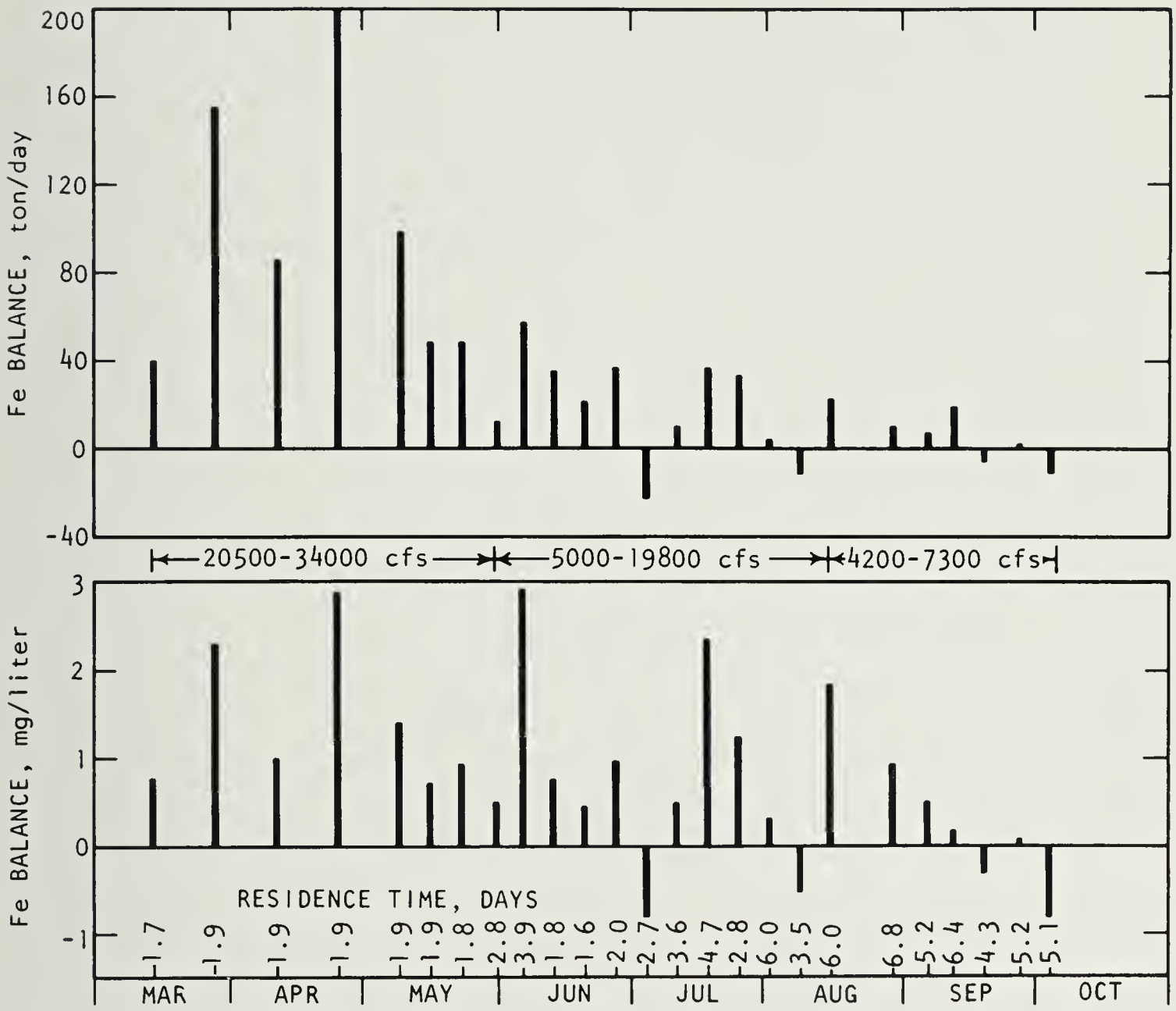

FIGURE 2. Iron budget in Peoria Lake.

The precise structure of particulate iron on the surface of particulate matter is unknown. As previously mentioned it can be in the form of a precipitate granule, film, or peptized sol (Shapiro, 1964). It would seem likely that a strong linkage exist between iron and particulate matter. Iron, being an electrophylic substance, is naturally inclined to attach to clay, a nucleophylic substance, the "backbone" of particulate matter in water.

Dissolved iron, mentioned earlier as not being truly soluble in Peoria Lake may be in the form of neutral salt or an electron-rich form which renders it free from electrostatic attraction to clay minerals or organic detritus.

Iron and Other Elements

A significant relationship between turbidity and particulate iron was observed. A similar relationship between turbidity and particulate silica was also found (Wang and Evans, 1969). It is thus logical to expect that particulate iron is also significantly correlated with particulate silica and phosphate. These relationships are shown in Figures 5 and 6 and summarized in Table 2.

The overall average concentrations

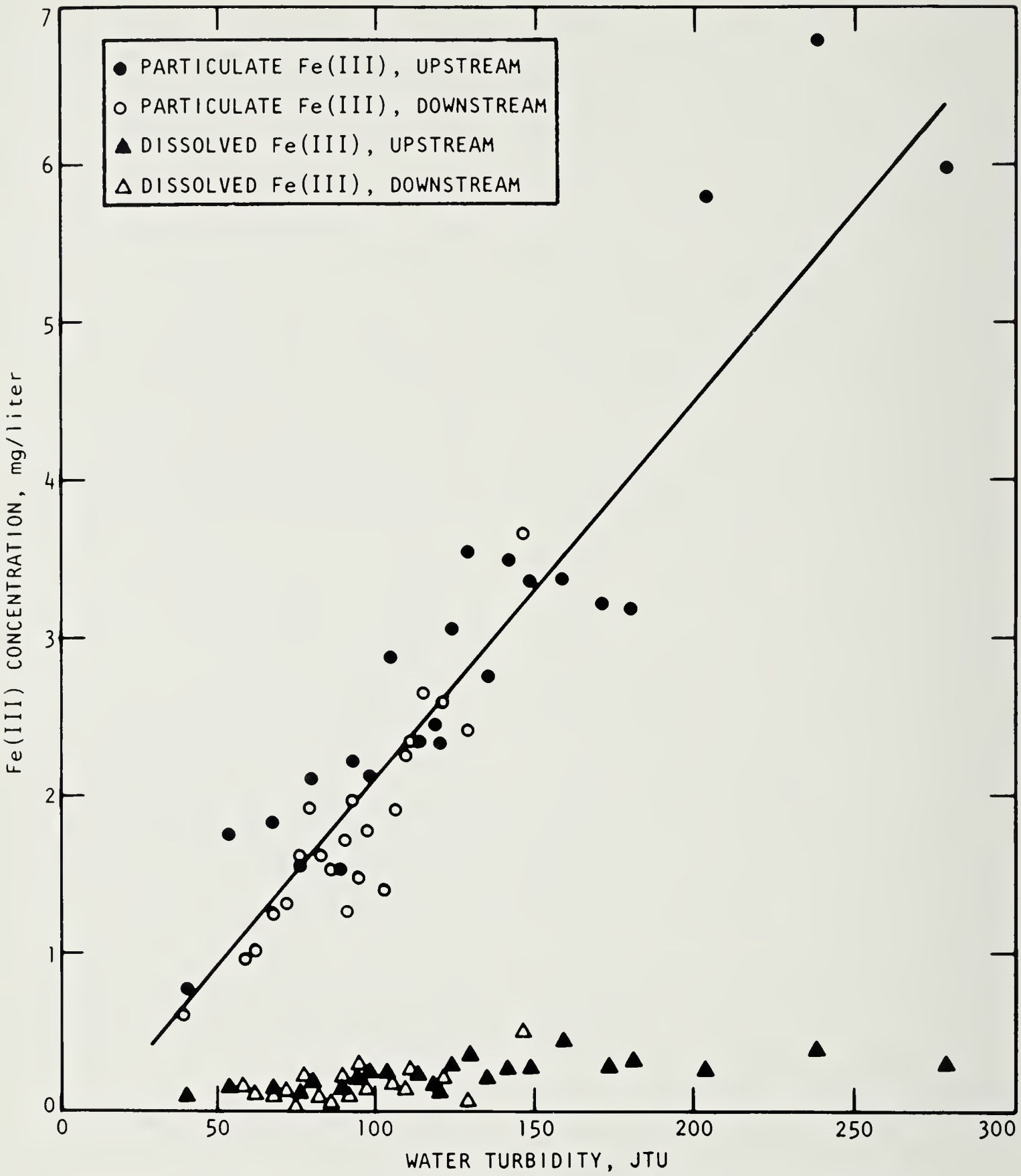

FIGURE 3. Relation between water turbidity and Fe(III) concentration.

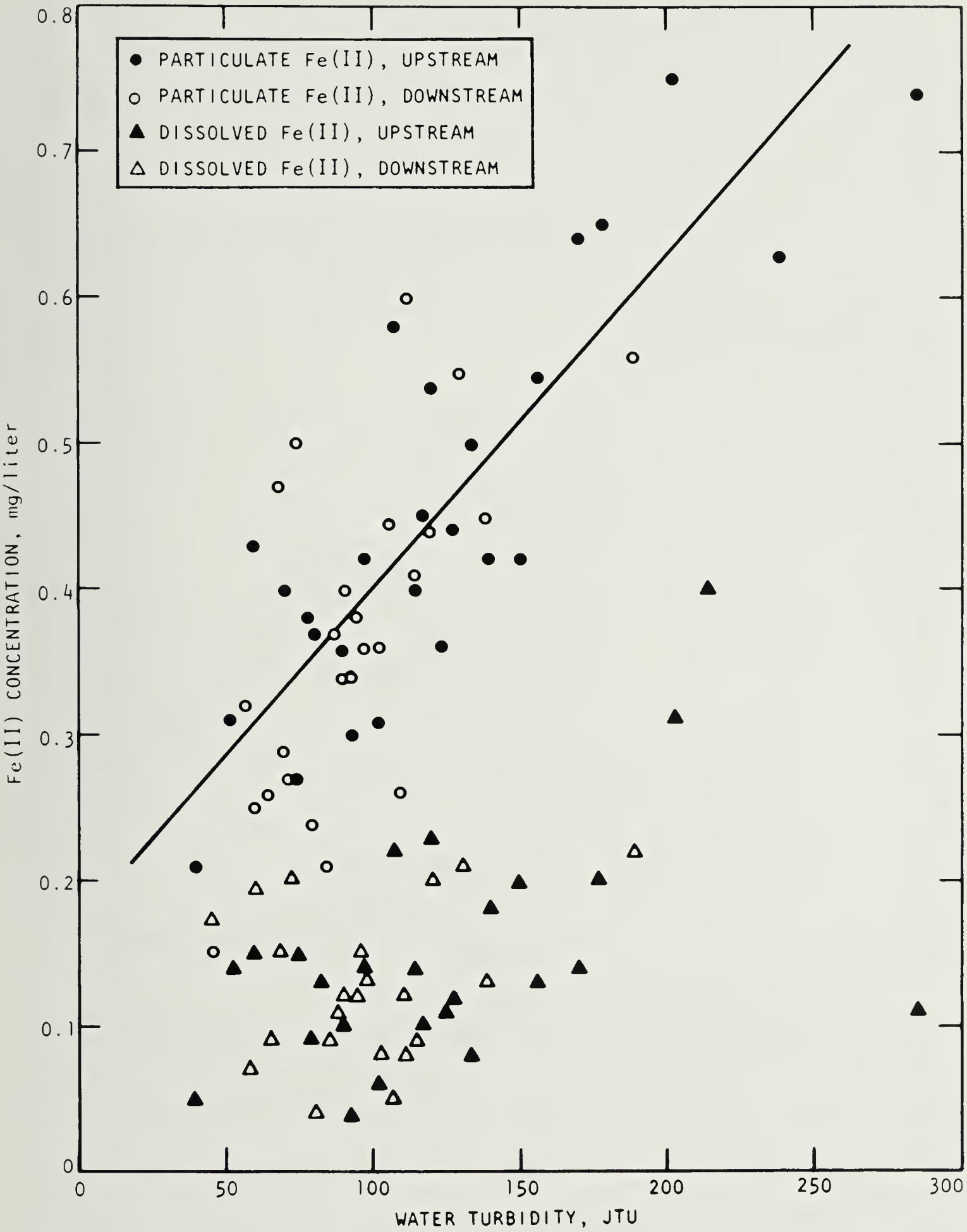

FIGURE 4. Relation between water turbidity and Fe(II) concentration.

TABLE 2.—Correlation Coefficients of Various Parameters in Peoria Lake

	Part. Fe(III)	Part. Fe(II)	Part. SiO_4	Part. PO_4	Turbidity
Part. Fe(III)	.....	0.587**	0.704**	0.850**	0.854**
Part. Fe(II)	0.587**	.....	0.218	0.257	0.751**
Part. SiO_4	0.704**	0.218	.....	0.444*	0.454*
Part. PO_4	0.850**	0.257	0.444*	.....	0.648**
Turbidity	0.859**	0.751**	0.454*	0.648**	.....

* = 95% significance level
** = 99% significance level

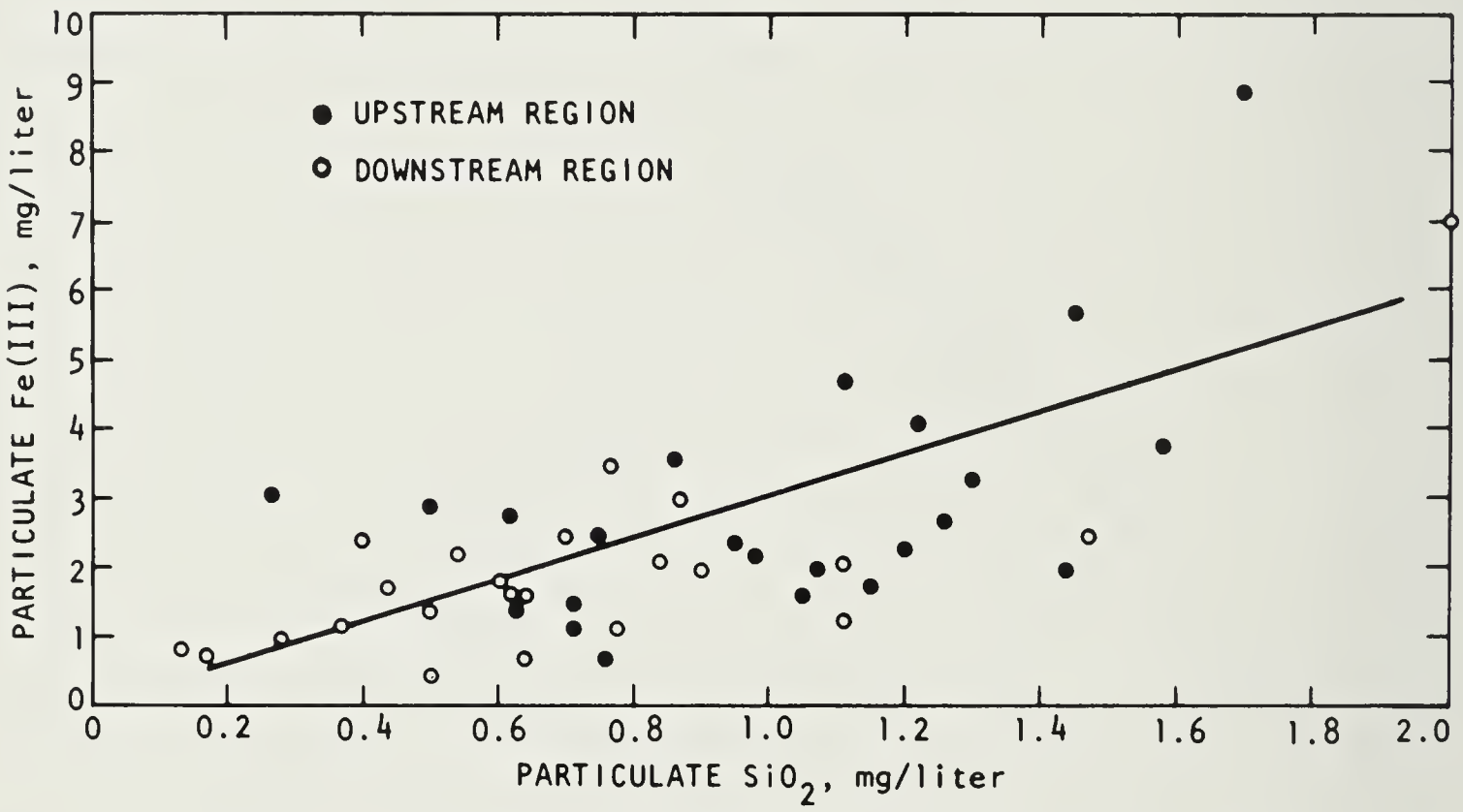

FIGURE 5. Relation between particulate silica and particulate Fe(III).

of particulate iron, silica, and phosphorus were 2.48, 1.80, and 0.42 mg/l, respectively. These values represent a molar ratio of 3.15, 2.15 and 1 in the same order. In other words, on particulate matter the computed sum of silica and phosphorus molecules was exactly same as the iron molecules. The result suggests a stoichiometric relationship among these three constituents.

The particulate matter in Peoria Lake is believed to be mainly silt and clay particles. These particles carry negative charges principally due to isomorphous substitution. In a water environment, these particles may associate with various elements through physico-chemical forces. Of the three constituents — particulate Fe(III), silica and phosphorus — particulate Fe(III) is the most likely one to attach to particulate matter. Silica and phosphorus may then link with particulate matter through the iron "bridge", a propounded mechanism in soil chemistry (Evans and Russell, 1959).

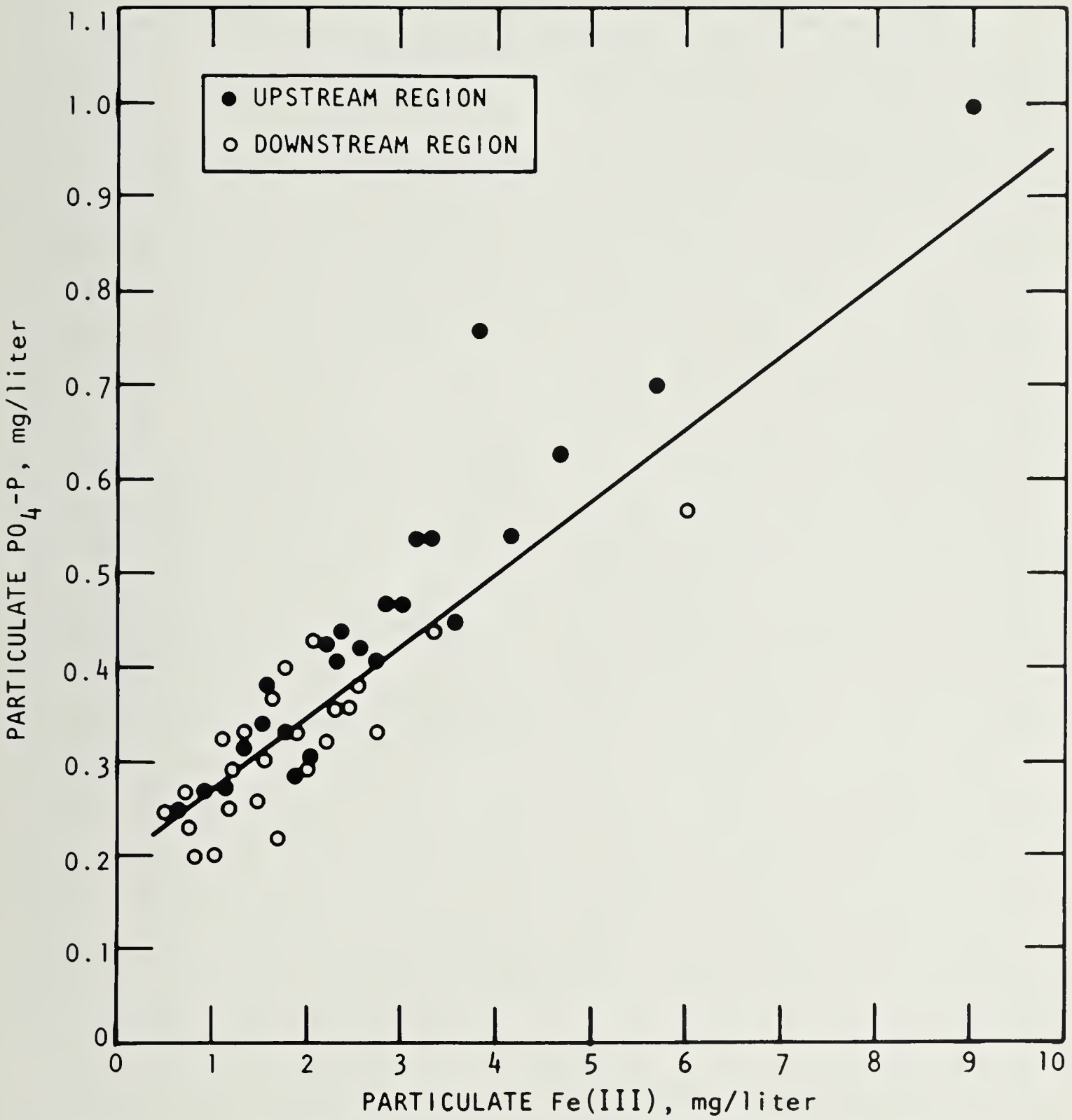

FIGURE 6. Relation between particulate Fe (III) and particulate PO_4-P.

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IMPROVED X-RADIOGRAPHY OF CYLINDRICAL SEDIMENT CORES

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ABSTRACT.—X-rays passing through the center of cylindrical sediment cores during radiography are absorbed to a greater degree than are X-rays passing through the sides of the core. Four methods have been devised to compensate for this effect. (1) The penetrating power of the X-ray beam may be increased. (2) A lead intensifying screen can be used to increase the intensity of X-rays passing through the center of the core. (3) A material similar to that in the core may be packed around the sides of the core. (4) The film density of the central region of the film may be reduced. These methods were quantitatively compared by testing the effects of each method on the exposure and sensitivity of the film. The results of the tests indicated that each method alleviated the problem but to a different degree. Method 3 produced the most even exposure across the film and showed the maximum detail of the internal features of the core.

When radiographs of cylindrical sediment cores are made, X-rays passing through the center of the core tube are absorbed by the sediment to a greater degree than X-rays passing through the sides of the core. The radiograph is unevenly exposed and the gradient of film density (darkness of film) increases from the center toward the sides of a radiograph. A film correctly exposed for the center of the core will be overexposed near the edges, resulting in a loss of detail.

We tested several methods to minimize this problem. Only a tentative solution has been reached because each method introduces factors that adversely affect the film sensitivity or the ability of the film to record detail.

METHODS

In each of the methods discussed below, a Picker Gemini Model 160 constant potential industrial radiographic machine was used with a 150kV Morris Be-window X-ray tube selected to have a 0.5 mm diameter effective focal spot. The X-ray output from the tube was collimated by a medical-type collimator attached to the tube. The focal-spot to specimen distance was fixed at 100 cm. Kodak AA industrial X-ray film was used exclusively. The film was developed in a Calumet Model 147 nitrogen-burst machine to insure that each film was uniformly processed.

The penetrating power of X-rays may be increased by increasing the potential, or kilovoltage, between the anode and the cathode in the X-ray tube. The "harder" X-rays that are produced at higher kilovoltages are less sensitive to varying thick-

nesses of a cylindrical core and, therefore, reduce the density gradient across the radiograph. Radiographs made from these hard X-rays show less detail of the core because harder X-rays are also less sensitive to differences of density in the object being radiographed.

A second method uses lead intensifying screens to increase the intensity of X-rays passing through the center of the core relative to those passing through the sides. The rays that have passed through the central region are "hard" X-rays of short wave length because the softer rays have been selectively absorbed. Toward the sides of the core, however, more soft rays can penetrate the core because the material is thinner. The intensifying effect increases as wave length decreases (Clark, 1955), so that rays of short wave length penetrating the central region of the core tube are preferentially strengthened. Unfortunately, the ability of the intensifying screens to reduce the film density gradient is somewhat limited.

In the third method, material is packed around the core tube presenting plane parallel surfaces to the incoming X-rays. Thus, X-rays passing through the sides of the core are absorbed to an equal degree as those passing through the center.

Several materials have been used as the absorbing material. Klingebiel *et al* (1967) immersed the cores in liquids of varying densities. The densities of the liquids were matched with the density of the material of the core so that the absorptive characteristics of both materials were the same. The liquids, however, were inconvenient and difficult to handle during preparation of the core for radiography.

Haase (1967) attacked this problem by placing the cores in molds of plaster or plastic. Plastic was preferred because the plaster contained air bubbles and other imperfections. Baker and Friedman (1970) placed cores in a machined aluminum block. The molds are convenient to handle, but their densities cannot be varied to match the absorptive characteristics of the sediment core. They also may be expensive or difficult to construct.

Bouma (1969) recommended placing the core in loosely packed fine sand. The absorptive effect of the sand could be varied by changing the thickness of the sand pack. A fair match between the absorptive characteristics of the sediment and the sand could thus be obtained. Even though a fine sand is used, however, the graininess of the sand would be shown on the film and would tend to mask detail in fine-grained sediment.

A method which alleviates these problems was devised by Nathan Ayer (1970) in 1967. He designed a plastic box (Fig. 1) containing small glass spheres, called glas-shot® or microshot, as the absorbing material. The box consists of an outer plexiglas shell fitted with an inner

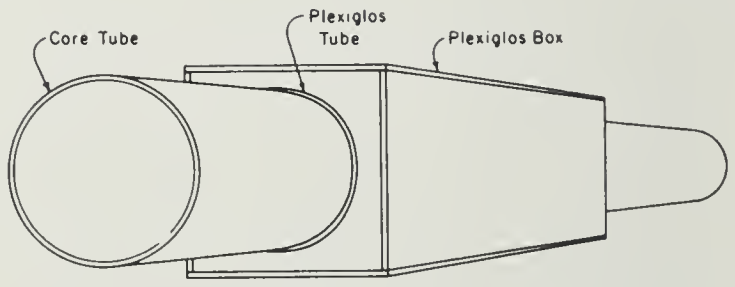

FIGURE 1. Glass bead box designed by Nathan Ayer. The core tube slides inside the plexiglas tube.

plexiglas tube that has an inside diameter only slightly larger than the 4.5-inch (11.4-cm) outside diameter of our core tubes. The microshot ranges from 10 to 53 microns in diameter. (Number MS-XL, Microbeads Division, Cataphate Corp., Jackson, Miss.) The box is convenient because the core can easily be fitted into the plexiglas tube without having to repack the microshot each time a new core is radiographed. The microshot is sufficiently small to prevent masking detail in fine-grained sediments, and the absorptive characteristics of the glass beads are similar to those of many types of unconsolidated sediments. In addition, the absorptive ability can be varied by increasing or decreasing the packing density of the microshot. The glass beads, however, cause some scattering of the X-ray beam that tends to blur the image slightly on the radiograph.

In method 4, the film density of the entire radiograph is decreased until detail can be seen along the edges of the film. This procedure, however, may cause the film density of the central region to become too low, causing the visibility of detail in that area of the radiograph to be reduced.

Comparison of Methods

The relative pros and cons of the four methods may be quantitatively compared by testing the effect of these methods on the density gradient and on the contrast of the film. A synthetic core was constructed for the tests by putting five strips of lead foil on half the width of a plexiglas plate and inserting the plate into a plastic core tube filled with the same microshot as those in Ayer's box (Fig. 2). A radiograph of the

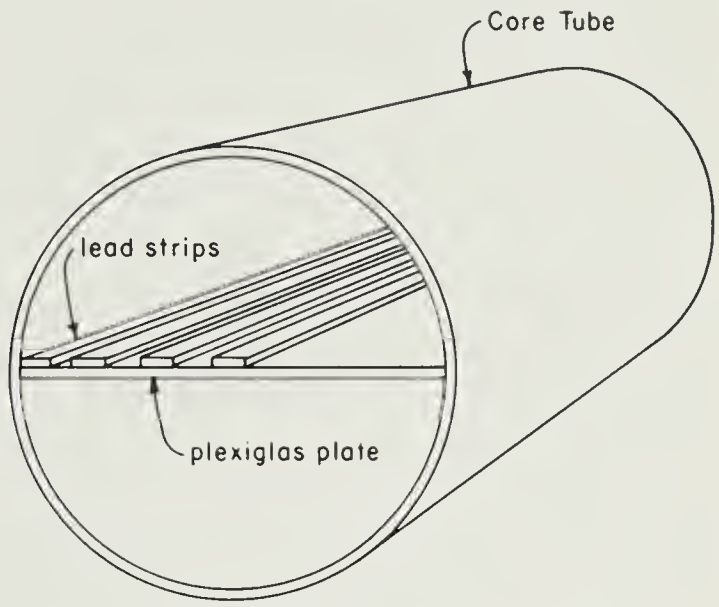

Figure 2. Synthetic core used to test the effects of the various methods on the film density gradient and contrast.

core showed the strips of lead as slightly lighter images against a darker background on one side of the radiograph and a gradient of film density progressively increasing from the center to the edge of the other side of the radiograph (fig. 3).

The film density of the images of the five lead strips and of the corresponding areas on the opposite side

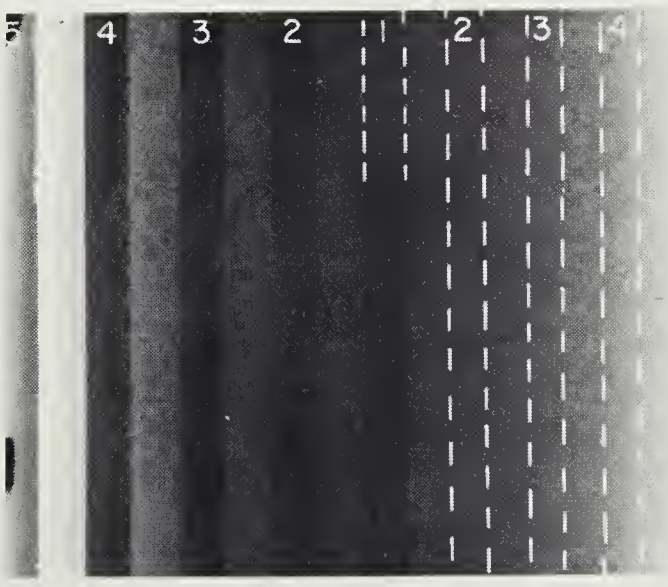

Figure 3. Typical test radiograph print. The darker areas represent the lead strips and areas enclosed by the dashed lines are the counterpart areas to the images of the lead strips.

of the film were measured. To compute the film contrast, the arithmetic difference was determined between the film density of the image of a lead strip and the film density of its counterpart area on the opposite side of the film. The film contrast also had a gradient from the center to the edge of the radiograph.

Three sets of graphs were prepared to show the effects of the various methods on the density gradient and the film contrast. The first set (Fig. 4) compares the film density gradient for each of the first three methods and for three different kilovoltages. The graphs are plotted with film density as a function of distance from the center line of the radiograph of the core tube. The initial film density (film density of the center line of the radiograph) was 2.00 in all cases. The angle of the slope of any of the plotted curves bears an inverse relation to the film density gradient.

The second set of graphs (Fig. 5) are plots of the film contrast gradient for the first three methods and for three different kilovoltages. The contrast is plotted as a function of distance from the center line of the radiograph with an initial film density of 2.00. The angle of slope of

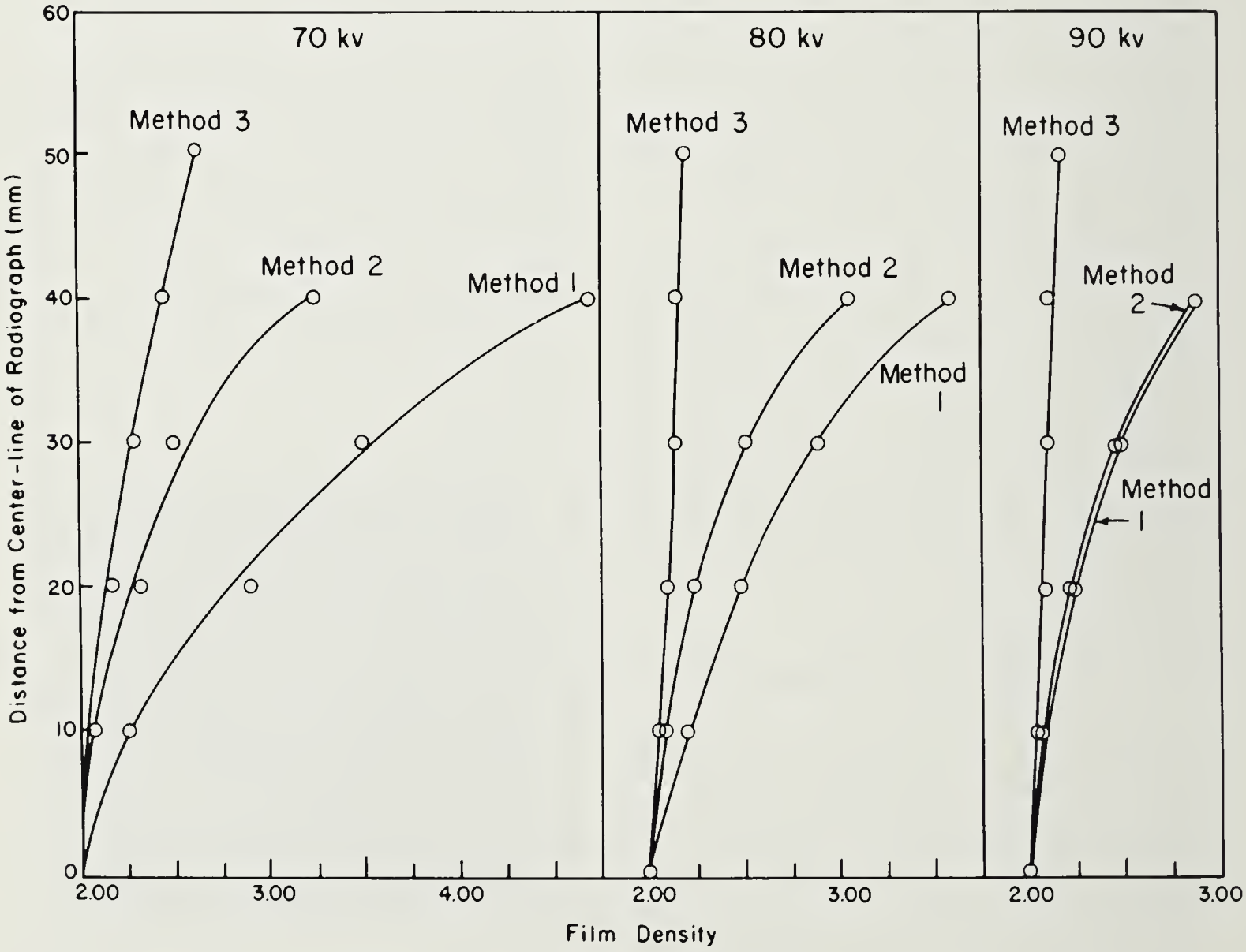

FIGURE 4. Plot of the film density as a function of distance from the center line of the radiograph for methods 1, 2, and 3 with three different kilovoltages. No film densities could be measured beyond 40 mm from the center line of the film for methods 1 and 2 owing to extreme blackening of the film.

any of the curves bears an inverse relation to the film contrast gradient.

The third set of graphs (Fig. 6) was prepared from data taken from radiographs in which the initial film density was set at 1.00, 1.50, and 2.00, while the kilovoltage was held constant at 70 kV. These graphs were prepared to show the effects of method four (decreasing the initial film density) on the density gradient and the contrast gradient.

Figures 4 and 5 show that the microshot box, method 3, is the best mechanism for reducing the film density gradient. This method, however, also has the most detrimental effect on the film contrast. In all graphs, the microshot box method showed the lowest contrast at the center line of the radiograph and also the slowest rate of increase from the center to the edge of the radiograph. Use of the lead intensifying screen in method 2 decreased the film density gradient slightly, but it was not as harmful to the contrast as method 3. Increasing the kilovoltage decreased the density gradient considerably but also adversely affected the contrast. The film densities at the edges of the radiographs made using methods 1 and 2 were too high to be measured, i.e., no light penetrated these areas when a high-intensity X-ray illuminator was used.

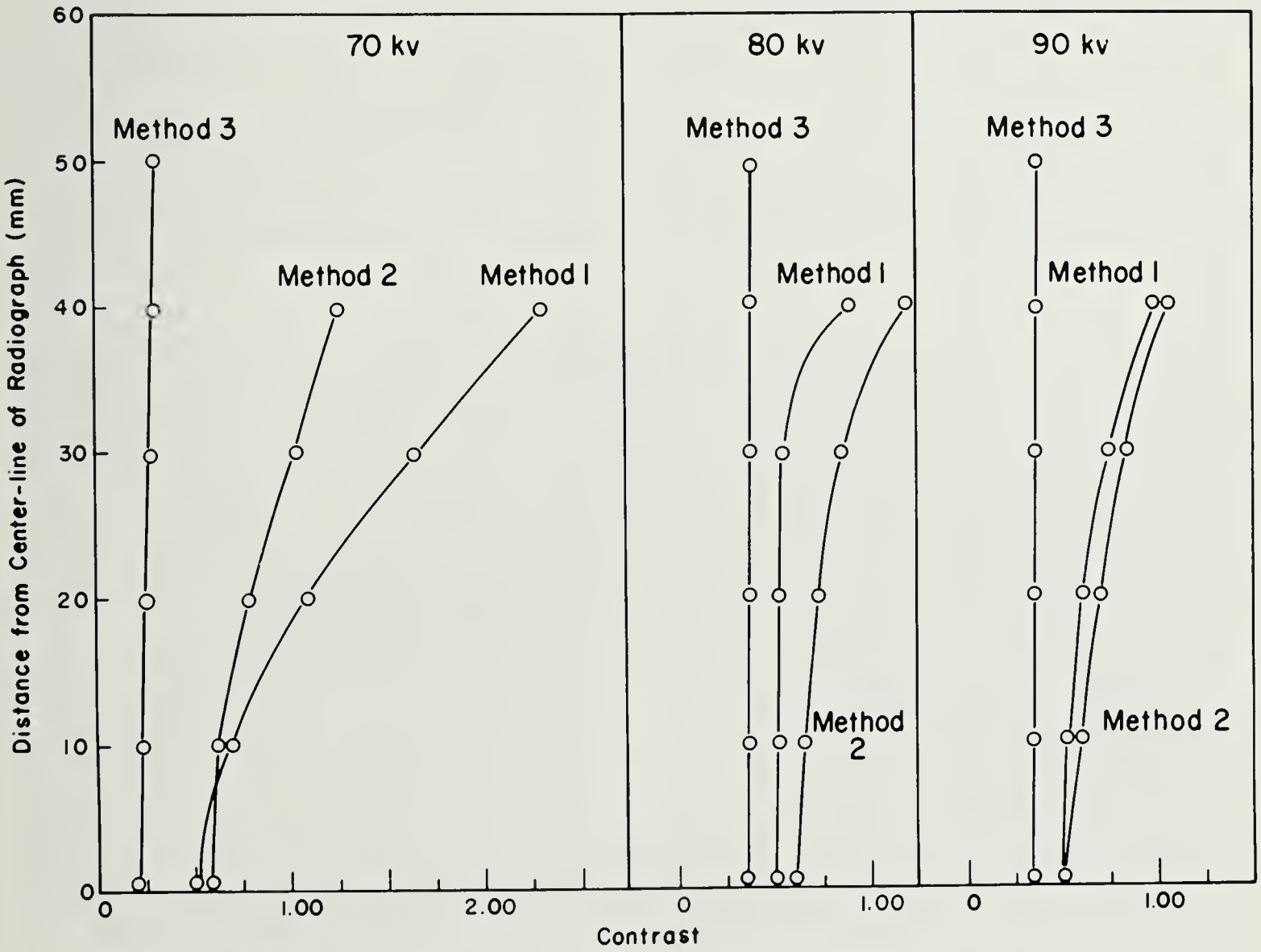

FIGURE 5. Plot of the film contrast as a function of distance from the center line of the film for methods 1, 2, and 3 using three different kilovoltages. No film densities could be measured beyond 40 mm from the center line for methods 1 and 2 because of extreme blackening of the film.

Figure 6 shows that with an initial film density of 1.00 the density gradient was the lowest and the contrast of the center portion of the radiograph was so low that the contrast gradient became negative. Increasing the film density to 1.50 increased the contrast but also greatly increased the film density gradient. An initial film density of 2.00 showed the best contrast and also the most extreme film density. In all tests, the edges of the radiograph were completely blackened, and it was impossible to measure the film density with a high-intensity illuminator.

Although the evidence of the graphs seems inconclusive, it should be pointed out that an extreme film density gradient is much more adverse to radiographic sensitivity than a steep contrast gradient is beneficial. A satisfactory compromise is found by decreasing the density gradient and accepting the slightly adverse effects produced on the contrast of the film.

Methods 1, 2, and 4 left 10 to 15 mm of a radiograph completely opaque when viewed on the available illuminator. Method 3 was the only method tested that produced acceptable film densities across the entire radiograph and showed the maximum detail of internal features on X-radiographs (Fig. 7).

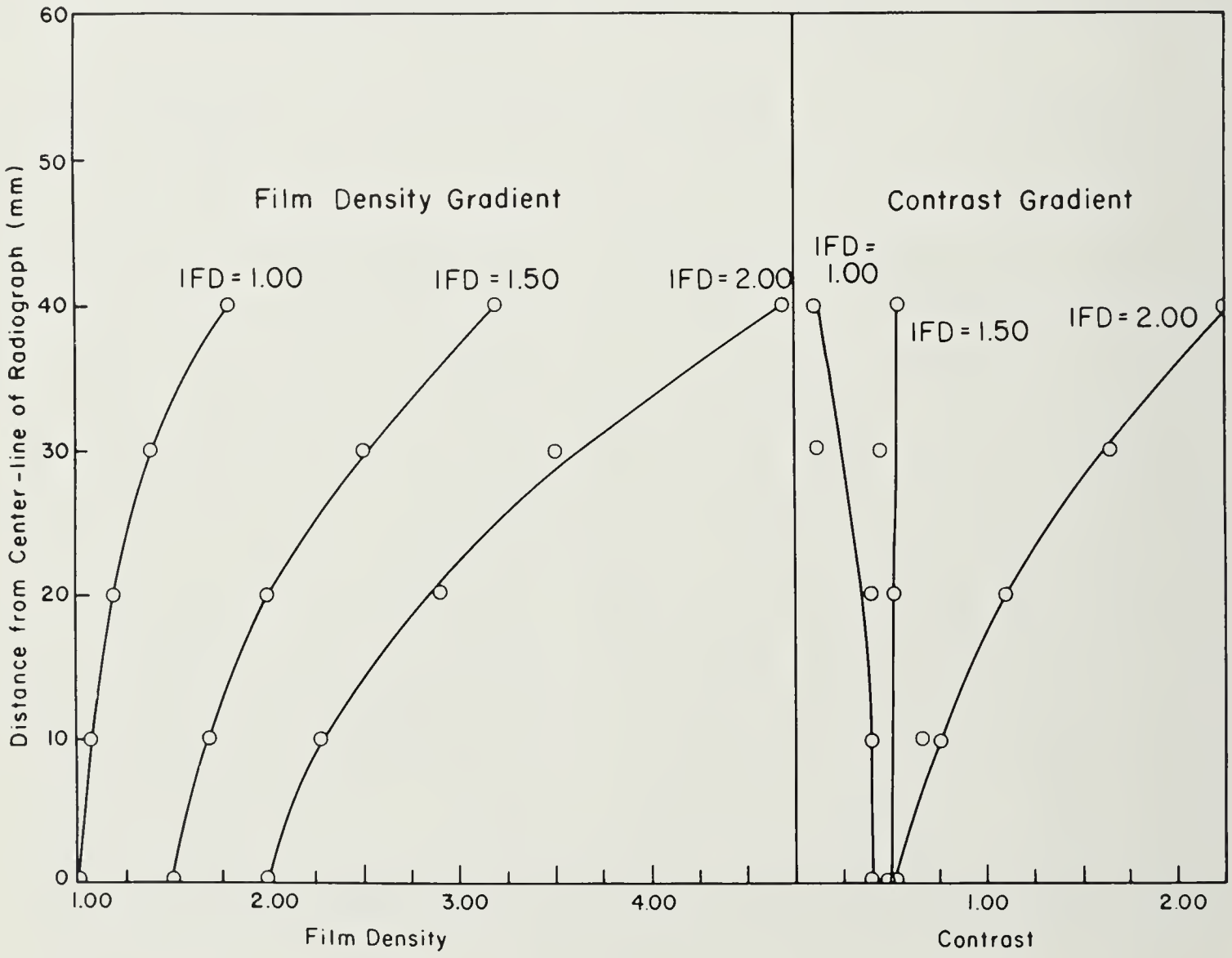

FIGURE 6. Plot of the film density and the contrast as a function of the distance from the center line of the film for three initial film densities (IFD), 1.00, 1.50 and 2.00. Blackening of the film beyond 40 mm from the center line produced film densities too high to be measured.

Discussion

A film density of about two is considered optimum in industrial radiography. Lower film densities lack detail, and higher densities are difficult to view with conventional high-intensity viewers. A film density of two is opaque to the eye when viewed without the use of a high-intensity illuminator. While optimum detail of the film negative is possible using the viewer, we have found that it is difficult to obtain satisfactory positive prints. The dynamic range of most photographic papers evidently is insufficient to satisfactorily record the detail present in the negative. Very long exposure times also were necessary to obtain suitable prints from the dense negatives. Small differences in film density caused by the geometry of the radiographic box used in method 3, in particular, resulted in unsatisfactory prints made by conventional photographic procedures. A number of methods were tried to solve this problem. The most satisfactory results were obtained using the Mark II Log-Etronic contact printer.

Acknowledgments

The studies reported were made using the X-ray facility of the Sedimentology Laboratories in the Department of Geology, University of Illinois, Urbana. The X-ray machine was acquired from funds provided by NSF Grant GK-1292 to A. F. Richards and J. E. Stallmeyer. Our work at the University of Illinois was supported by Office of Naval Research Contract NONR 3985 (09), NR 081-260. The manuscript was partly prepared under Office of Naval Research Contract N00014-67-A-0370-0005, NR 083-248. The positive print shown in figure 7 was kindly made for us at the Waterways Experiment Station, Vicksburg, Mississippi, through the courtesy of E. L. Krinitzsky.

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Figure 7. X-radiograph print using the microbead box of a sediment core within a 11-cm-diameter core barrel of Delrin plastic. A gastropod shell is the dominent feature in the core. Smaller features probably are clam shells.

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THE SPATIAL DISTRIBUTION OF LAKE-EFFECT SNOWFALL WITHIN THE VICINITY OF LAKE MICHIGAN

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ABSTRACT.—The average monthly and annual snowfall patterns are analyzed for the region within 100-150 miles of Lake Michigan. From these patterns the distribution of lake-effect snowfall is determined for the study region.

During the last few years much attention has been given to Great Lakes Climatology. Among the many parameters being studied is snowfall. It has been illustrated by several authors that excessive amounts of snowfall occur along the shorelines of the Great Lakes, especially along the lee shore of the lakes (Falconer, Lansing, and Sykes, 1964; Sheridan, 1941; Pack, 1963; Namias, 1960; Bolsenga, 1967; Johnson and Mook, 1953; and Williams, 1963). Muller (1966, p. 256) developed a map for mean seasonal snowfall in the Great Lakes region and surrounding areas. The most outstanding features of this map are the snowbelts associated with the frequently recurring lake squalls coming off the Great Lakes.

These excessive amounts of snowfall along the shores of the lakes have been explained to be an overt manifestation of lake-effect snow showers. Lake-effect snowfall by definition occurs as a result of cold air being advected over the warm moist surface of a lake. The air in contact with the surface warms rapidly, gains moisture and under superadiabatic lapse rate conditions rises rapidly forming convective clouds and precipitation. During the periods of lake-effect snowfall it is assumed that the snowfall is generated specifically from the vast reservoir of heat and available moisture in the lake, and that there is no direct influence from either cyclonic or frontal systems.

PURPOSE OF THE STUDY

It is the purpose of this study to conduct an analysis of the phenomenon lake-effect snowfall for a single lake in the Great Lakes basin. Lake-effect snowfall is studied indirectly by analyzing the spatial variation of snowfall within the vicinity of Lake Michigan. Average monthly and annual snowfall amounts are determined for the study region and the patterns analyzed.

THE STUDY AREA

A boundary was chosen for this study to average between 100 and 150 miles inland from the shoreline of Lake Michigan. Lake Michigan offers several advantages which do not exist for any of the other Great Lakes. There is a dense network of reporting stations along all sides of the lake. There is topographic uni-

formity throughout most of the study region. And, there is little interaction between Lake Michigan and the other lakes in the Great Lakes basin.

Sources and Utilization of Data

The U. S. Department of Commerce's data records for each of the states within the region of study were examined. A list was compiled indicating all reporting stations in the study area that kept systematic monthly and annual records of snowfall. A total of 145 stations were included in the study.

Monthly and annual snowfall amounts were tabulated for the stations in the study region for 10 successive snowfall seasons beginning October 1959 and ending March 1969. Monthly snowfall amounts were tabulated for the four months November to February of each snow season. An average monthly and annual snowfall amount was computed for each of the 145 stations in the study.

These averages were compared to the long-term averages which were available in the "Climatography of The United States" series. It was noted that the 10 year averages obtained in this study were significantly higher than the long-term averages. The average increase over the study region was from 10% to 15%. In his recent study, Eichenlaub (1970) found that there has been a 100% increase in the average snowfall amounts of western Michigan. There is no definite answer to the question of what is causing this increase in snowfall and lake-effect snowfall. There are however several possible explanations for this climatic change which warrant further research. Namias (1960) suggested that an increase in snowfall can result from a shift in the position of the mean troughs and ridges over North America. It has also been suggested that a general cooling of the atmosphere has been occurring since the 1930's. Finally further research should investigate the role of atmospheric pollution in increasing the amount of snowfall (especially near the large industrial complexes like Chicago-Gary, Milwaukee, and Muskegon).

Analysis of The Average Annual Snowfall Pattern

The average annual snowfall was portrayed on the map of the study region using an isopleth interval of 10 inches (Figure 1). In an analysis of the annual as well as the monthly snowfall patterns it should be emphasized that the positioning of the isopleths across the lake is based upon estimation. Although there are values of snowfall at the shoreline of each side of the lake it is difficult to determine the correct gradient of isopleths across the water. According to Changnon (1968, p. 23) "When lake-effect snowfall develops over the lake it begins somewhere within 20 miles of the eastern shoreline." On the annual snowfall map and all maps of monthly average snowfall, it was found in this study that the isopleths generally tended to parallel both the eastern and western shorelines, so it seemed most likely that the isopleths over the lake would also tend to follow a north-

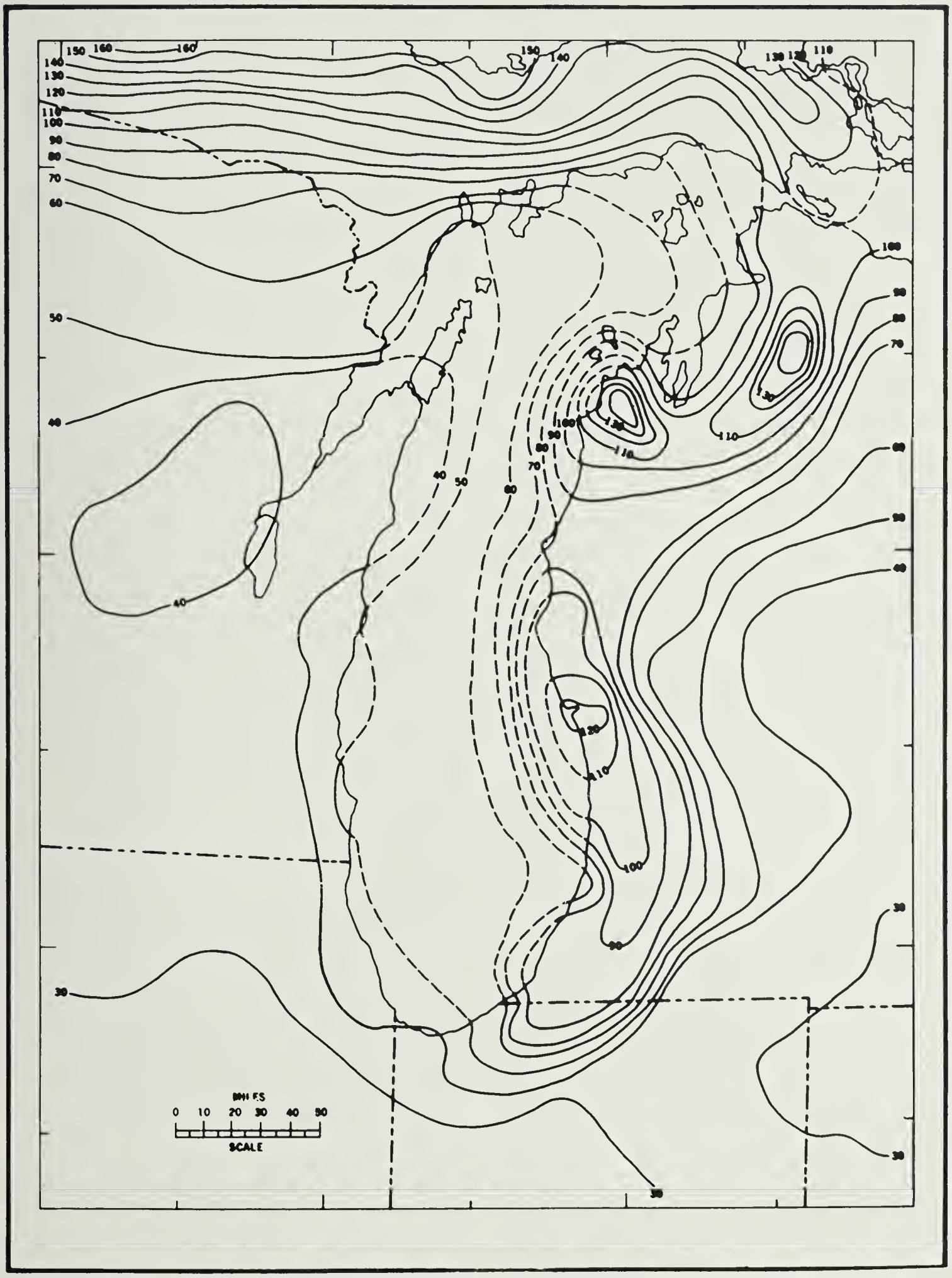

FIGURE 1. Average Annual Snowfall In Inches.

south direction. However, it was also assumed that the isopleths of snowfall are more tightly spaced along the eastern shore than the western shore.

Were there no Lake Michigan one would expect the isopleths to be oriented in an East-West direction across the study region, with snowfall increasing to the north. However, this latitudinal positioning of isopleths occurs only within 75 miles of the western edge of the study region, within 50 miles of the southern boundary of the study region, and at a maximum, within 50 miles of the eastern edge of the study region. The remainder of the study region has a snowfall pattern which could not exist without the presence of Lake Michigan.

Along the western side of the lake, the gradient of snowfall is small. For example, the distance between the 30 inch and 40 inch isopleth is 200 miles. The gradient becomes steeper near the northern margin of the study region, but this is a result of the additive influence of lake-effect snowfall from Lake Superior. The extent of lake-effect snowfall averages 10-15 miles inland along the western shore and the magnitude averages 10-15 inches annually. Lake-effect snowfall therefore increases the annual snowfall along this side of the lake by 30-40%.

The additive factor of lake-effect snowfall is dramatically illustrated along the lee side of the lake. Here the isopleths are packed quite closely together, their high gradient indicating a region of copious snowfall. The penetration inland of lake-effect snowfall averages 50-75 miles in Michigan. Some areas receive more than 60 inches of lake-effect snowfall annually, which is 200% more snowfall than at the same latitude in the interior of the state.

There are three significant features in the pattern of isopleths along the eastern shoreline of Lake Michigan. First, there are three cores of extremely heavy snowfall each averaging over 130 inches. The first two cores, centered on Gaylord-Vanderbilt and Maple City in northern Lower Michigan are a function of elevated topography, as well as a peninsular effect. The land area of this region is surrounded on three sides by the close proximity of water bodies therefore increasing the magnitude of lake-effect snowfall. Elevations above sea level for these two cores respectively are approximately 1435 feet and 850 feet, or 853 feet and 268 feet respectively above the mean level of Lake Michigan (582 feet).

The effect of these hills is twofold, one effect is to provide lifting, and the other is to provide increased surface friction for the bands of snow as they move inland from the lake. In an article by R. L. Peace and R. B. Sykes (1966) which examined the conditions attendant upon lake-effect storms at the eastern end of Lake Ontario, it was made evident that the lake-effect snow bands are characteristically shallow systems with radar-detectable tops usually lying below 10,000 feet. Extreme instability is also attendant with these bands. While the relief of these two areas in northern Lower Michigan may be insignificant for orographic snowfall during cyclonic and frontal

situations, it is significant for orographic snowfall during lake-effect snowfall situations.

The third core centered on Muskegon could be related to topography but not with as much confidence. Instead, the primary factors causing excessive snowfall over the Muskegon area are hypothesized to be pollution and urban influence. Several recent studies have indicated that there has been a considerable increase in the amount of air pollution downwind from Great Lakes metropolitan centers. The presence of high concentrations of ice nuclei and crystals in the Great Lakes area and the availability of moist air from off the lake is favorable for the occurrence of increased lake-effect snowfall. No significant evidence is available at the present time to confirm such a link. Therefore, with pollution becoming a more urgent problem there is the need for further research into the role of pollution in lake-effect snowfall. The urban effect results from heat being added to the bands of snowfall as they move inland from the shoreline. The heat added to the bands of snowfall increases the instability of the air masses and subsequently the fall of snow for the area surrounding Muskegon.

The hills of western Michigan increase the surface friction greatly as these bands of snowfall move across the region and this creates the second significant feature of the pattern of isopleths. This increased surface friction for areas of hills, and conversely, the relative lack of surface friction for areas of little local relief, creates a phenomenon which can be termed "avenue of penetration". It is hypothesized that where there are hills close to the shore of the lake, large amounts of snowfall result from the fact that the increased surface friction slows the storm bands down so that much of the energy is released near the shoreline and little residual energy or moisture remains by the time the band has crossed the hilly region. Conversely, where there are no topographic barriers along the shore, the bands of snowfall can progress inland with only a gradual loss of moisture and most likely extend further inland creating avenues of penetration.

The third significant feature in the pattern of isopleths as illustrated on Figure 1 is the occurrence of the maximum amounts of snowfall inland away from the shoreline. The maximum amount of lake-effect snowfall is felt at the shoreline of the western side of the lake with decreasing amounts of snowfall occurring inland. However, in Michigan (not including the Upper Peninsula) the maximum amounts of snowfall occur approximately 25 miles inland. This is in agreement with the findings in Changnon's (1968a) study of annual snowfall in the Lake Michigan basin. In his study, Changnon found that the maximization of lake-effect snowfall occurs anywhere from 10 to 25 miles inland, with 10 to 40 more inches of snow there annually than at the immediate eastern shoreline of Lake Michigan.

It is hypothesized that this phenomenon exists as a result of the increased surface friction as the air passes from the lake onto the land surface of Michigan and Indiana, causing the air masses to slow down

and begin to pile up inland some distance from the shoreline. The increased friction resulting from rough topography can enhance this process of convergence of air masses as the bands of lake-effect snowfall move inland.

Comparison of The Average Monthly Snowfall Patterns with The Annual Pattern

The average monthly snowfall patterns for each of the four months under investigation were portrayed on Figures 2-5 using an isopleth interval of 5 inches. As in the case of the annual average snowfall, the positioning of the isopleths across the lake was based upon estimation.

The three cores of extremely heavy snowfall which appeared on the map of annual snowfall appear only from December through February. During November the second core of heavy snowfall around the vicinity of Maple City has only begun to develop. The third core of heavy snowfall centered over Muskegon is well developed by this time. There is the development of a fourth core of snowfall south of Muskegon for all of the months under study except January. The significant feature of this core of snowfall is that the diameter is considerably larger, averaging three times the size of the largest of the other three cores. During January the isopleths of snowfall around the core of snowfall centered on Musekgon curve southward and are elongated to such a degree that the area represented by the fourth core might be considered to be a part of the Muskegon core.

The distributions of the average monthly snowfall also dramatically display the avenues of penetration. For all months under investigation, the tendency for fingers of heavier snowfall to extend inland is quite definite. The eastward extent of the avenues of penetration remains almost identical throughout the remainder of the snow season. The only variance from month to month is in the intensity of snowfall or amount of snowfall represented by the avenues of penetration.

As in the distribution of the average annual snowfall, there is the tendency for the heaviest amounts of snowfall to occur inland some 20 to 30 miles from the eastern shoreline for each of the months under investigation. It is significant to note that whether the month under investigation averages little snowfall or copious amounts of snowfall, the heaviest snowfall area remains identical.

Conclusions

An analysis of the annual and monthly snowfall patterns within the vicinity of Lake Michigan revealed an uneven spatial distribution of snowfall. This uneven distribution of snowfall results from the additive influence of lake-effect snowfall. The average distribution and magnitude of lake-effect snowfall was determined from these patterns. There were three significant patterns of lake-effect snowfall which appeared on all the maps of snowfall: Cores of excessive snowfall; avenues of penetration; and, a tendency for the heaviest amounts of snowfall to occur inland 20-30 miles from the shoreline.

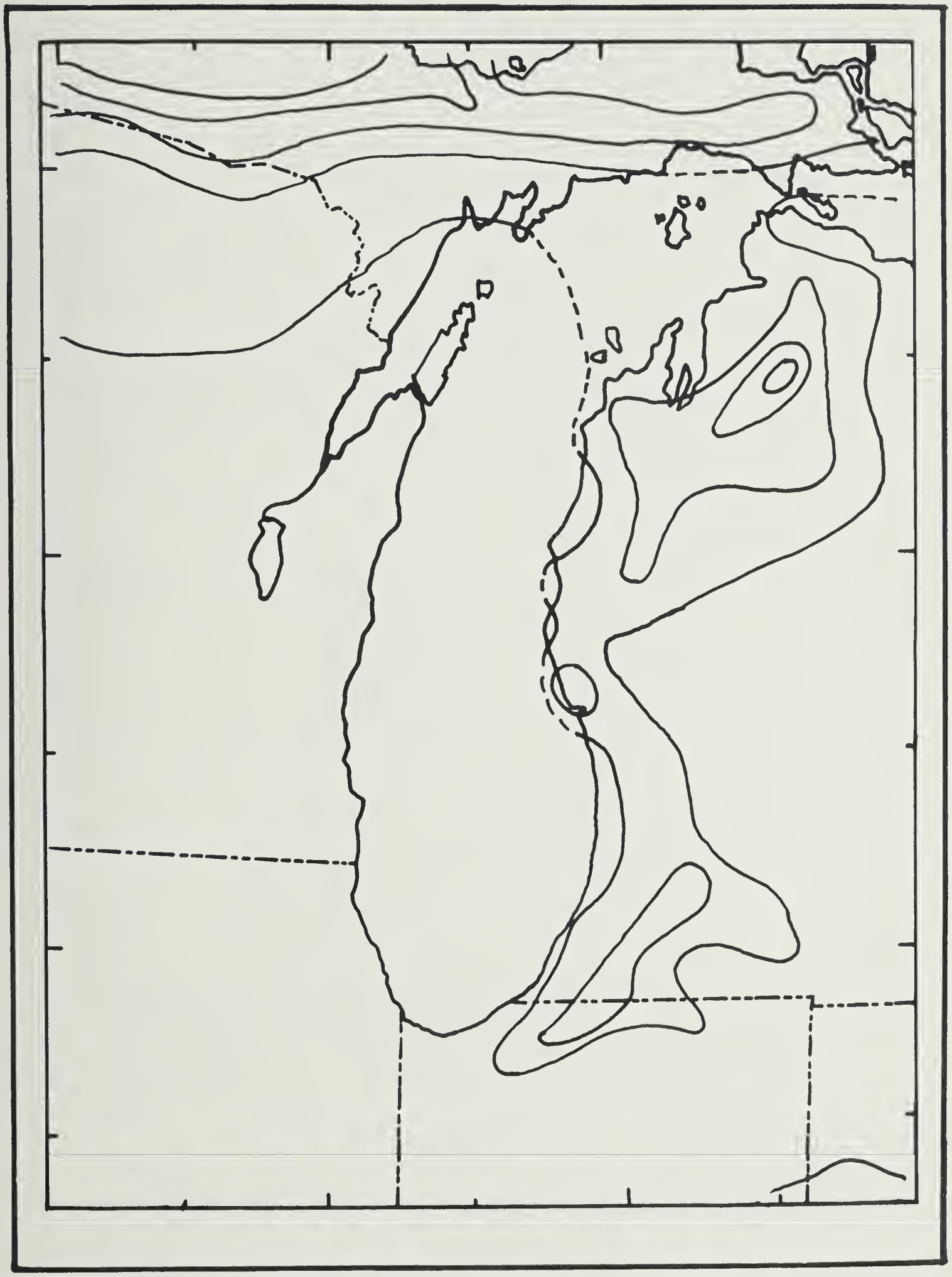

FIGURE 2. Average November Snowfall Pattern (5 inch interval).

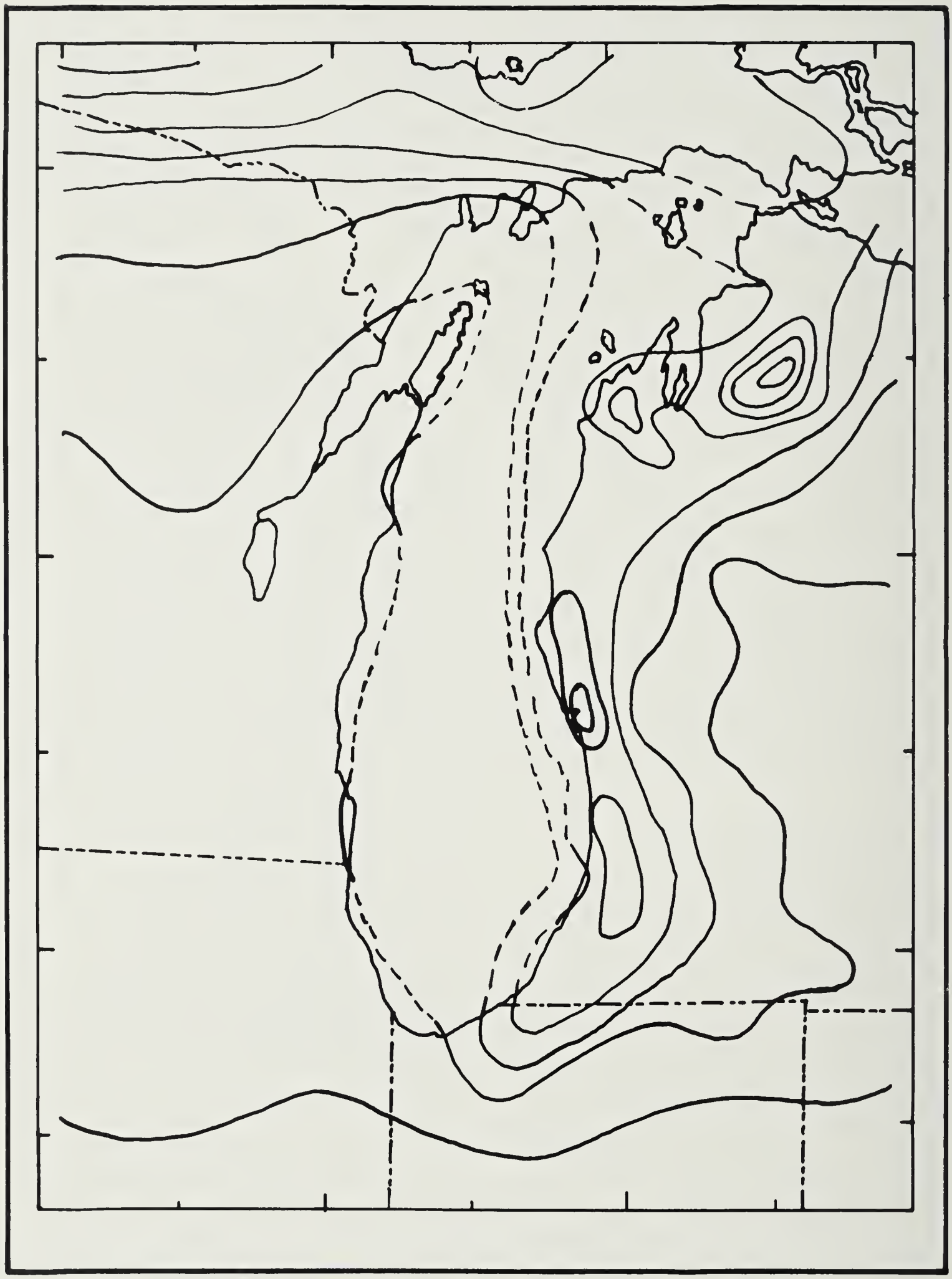

FIGURE 3. Average December Snowfall Pattern (5 inch interval).

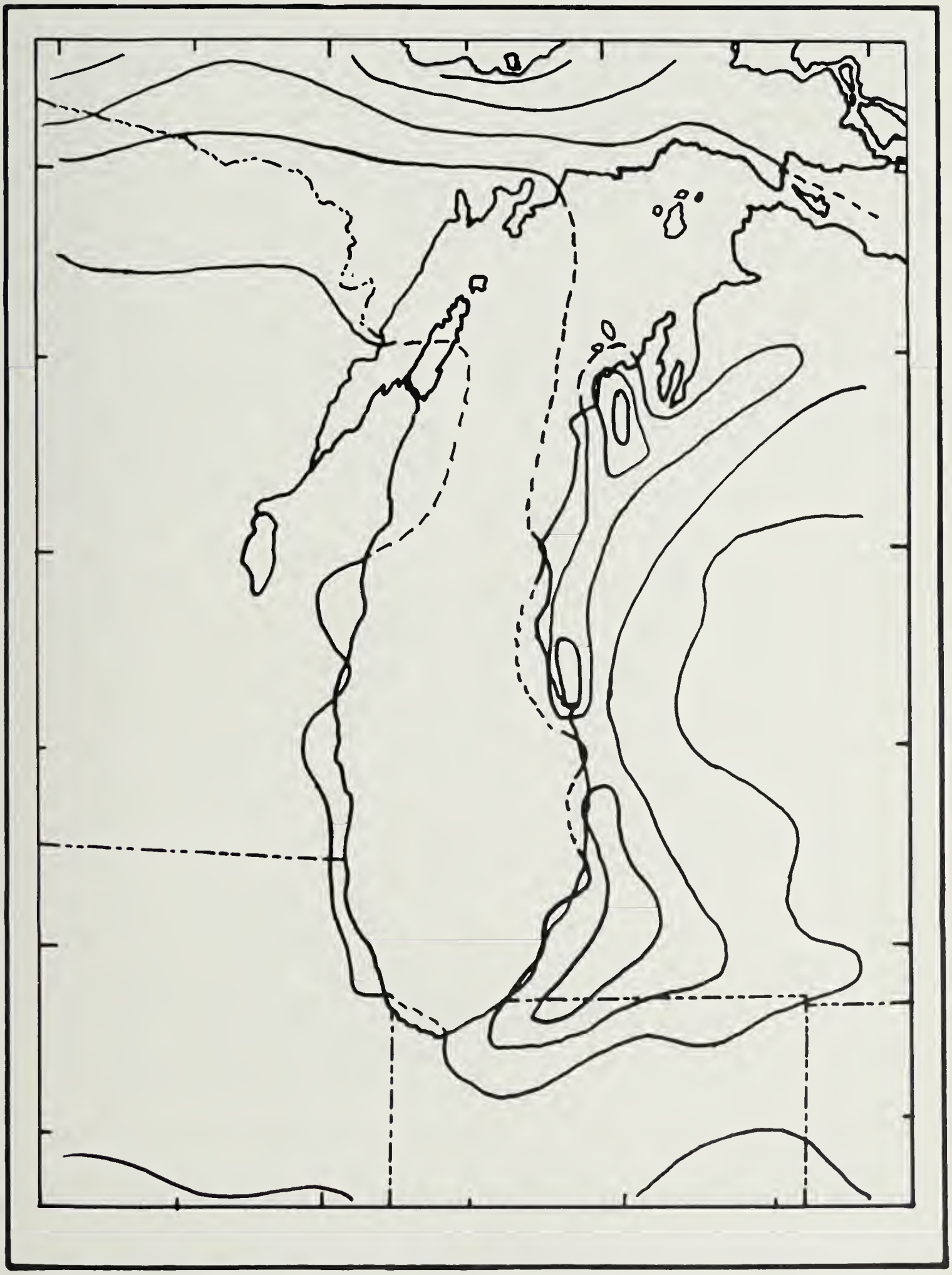

FIGURE 4. Average January Snowfall Pattern (5 inch interval).

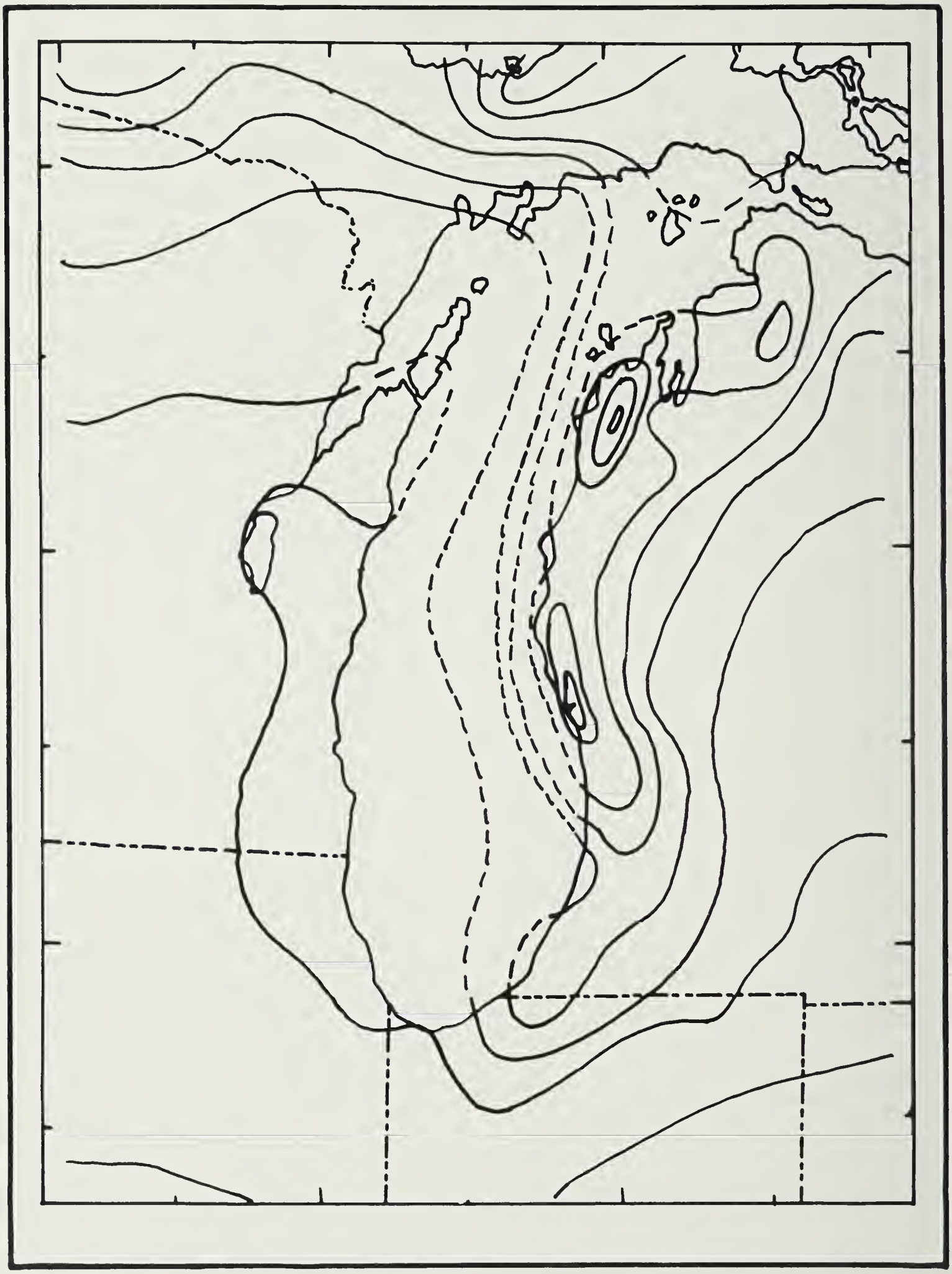

FIGURE 5. Average February Snowfall Pattern (5 inch interval).

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THE COUPLING OF ENERGY PRODUCTION TO SYNTHESIS IN THE ORIGINAL OPERATION OF LIVING SYSTEMS

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ABSTRACT.—The development of the self duplication of macromolecules into a living system is usually considered either to be due to heat energy, or without any explicit regard for the required energy supply. Simplified thermodynamic models are presented to illustrate that kinetic energy alone may not have been able to provide the necessary characteristics for the development of the synthesis of macromolecules into life processes. Reasons are presented for believing that the synthesis could only have developed in living systems after coupled to exergonic reactions.

Investigators concerned with the various phases of the origin of life processes, such as primitive photosynthetic reactions or the development of replication, have not generally related them to a coupling of endergonic with exergonic reactions. It is quite common to discuss the development of self duplication of macromolecules into primitive biological systems without explicit regard for the energy supply necessary for the process (Gaffron, 1965). In this communication, I shall consider some reasons why the coupling of energy producing reactions to synthesis or reproduction may have been a primary prerequisite for the emergence of original life processes. Justifications for the explicit inclusion of this coupling in the definition of a living system will be examined.

This problem might be approached by attempting to derive the most simple systems which still retain certain characteristics which are essential for present forms of life. A description of primary, essential characteristics (and their distinction from secondary ones) is difficult to make in an objective or unarbitrary manner. Nevertheless, the following three features may appear sufficiently reasonable for use as a tentative hypothesis for the present purpose:

1. *The direct or indirect collection and storage of solar energy.* Photosynthesis could be regarded as a direct collection, while the consumption by non-photosynthesizing or heterotrophic organisms of photosynthetic products would then constitute an indirect collection. Insofar as the products of photosynthesis have a higher potential energy than the reactants, they represent a form of stored energy which can later be released by exergonic reactions.
2. *The coupling or controlled use of this energy for synthesis of additional energy collecting structures*: i.e., reproduction or growth.
3. *The necessity of a specific mo-*

lecular geometry or structure for the above processes. A specifically ordered molecular geometry (as in chloroplasts, mitochondria, etc.) is generally recognized as a universal characteristic of present forms of life (Dean & Hinshelwood, 1966).

Attempts to derive the most simple system which still retains these three characteristics might lead to thermodynamic models (without specifying reactants) such as those in Figure 1. In Figure 1a, for example, conformation I consists of one or more substances whose molecules are in a particular geometrical arrangement essential for the utilization of solar radiation. When such radiation occurs, conformation I stores this energy by changing to a different conformation of higher potential energy, I′. When the conformation returns from I′ to I, the energy liberated is used for (or coupled to) the synthesis of more of the substance in conformation I (from constituents in the surroundings). This synthesis requires the initial presence of the structure being synthesized, which acts both as an autocatalyst for its self reproduction and as an acceptor and storer of light energy for this purpose.

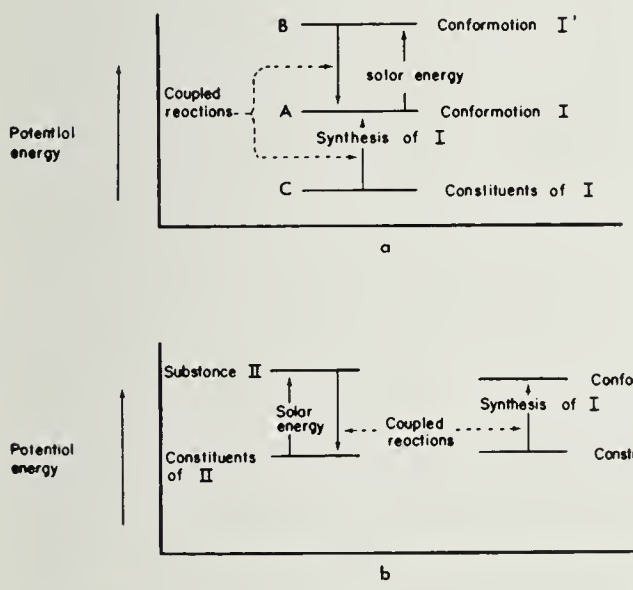

FIGURE 1. a. Energy for coupling stored by a photo-induced high energy conformation. The potential energy level A is that of a structure whose molecules are in a particular geometrical arrangement necessary for acceptance and storage of light energy. This is done by a light energy induced change from conformation I to a conformation of higher potential energy, I′, (at potential energy level B). Upon reverting back to conformation I, the energy liberated is used for autocatalytc synthesis of more material of structure I, from constituents of potential energy C.

b. Energy for coupling stored by the photo-induced formation of intermediate substance II, of higher potential energy than that of the reactants. The energy liberated by the exergonic reactions (or metabolism) of II is coupled to the synthesis of additional material of structure I.

In analogy with some concepts of present forms of photosynthesis, the conformational change from I to I′ could be associated with other conformation — dependent processes, such as oxidation-reduction reactions, or cation-proton exchanges. A photooxidation-reduction reaction, for example, could depend upon a conformational change if the change brings the bound groups which become oxidized and reduced close together.

Figure 1b illustrates a slightly more complex model. The solar energy is stored by the light-induced, endergonic formation of a separate compound, substance II, such as carbohydrate or ATP. Substance II is thus a form of stored energy, since it has a greater potential energy than the reactants. The reverse reaction, or metabolism of substance II, liberates the energy, which is coupled to the synthesis of the material (in conformation I) with the specific structure necessary to catalyze both the photoreaction and its

self reproduction.

These models retain the required characteristics in simplified or primitive systems. Although not essential to the main argument of this paper, the energy source has been represented as solar radiations, utilized via endergonic photoreactions. Daylight has been the only continuous source of useful energy, and is commonly considered the most probable energy source for original life systems (Gaffron, 1962).

A specific molecular geometry or arrangement may be essential for various reasons. It is difficult to demonstrate endergonic photoreactions with a high quantum yield in the laboratory. Gaffron (1965) has suggested that living organisms are uniquely able to obtain high quantum yields in such reactions because of the specific structural arrangement of the molecules. These molecular arrangements may serve to prevent the reversal or rapid recombination of reaction products, which would occur in a homogenous solution. Such a molecular arrangement can be considered metastable, due to its relatively high potential energy.

The models, or others that might be proposed, are meant only to illustrate how the coupling of exergonic reactions to the energy requiring synthetic reactions can be essential for the most primitive systems which still retain the components of a reasonable definition of life. Let us consider how this coupling can lead to an energy source which is utilized in a controlled manner; i.e., so that the extent of the energy collection and storage, energy liberation, and endergonic synthetic reactions are each correlated with the others according to the overall requirements of the system. If the molecules of the system are all in conformation I′ in the case of Figure 1a, or if all of the binding sites for substance II are occupied in the case of Figure 1b, then further energy cannot be stored beyond this maximum value, until some of the stored energy is utilized. The stored energy can only be used when the availability of the necessary constituents in the medium permits the energy-requiring synthesis to take place. The coupled use of the stored energy for this purpose would result in the reformation of conformation I from I′ in the case of Figure 1a, or the vacating of some of the binding sites for substance II in the case of Figure 1b. Further energy storing photoreactions can now take place. The coupling thus serves to limit the energy storage and liberation according to the requirements, which are determined by the extent of synthesis. Without the coupling, the energy uptake and liberation might proceed independently at maximum rates at a time when no substrates necessary for synthesis are available. In this case, the correlation of activities which is observed in present forms of life would not exist.

The mechanism of such coupling, i.e., the means of energy transfer from the site of energy liberation to the site of utilization, is also a primary consideration. When photosynthesis occurs in presently existing organisms, there is evidence that the radiant energy is transferred to, and used at, the reaction centers by means of a resonant transfer mechanism (Rabinowitch, 1963). Reso-

nant energy transfers are also a possible mechanism in the coupling of energy liberating reactions with synthetic ones. Various characteristics of resonant energy states make this possibility of interest (Rabinowitch, 1963; Ressler, 1969). The efficiency of energy transfer by a resonant mechanism depends upon the molecular geometry, which is consistent with the necessity for a specific molecular geometry or structure in living systems. The efficiency also depends upon the overlapping of the energy levels of the energy donor and acceptor. This provides the opportunity for energy "switches", or activation of reactions in a specific sequence. Resonant energy transfers can occur at both electronic and infra-red frequencies, so that steps with various energy requirements might be activated. Since this type of transfer does not require molecular contact or heat, it can occur with little increase in entropy. The possibility of this mechanism playing a role in biological processes such as enzyme activation and the control and coordination of energy transduction, has recently been discussed by the present author (1969).

The coupling of energy liberating and energy requiring reactions could, of course, also involve other factors. In Figure 1b, for example, the metabolism of substance II might be required because it liberates one of the substrates involved in the synthesis of the substance in conformation I. Whatever mechanisms may be involved, the energy requiring synthetic reactions must be coupled to an energy source in order for reproduction to occur. Without a regular source of energy, the reproduction of macromolecules would only be a temporary or transient phenomenon, without permanent consequences.

It might be assumed that the energy for initial life systems could have been provided by heat, without coupled, energy producing reactions. Even though a certain amount of kinetic energy, i.e., a given temperature range, may be necessary, this explanation still has certain limitations. Hulett (1969) has discussed reasons why the prebiological synthesis of intermediate substances, later used for development towards living systems, probably required a coupled energy source, rather than heat. The same considerations would be relevant when such substances started to become synthesized into biological systems. Biological structures involve a high degree of order, and a relatively high potential energy state. Heat or kinetic energy would increase the entropy or disorder. The stability of such a metastable state, and the accuracy of the reproduction would therefore tend to decrease at higher temperatures. The requirement of a specific molecular geometry for heat activated reactions is not apparent. It would also be difficult to account for the correlation and adaptation of different activities with changes in the environment, if heat were the sole energy source.

Theories concerned with the development of the synethesis of macromolecules into primitive life systems, which do not explicitly consider the energy source required, generally involve an implicit assumption that the reactions occur by virtue of heat energy. The large majority of such

theories do not specifically include energy-producing reactions coupled to this synthesis. Since this coupling would appear to be necessary before such synthetic reactions could develop into biologically functioning systems, it is hoped that this communication may be of value.

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NOTES

GIDEON HERMAN BOEWE

1895-1970

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G. H. Boewe in 1963

Gideon Herman Boewe, associate plant pathologist, Illinois Natural History Survey, and a member of the Illinois State Academy of Science, died suddenly in his home in Champaign, Illinois, on Saturday, December 19, 1970, at the age of 75 years.

Mr. Boewe was born October 3, 1895, in Parkersburg, Richland County, Illinois, a son of Henry M. and Augusta C. Maas Boewe. He spent his boyhood years on a farm and attended grade schools in Richland County. He graduated from the Olney Township High School. From 1918 to 1924 he taught in grade schools in Lawrence and Richland Counties. On June 12, 1921, Mr. Boewe married Isabelle Shafer in Olney. For nearly 50 years the Boewes were a devoted couple.

Mr. Boewe attended Illinois State Normal University and Eastern Illinois State Teachers College (now Eastern Illinois University), receiving a Bachelor of Education degree from the latter in 1928. He then enrolled in the University of Illinois and obtained a Master of Science degree in 1930. Mr. Boewe joined the staff of the Illinois Natural History Survey as field botanist on March 17, 1930. He became assistant plant pathologist in 1947 and associate plant pathologist in 1955. His work was primarily concerned with the distribution, severity, and incidence of field, forage, fruit, and vegetable crop diseases. He was, however, interested in plant diseases in general and collected numerous specimens which form a large part of the plant disease survey herbarium of the Survey. From 1933 to 1946, the accumulation of Illinois vascular plants for the Survey herbarium was added to the duties of the plant pathologists on the staff. Mr. Boewe conscientiously assumed this duty and collected numerous samples in conjunction with his work on the plant disease survey. Mr. Boewe retired in 1966, after 36 years in plant pathology at the Survey.

In the course of his field work, Mr. Boewe discovered a number of plant diseases new to Illinois. Among these were the downy mildew of soybean in 1929, a tiny toadstool on crop plants in 1935, charcoal rot of potatoes in 1941, and Helminthosporium blight of oats in 1947. In 1964, his report on plant diseases new to Illinois during the period of 1922-1964 included 123 organisms that attack 101

host plants. Plant pathologists in Illinois, and those in the corn belt, perhaps remember him best for his publications on field crop diseases and for his annual forecasts, beginning in 1948, on the late-season or leaf-blight stage of Stewart's disease of corn.

Mr. Boewe was active in his church, the First United Methodist Church of Champaign. He and his wife were members for 40 years. He not only attended regularly but served as an usher for 25 years, was a Church School teacher for many years, and, during the past 10 years was secretary of the Church School. For many years he served on the Official Board. He was also an active member of the Men's Club of the church. Those of us who associated with Boewe at work, soon learned that his religion was not a "Sunday cloak" but a vital part of his life which was exemplified daily by his kindness and generosity to his fellow men.

Mr. Boewe was a member of the Illinois State Academy of Science for many years and he attended the annual meetings regularly. In 1944, he was appointed to the Membership Committee, serving as a member until 1950 when he was appointed chairman. He served as chairman until 1959 when, at the annual meeting and at his request was retired from the position. Under his chairmanship the Academy grew, reaching the highest membership in its history. He served on the Council of the Academy from 1960 to 1964, was a member of the Nominating Committee in 1962-63, and a member of the Resolutions Committee for a number of years, beginning in 1963. He truly was devoted to the work of the Academy.

In addition to the Academy, Mr. Boewe was a member of the American Association for the Advancement of Science, the American Phytopathological Society, the Illinois State Horticultural Society, and the Illinois Vegetable Growers Association. He was also a member of the men's division of the W.C.T.U., serving as Treasurer of the local union and the county unit since 1967.

He is survived by his wife, and brother, Ellis, of West Salem, Illinois. He was preceded in death by his parents, one sister, and three brothers.

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1945

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A LEUCISTIC LITTLE BROWN BAT (*MYOTIS L. LUCIFUGUS*)

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ABSTRACT.—Review of the known records of albinism and leucism in Chiroptera, with remarks on a specimen of leucistic Little Brown Bat (*Myotis l. lucifugus*) from Illinois.

Few records of albinism and leucism of bats have been recorded. Allen (1940) and Setzer (1950) in reviewing the literature cited few instances, listing species of only nine genera. Since that time only twenty-three reports of albinism and leucism in bats have been published. This is a small number when one considers several million bats having been handled during banding operations (Table 1).

While recording recoveries of bats at Blackball Mine, 1.75 miles West of Utica, LaSalle Co., Illinois, on 9 January 1971, an unusual leucistic male, Little Brown Bat, (*Myotis l. lucifugus*) was taken from a cluster of 107. The aberrant individual

TABLE 1.—Records of albinistic and leucistic traits cited in bats. Numbers represent individuals.

Species	Albinism	Leucism	Author
Nycteris nana	1		Verschuren (1955)
Rhinolophus euryale	1		Dorst (1957)
Anoura caudifera		1	Linares (1967)
Glossophaga longirostris	1		Setzer (1950)
Antrozous p. pallidus	1		Setzer (1950)
Barbastella barbastellus		Numerous	Palasthy (1968)
Eptesicus capensis	1		Allen (1940)
Lasiurus borealis	2		Allen (1940)
Miniopterus schreibersi	1	1	Hamilton-Smith (1968) Dorst (1957)
Myotis daubentoni		1	Haensel (1968)
Myotis grisescens		3	Tuttle (1961)
Myotis lucifugus	1	1	Dubkin (1952) and this report
Myotis sodalis		Numerous	Metzger (1956) Barbour and Davis (1970)
Myotis velifer incautus		1	Rogers (1965)
Nyctalus noctula	1		Dulic and Mikuska (1968)
Nycticeius humeralis		1	Easterla and Watkins (1968)
Pipistrellus pipistrellus	1		Allen (1940)
Pipistrellus s. subflavus		1	Blair (1948)
Mollossus fortis	1		Heatwole et al. (1964)
Molossus tropidorhynchus	1		Allen (1940)
Tadarida b. mexicana	1	Numerous	McCoy (1960) and Glass (1954), Herreid and Davis (1960)
Tadarida (Chaerephon) plicatus	3		Allen (1940)
Tadarida femorosacca		1	Mitchell (1963)

(Figure 1) exhibited a completely snow white ventral coloration, with an extensive white patch extending up over the right shoulder and covering approximately half the dorsal surface of the body. The interfemoral membrane, head, and wings are of normal pigmentation. The eyes of this individual are black. The specimen is preserved as a skin and skull (HDW 1102). Standard measurements (mm) 80; 33; 10; 14.

I have been unable to find any similar reports for *Myotis l. lucifugus*, although Dubkin (1952) cites a completely albinistic specimen. Of some 20,000 *M. lucifugus* handled in northcentral Illinois, only one individual exhibited this trait.

It is interesting to note that leucism occurs rather frequently in three species, *Tadarida brasiliensis mexicana* (Glass, 1954); *Barbastella barbastellus* (Palasthy, 1968) and *Myotis sodalis* (Barbour & Davis, 1970).

FIGURE 1. Leucistic Little Brown Bat (*Myotis l. lucifugus*).

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THE FIRST RECORD IN ILLINOIS OF A POPULATION OF *STETHAULAX MARMORATUS* (SAY) (HEMIPTERA: SCUTELLERIDAE) WITH INFORMATION ON LIFE HISTORY

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ABSTRACT.—The first record in Illinois of a population of *Stethaulax marmoratus*, and life history information are presented.

The genus *Stethaulax* contains at least one species in America north of Mexico, *S. marmoratus* (Say). However, *Aulacostethus simulans* Uhler, found in Arizona and California, may be a second species of *Stethaulax* (Blatchley, 1926).

S. marmoratus has been collected in several states including New York, New Jersey, Maryland, North Carolina, Georgia, Missouri, and Texas. Hart (1919) did not list the species for Illinois. However Malloch, in editing Hart's posthumous publication, added that he had collected one female specimen at Cobden, Union Co., May 9, 1918. Since only one specimen has been listed for the state, there is some doubt whether or not the species is established in Illinois.

Little is known of the biology of this insect. It has been collected from cedar (species unknown) and *Rhus aromatica* Ait. It has also been found on *R. canadensis* Marsh. (*R. aromatica*?). The adult is mottled in appearance (Fig. 1), measuring 4.5-5.5 mm in width, 6.0-7.5 mm in length (Blatchley, 1926; Froeschner, 1941).

On October 17, 1970, we found 23 individuals of this species feeding on drupes of *R. glabra L.* in Jackson Co. on and near the Southern Illinois University campus, Carbondale. These sites were approximately 14 miles north of Malloch's site of collection. The animals were well camouflaged and thus, difficult to detect (Fig. 2).

There are five nymphal instars as determined by rearing in the laboratory. In the field, evidently, the entire developmental period is spent on the drupes. Eggs and first-instar nymphs were not collected. However, egg shells found on October 31 indicated the eggs had been laid in clusters, typical of scutellerids studied thus far, on the twigs and surrounding drupes. It is very probable, based on work previously done on related species, that the first-instar nymphs are also found here.

Second-instar nymphs through adults were found continuously October 17-31, actively feeding. On October 31, approxi-

FIGURE 1. Female (left) and male of ***Stethaulax marmoratus*** in copulo.

mately 80% of the 81 individuals collected were adults. By mid-November, the insects had left the host plants.

The length and width of 10 adult males and females were measured (Table 1). Length was from tip of tylus to hind abdominal margin; width was across the humeral angles. Measurements approximated those previously reported. Also, using Student's t-test, the lengths and widths of females were found to be significantly larger than those of males ($P < .025$).

Our collection dates are unique because specimens taken by other investigators have been between May 7 and September 16. Also, it was unusual to find individuals still developing at this time since, to our knowledge, individuals of other species of Scutelleroidea in this area had completed development by the end of September of the same year. It is impossible to state whether this species is uni- or bivoltine.

Based on the above information, this species appears to be well established in southern Illinois.

TABLE 1.—Measurements of adult *Stethaulax marmoratus* in mm.

	Male		Female	
	Length	Width	Length	Width
Number	10	10	10	10
x ± S.E.	6.7 ± .2	4.5 ± .1	7.6 ± .1	4.9 ± .1
Range	5.7–7.3	3.8–4.9	7.1–8.3	4.4–5.2

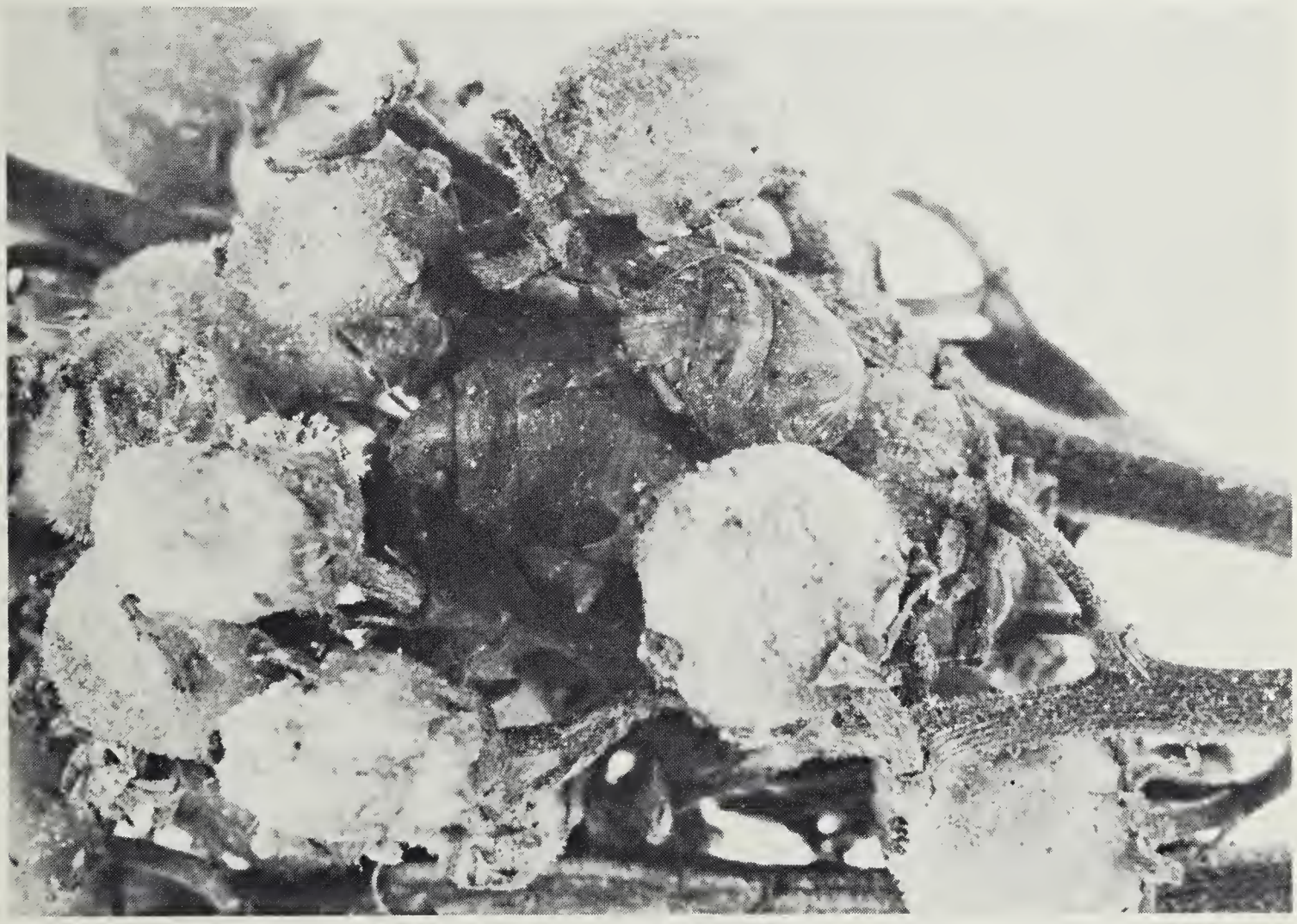

FIGURE 2. Two fifth-instar nymphs hidden among drupes of *Rhus glabra*.

Acknoweldgments

We wish to thank Dr. H. I. Fisher, Southern Illinois University, for reviewing the manuscript and Dr. R. C. Froeschner, U. S. National Museum, for confirming our identification of this insect. We also are grateful to Mr. John A. Richardson, Southern Illinois University, who provided the photographs.

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Manuscript received February 2, 1971

MR. STOVER

HIRAM F. THUT AND JOHN EBINGER
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Professor Ernest L. Stover, died November 30, 1969. He taught at Eastern Illinois College and University for 37 years, was a past president of the Illinois State Academy of Science, served on its council for ten years, and served on various committees concerned with teaching.

Mr. Stover, the son of a Methodist minister, was born in Belbrooke, Ohio, on August 28, 1893. He grew up in southwestern Ohio, attended elementary school in Jeffersonville and Mechanicsburg and graduated from the Richwood High School. After a year at Adrian College, Mr. Stover finished his bachelor's degree at Ohio State in 1917.

As a college student, Mr. Stover played a baritone horn in the marching band. World War I interrupted his graduate work. He served with the Engineers in 1918 and saw limited action. Soon after the Armistice, he asked to be transferred to the band as an entertainer. For some months in 1919 he was stationed in Paris, entertaining soldiers. Recently Mr. Stover received his 50th year citation as a Legionnaire. He also had the opportunity to study briefly at the Sorbonne and the Pasteur Institute. His study at the Pasteur Institute made him keenly aware of the opportunities in science, especially botany. While in Paris, he bought a violoncello and took private music lessons. This violoncello was a pride and Joy to Mr. Stover for the rest of his life.

On returning to the states, Mr. Stover became an instructor in botany at the Ohio State University, 1919-1923. At that time he was not sure whether it would be botany or music. He was playing cello quite successfully with a theatre orchestra and some hotel bands. However, in time he was influenced by Dr. E. N. Transeau to give botany his primary effort. He obtained his Master's degree in botany in 1921. That was followed by graduate work at the University of Chicago, where he obtained the Ph.D. degree in botany in 1924.

Mr. Lord, President at Eastern, needing a botanist, asked his very good friend Dr. Transeau, who had taught at Eastern from 1908-1915, to recommend a likely candidate for the position. Dr. Transeau recommended the man he had persuaded a couple of years earlier to become a botanist. In September 1923 Mr. Stover joined the faculty of the Eastern Illinois State Teacher's College at Charleston. Mr. Stover served as botanist and head of the department until 1960.

Mr. Stover, as a botanist, had two primary interests — good teaching and plant anatomy. His 30 some odd scientific articles and books were published almost alternately between teaching and plant anatomy. As a teacher Mr. Stover wanted his students to know plants. He had a way of making a student feel that they had discovered something together while being helped through the recognition keys. Early in his teaching career he affiliated himself with the Illinois Academy of Science and served on several committees. His particular interest was in the committees that had to do with teaching.

The Botanical Society of America became much interested in good teaching, and in 1936 appointed a committee on the teaching of botany in colleges and universities. Mr. Stover was appointed chairman of that committee and with the help of a grant from the General Education Board and the research assistance of Dr. Clark W. Horton, they published two bulletins. In 1941 he was chairman of a committee on instruction in the biological sciences for the National Research Council.

Besides his botanical work, Mr. Stover had many other interests. He was a member of the National Education Association, a member of the Illinois Education Association and was President of the Eastern Division of the Illinois Education Association in 1943. He held membership in the A.A.A.S. (fellow), in Sigma Xi and Gamma Alpha. He retained his interest in music and was a member of Eastern's College Orchestra for years.

Mr. Stover married Patsy H. Lupo in

1923. They had been graduate students together at the University of Chicago and later she was teaching botany at Rockford College. She died in 1956. In 1959 he married Ethel Hanson, a music instructor at Eastern.

His death removed from our midst a scholar, a skilled teacher, a sensitive person, a successful administrator and most of all a friend. That was Mr. Stover.

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Manuscript Received December 21, 1970

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December 1, 1970

CONTENTS

CARDIAC OUTPUT AND CENTRAL BLOOD VOLUME AS A FUNCTION OF BODY WEIGHT IN THE BABOON

G. S. MOSS, L. D. HOMER, C. M. HERMAN, DONALD SIEGEL, ALAN COCHIN, AND VIDAL FRESQUEZ
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ABSTRACT. — The relationship between cardiac output and central blood volume as a function of body weight was investigated in tranquilized adult baboons. Cardiac output was determined by the dye dilution method. Central blood volume was calculated as the product of cardiac output and mean transit time. It was concluded that weight raised to the .62 power offers a slight improvement in comparing cardiac outputs between animals of different sizes. A simple weight index for central blood volume should be a satisfactory expression for the parameter.

To compare changes in cardiac output and central blood volume observed in baboons with those observed in other animals of varying sizes, it would be helpful to have an index for the basal value of cardiac output and central blood volume in the baboon, based on weight or some function of weight. For the purposes of this report, Central Blood Volume (CBV) is defined as that volume of blood contained between the tips of the sampling catheters in the right atrium and the arch of the aorta. An examination of the relationship between weight and these variables in the baboon is the subject of this report.

METHOD

Adult baboons (*Papio doguera*) weighing between 13.7 and 27.5 kg were used in this study. The basal cardiac output in 48 animals and the central blood volume in 19 were determined according to a technique published elsewhere (Moss et. al., 1968). Briefly, in each animal, a siliconized heparin-filled plastic catheter was implanted in the thoracic aorta and another in the pulmonary artery through a left thoracotomy two to three weeks prior to study. The ligated free ends of the two catheters were implanted under the skin of the left chest and the animal was then returned to his cage. On the morning of the study, the baboon was tranquilized with 1-(phenylcyclohex) piperidine HCL (Sernylan, Parke-Davis), 1 mg/kg of body weight, based on the animal's original weight. The animal was then reweighed and the second weight was used for this report. Each animal was then placed in the prone position, with its anterior chest wall protected by padded axillary supports and the extremities loosely restrained.

After a one-hour basal period, two dye dilution curves were obtained in the following fashion. Five milligrams (1 ml) of indocyanine green dye were injected into the pulmonary artery catheter, followed by a forceful 7 ml bolus of isotonic saline. Arterial blood from the aortic catheter was simultaneously aspirated through the cuvette of a cardiodensitometer (Beckman) at a rate of approximately 20 ml/min by means of a withdrawal pump (Gilford, Model 105-S). The cardiodensitometer recorded the resultant indicator dilution curve and also mechanically integrated the area under the curve, including recirculation. Correction for recirculation was made by extrapolating the exponential downslope to the base line, or, in most cases, by using a logarithmic nomogram provided by the manufacturer for this purpose. Central blood volume was calculated as the product of cardiac out-

put and mean transit time (Hamilton et. al., 1932, Kinsman et. al., 1929, Stewart, 1921-22). Mean transit time was obtained from the least squares fit of a gamma variate to the dye curves (Thompson et. al., 1964).

Statistical Analysis: The relationship between the average of the two cardiac output measurements and weight was examined by the least square method after taking the natural logarithms of both sets of numbers. Central blood volume versus weight was examined in a similar fashion. The general formula for this regression is log $Y = b_o + b_1 . W$, where Y = either cardiac output or central blood volume, b_1 = slope, b_o = intercept, and W = weight. The 95% confidence limits were calculated with a table of t.

The estimating equation for cardiac output was log C. O. = −0.70 + 0.62 log weight. The standard error of the coefficient of log weight was 0.21.

The estimating equation for central blood volume was CBV = 1.80 + 1.19 log weight. The standard error of the coefficient of weight was 0.44.

Results

Figure 1 shows the scatter diagram of cardiac output and weight plotted on a double logarithmic scale. Also shown in the diagram is the regression line fitted by least squares and the 95% confidence limits for Y. The estimating equation for the best fit is log cardiac output = −0.70 + 0.62 log weight. Although the relationship between cardiac output and weight is statistically significant ($p < 0.01$), it is at best only approximate. This is evidenced by a low coefficient of correlation, R = 0.39; wide 95% confidence limits of b^1, 0.19 to 1.04; and wide 95% confidence limits of b_o, 0.57 to − 1.97.

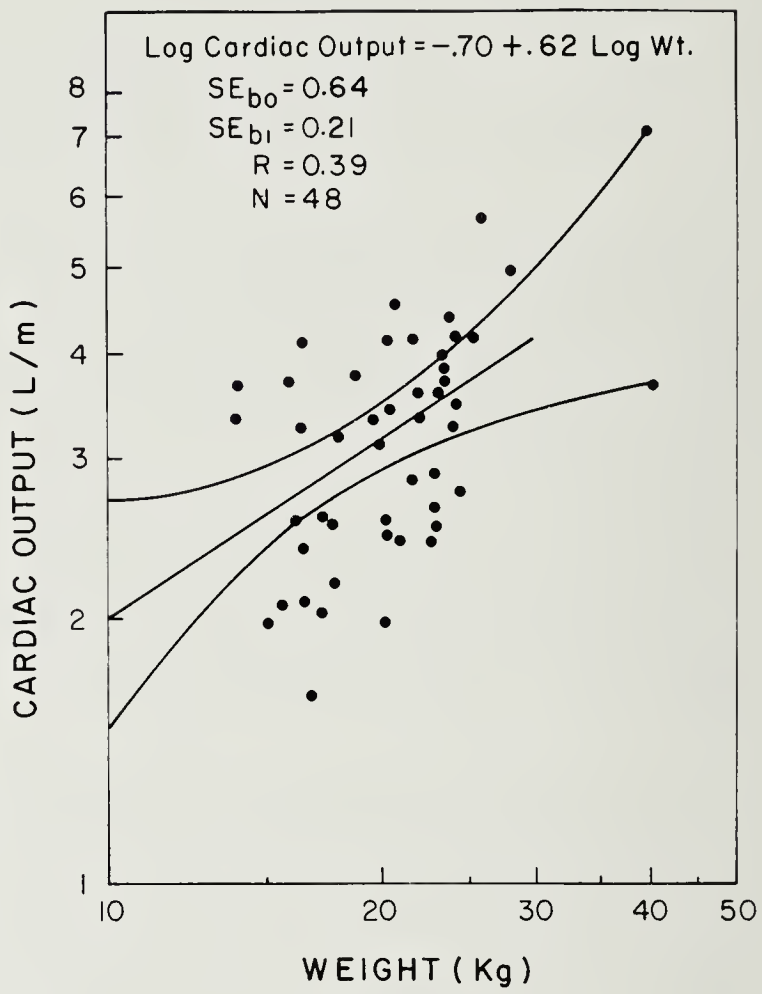

FIGURE 1. The relationship between cardiac output and weight in 48 tranquilized baboons. SEbo = the standard error of the intercept, and SE b_1 = the standard error of the slope.

The mean cardiac output (liters/minute) for the 48 baboons was 3.80 ± 0.82. The coefficient of variation was 26%. The index obtained when the cardiac output of each animal was divided through by $(\text{weight})^{0.62}$ had a mean value of 0.50 ± 0.10. The coeffiicient of variation was 20%. The reduction in the coefficient of variation gained in the latter expression is only a minor improvement in expressing cardiac output.

Figure 2 shows the relationship between CBV and weight on a double logarithmic plot. Also shown are the least squares line, 95% confidence limits of Y, standard error of the slope and intercept, and the coefficient of determination. Despite a reasonably high R value, there is a substantial degree of scat-

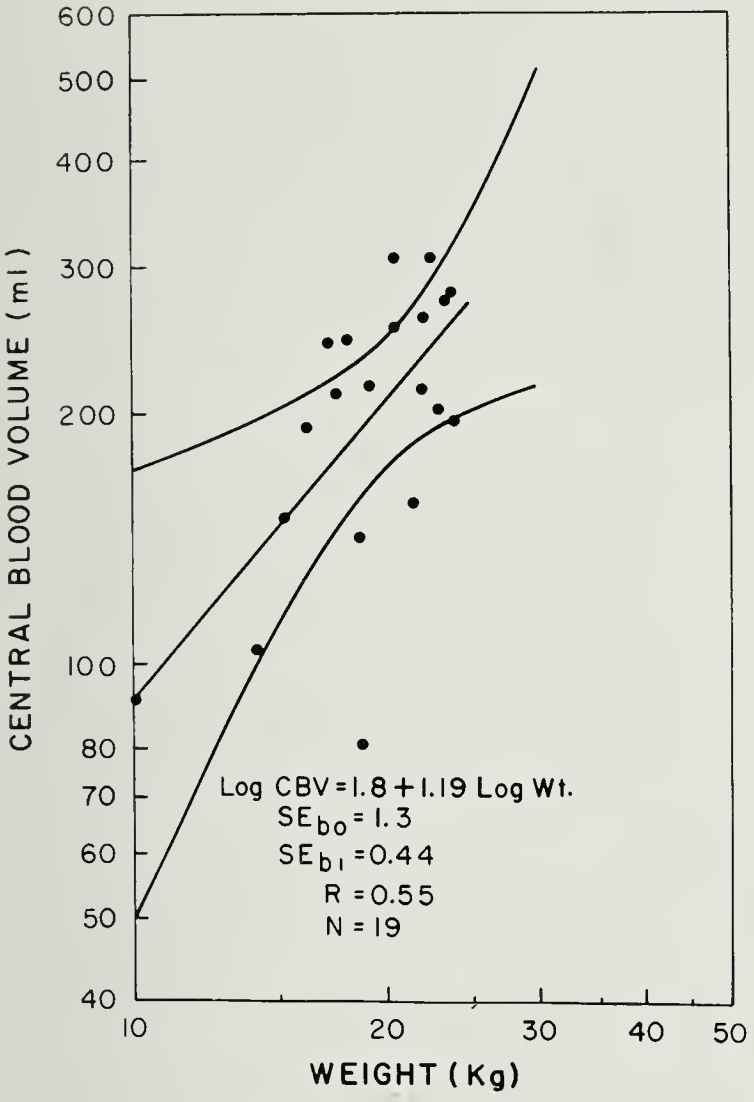

FIGURE 2. The relationship between central blood volume and weight in 19 adult tranquilized baboons. SEbo = the standard error of the intercept, and SE b_1 = the standard error of the slope.

ter in the relationship. The slope of the line is not significantly different from 1.0. The central blood volume index for the 19 baboons is 10.7 ± 2.9 ml/kg.

DISCUSSION

A number of investigators have studied the relationship between cardiac output and some function of body weight in animals and man (Brotmacher et. al., 1956, Cournand et al., 1945, Stead et al., 1945, Tanner, 1949, Taylor et al., 1952). Most have reported a significant but low correlation, as found in this study. A high degree of scatter was observed consistently. Some have advocated using body weight raised to the two-thirds power, although others have favored the three-fourths power. In this study of baboons, cardiac output was best correlated with weight raised to 0.62 power, which is quite close to the two-thirds power. However, because of the large standard error of the exponent, either number could have been selected in calculating an expression for cardiac output for the baboon, based on weight. Indeed, it is not clear that either method is superior to simply dividing cardiac output by weight.

Guyton (1963) recalculated a similar expression for animals of all sizes, from the rat to the horse, using weight raised to the two-thirds power. He found that in general the value for cardiac output varied directly with body weight. The rat cardiac output was 0.15 L/kg$^{2/3}$, the dog cardiac output was 0.31 L/kg$^{2/3}$, and the horse cardiac output was 0.46 L/kg$^{2/3}$. Our reported baboon cardiac output of 0.50 L/kg$^{0.62}$ is consistent with this general scheme in that it lies between the values for rats and horses and not far from that of the dog.

For making rough comparisons between the results in baboons and other animals of varying sizes, the baboon cardiac output divided by (weight)$^{0.62}$ may prove helpful. However, in baboons of a similar size to those studied in our investigation, the simple value for cardiac output alone or cardiac output divided by weight should be satisfactory for comparison.

Abel, Waldhausen, Daly and Pearce (1967), in a study of hemorrhagic shock in five young baboons weighing 8.8 to 10.5 kg. reported basal cardiac outputs of 84 ml/min per kilogram. This figure is substantially lower than the 150 ml/min per kilogram we would calculate for a 20 kg. baboon in our study, and well below the 95% confidence region of our estimating equation extrapolated to 10 kg. The upper 95% limit of these investigators was about 140 ml/kg.

The basal cardiac output of the dogs in the study of Abel et al., (1967), when adjusted to the two-thirds power of body weight after the manner of Guyton, was 0.26 $l/kg^{2/3}$. For the baboons in their study, the figure would be approximately 0.17 $l/kg^{2/3}$.

We do not know why our estimate of cardiac output differed from that of Abel and co-workers. We did note, however, that their animals were referred to as "young" and that their anesthetic consisted of a mixture of barbiturates, given both intramuscularly and intravenously, together with morphine given intramuscularly. In contrast to the disparity in cardiac output estimates, their CBV estimate of 10.2 ml/kg closely agreed with our estimate.

The validity of expressing central blood volume as the product of cardiac output and mean transit time was confirmed by Schlant and his associates (Schlant et al., 1959). They compared this measurement with results observed when the actual amount of blood in the heart and lungs was calculated from the total radioactivity in these organs in dogs previously treated with chromium-51 labeled red blood cells. They found a high correlation ($R = + 0.88$).

Since the slope of the line shown in Figure 2 is not significantly different from 1.0, dividing central blood volume estimates by body weight should provide a means of reporting this variable that would make approximate comparisons with other baboons convenient. It is not at all certain, however, that the same index would be suitable for comparison with other species. If, in fact, CBV increases linearly with weight, while cardiac output does not, then the mean pulmonary transit time of indicator should be greater in animals of large species than in those of small species.

Acknowledgements

This study was supported by Research Task No. MR005.19-0004, from the Bureau of Medicine and Surgery, U.S. Navy Department. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as offiicial or as reflecting the views of the Navy Department or of the Naval Service at large.

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EVALUATION OF THE DIGESTION—BAERMANN TECHNIQUE FOR THE DETECTION OF DEAD TRICHNAE

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ABSTRACT.—The value of the digestion-Baermann technique as a diagnostic tool for the detection of dead *Trichinella spiralis* larvae was reinvestigated. Quantitative studies showed this method to be only 6.0 per cent efficient and that as high as 31.0 per cent of the trichinae failed to pass through the opening of the screen. Examination of the filtrate only would have failed to detect a significantly large number of dead larvae. A decrease in the number of larvae recovered appeared to be directly proportional to the length of the digestion period.

It is generally agreed that the digestion-Baermann technique is rather dependable for detecting live larvae of *Trichinella spiralis* (Owens, 1835) Raillett, 1895. However, in surveying the prevalence of *T. spiralis* in wild animal populations it is not always possible to examine fresh tissue and some carcasses must be frozen for inspection at a later period. The efficiency of the digestion-Baermann technique as a diagnostic tool for detecting dead larvae has for the most part been evaluated on the results of comparative studies utilizing data obtained from tissue examined both by the direct microscopic method and by the digestion-Baermann technique. In view of the fact that some workers continue to utilize the digestion-Baermann method only for examining either frozen tissue or tissue removed from the dried skins of animals, the purpose of the present investigation was to reevaluate the digestion-Baermann method on the basis of data obtained from studies containing quantitative elements.

MATERIALS AND METHODS

Dead trichinae were obtained from the diaphragms and skeletal muscles of experimentally infected rats which had been frozen for two months and subjected to a modified artificial digestion-Baermann technique of Kerr *et al.* (1941) in that a 60-mesh screen and a digestion period of 2.5 hr were substituted for an 80-mesh screen and an 18 hr digestion period. Approximately 200 dead larvae were isolated, examined, transferred to a watch glass, and counted four times. The dead larvae were then added to a trichinae-free 40 gm sample of skeletal muscles of frozen white-tailed jack rabbits, *Lepus townsendii* Backmann, 1839, which had been artificially digested in 2 liters of a mixture of 1.0 per cent pepsin solution and 0.7 per cent hydrochloric acid at 37° C. for 4 hr under constant mechanical agitation. After settling for 1 hr, two-thirds of the mixture was siphoned off and examined for the presence of larvae. The remaining material was poured through an 80-mesh screen into a 3-liter funnel and enough water added to cover the screen. The filtrate was then drawn into a ruled petri dish for examination and counting of trichinae under a dissecting microscope. The screen was transferred to a large container, inverted, washed several times with a strong stream of water and the wash transferred to a funnel with a short neck closed by means of rubber tubing and a Hofmann clamp. The wash was then drawn into a ruled petri dish for examination and counting of trichinae.

TABLE I.—Recovery of *Trichinella spiralis* added to digest rabbit muscle.

Trials	No. Larvae Added	Recoveries			
		Filtrate No.	Screen No.	Total No.	Total %
1	200	14	53	67	33.5
2	202	12	8	20	9.9
3	198	24	61	85	42.9
4	199	11	53	64	31.8
5	197	9	74	83	41.6
6	200	12	102	114	57.0
7	200	14	56	70	34.8
8	200	6	39	45	22.5
9	200	10	79	89	44.5
10	200	9	91	100	50.0
11	199	13	60	73	36.7
Mean	200	12	62	74	37.0

RESULTS

Examination of the supernatant showed it to be trichinae-free. The results of 11 trials to determine the efficiency of the digestion-Baermann technique are shown in Table I. An average of only 12 (6.0 per cent) of 200 larvae added to digested rabbit muscle was recovered in the filtrate, and 62 larvae (31.0 per cent) failed to pass through the openings in the mesh. An average of 63.0 per cent of the 200 larvae added to digested rabbit muscle were not recovered.

DISCUSSION

Examination of the filtrate only would have failed to detect a significantly large number of dead larvae added to the digested rabbit muscle. In routine procedure the screen would not be examined with the high probability of missing light infections due to the failure of dead larvae to pass through the openings of the screen. Hence, the digestion-Baermann technique is inadequate as a diagnostic procedure for the detection of dead trichinae. These findings agree with those of Hall and Collins (1937, p. 471) who stated that . . . "since the efficiency of the Baermann apparatus depends for its effect on the movement of live worms and the effect of gravity in bringing down these moving worms, the digestion method is of little value for the detection of dead trichinae unless these are present in numbers large enough to insure that some of them will land directly on the screen and fall through." Zimmermann *et al.* (1961) in a study on the occurrence of *T. spiralis* in pork sausage also emphasized that the digestion-Baermann technique is of little value for detecting dead larvae.

Preliminary observations revealed that the internal organs of a few larvae were ruptured following a digestion period of 2.5 hr and that the number of larvae injured as well as the decrease in the number of larvae recovered appeared to be directly proportional to the length of the digestion period. However, these observations were not quantified as neither the percentages of larvae injured nor recovered were recorded at this time.

Ransom (1916) demonstrated that artificial digestion for 24 hrs or less had no appreciable effect upon the viability of trichinae. Gursch (1948) studied the recovery of *T. spiralis* from the digestive system of experimentally infected rats previously exposed from 4 to 12 hr of artificial digestion and 0 to 72 hr of refrigeration (5° C.). Ac-

cording to Gursch, 4 to 8 hr of digestion and up to 24 hr storage in the refrigerator did not impair the infectivity of the trichinae, but that a 12 hour period of digestion decreased the percentage of worms recovered from test infections. He also found that exposure to 72 hr of refrigeration was definitely injurious to the larvae even in combination with a few hours of digestion. From the findings of Gursch and the preliminary observations in the present study, it is highly probable that the reverse procedure (exposure to freezing followed by even a short period of digestion) may result in the complete destruction of some larvae. These factors may largely account for the low combined recoveries (filtrate and screen) of trichinae utilizing the digestion-Baermann technique.

Acknowledgement

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SYMPOSIUM: A NEW ROLE FOR THE ACADEMY—UNIVERSITY OF ILLINOIS, CHICAGO CIRCLE CAMPUS, APRIL 24, 1970

SYMPOSIUM INTRODUCTION

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During the past two decades, I have formed an opinion that most of my colleagues believe the state academies of science are ineffective organizations for professional activities of senior scientists and for improvement of life in our society. They assign to the academies the roles of being the training ground for graduate students to present research papers; the caretaker of a state-wide high school science club program; a science fair organization; the organizer of a journal that has little, if any, recognized value in professional circles, even within the state; and, the organizer of ineffective committees that may discuss problems of limited interest and have trouble getting an agreement on the wording of a resolution to bring before the annual meeting. This view of the state academies changes when persons are asked about the New York Academy of Science. Senior scientists are aware of monograph publications or special topic symposia conducted by that academy. To many of us, the question of defending the Academies does not arise, for all of the mentioned activities are essential. The question that does arise is - What else should the Academies be doing?

With regard to the Illinois Academy, I have often suggested a break from the traditional annual meeting and annual field trip. Some changes have occurred under the direction of recent officers. But, as chairman of the Botany section in 1968, I found it impossible to change the member's habits without changing the traditional role of the section chairman. A change in the manner that the annual meetings are arranged appears necessary. The section chairmen are wasting time trying to do their work at the council meetings. Thus, a new approach within sections, possibly to commit the section to long range year-round planning, may be the first break in the activity cycle now supporting the annual meeting concept. To a Botanist, it would mean Academy organized field trips to botanical study areas at the appropriate times of the year. I believe agricultural and industrial facilities could also be visited. Agriculture and environmental section meetings should be encouraged. I believe these changes are presently possible within the organization of the Academy. A more independent annual meeting committee could operate separately from the council meeting freeing the section chairman to prepare more interdisciplinary programs instead of waiting long hours at the council meetings to state he needs two rooms and one projector. But a more important idea can be presented. I suggest a new role for the Academy Council; the planning and promotion of additional symposia type programs to benefit sections or the entire Academy.

Many members have said they would like senior scientists to publish research in our transactions. This would mean a greatly expanded

journal and an increased professional value for the transactions. It seems to me we could obtain this goal by first adopting the organized symposium with published papers involving senior scientists in Academy activities. I believe we could attract these leaders in the state, region and nation both as participants and as readers. In time, I am confident that many would become active in the year-round activities of the Academy and increase the effectiveness of the entire Academy program.

This new role for the Academy, organizing and publishing the results of symposia, may make it attractive to others seeking advice on the social implications of science and technology. By this I mean that some symposia may be helpful to the state and city governmental agencies, especially in developing long range plans for action in scientific and technological areas to benefit society. The Academy could be an information gathering group, inviting persons from educational, industrial and governmental units to participate. It appears appropriate to suggest a new affiliation of the Academy with the state government. Precedence for this affiliation already exists. The Director of the State Museum Division of the Department of Registration and Education of the State of Illinois functions as Librarian of the Academy, has charge of the distribution, sale, and exchange of publications of the Academy and is a permanent member of the Academy Council.

I have invited the panel members to present their views on the topic of a new role for the Academy, especially the possibility of acting as an advisory group to the governor. I will introduce the panel, proceed to their presentations, and invite discussion and comments from the panel and from the floor. Comments or questions from the floor will be invited after a preliminary round of discussions by the panelists.

The panel consists of Senator Alan J. Dixon, Mr. Milton Thompson and Dr. Andreas Paloumpis.

Senator Dixon is an attorney, businessman, banker and leader of his party on the floor of the State Senate. In 1950, at 23, he was elected to the State House of Representatives. He was re-elected to the House for five consecutive terms (1952 to 1960) and served on every major committee. He was Chairman of the House Judiciary Committee in 1959 and 1961. In 1962, he was elected to his first term in the Senate and was re-elected in 1966. After only two years in the upper house, he was named Minority Whip. He became identified with a particular brand of legislation such as the new criminal code; minimum wage bills; judicial and election reform and consumer fraud. Senator Dixon will be our main speaker.

Mr. Milton Thompson received his B.S. degree in Zoology from the University of Minnesota and has been a museum worker since 1937. In 1951, he was appointed Assistant Director of the Illinois State Museum, and Director in 1963. In 1952, he was assigned the duty of Librarian for the Illinois State Academy of Science. In 1968, he was elected President of the Academy. We all appreciate the fine leadership given by Mr. Thompson.

Dr. Andreas Paloumpis received his Ph.D. degree from Iowa State University in 1956 with specialization in fisheries research. He was appointed to the Faculty of Biological Sciences, Illinois State University, Normal and, reached the rank of Professor in 1966. In that year, he left his position to become the first President of Winston Churchill Junior College, Pontiac,

Illinois. In July 1969, he accepted the responsibility of Dean of Instruction, Illinois Central College, Peoria, Illinois. Dr. Paloumpis has served as Secretary of the Illinois State Academy of Science from 1962 through 1964.

It is with great pleasure that I present to you the main speaker, Senator Alan Dixon.

A NEW ROLE FOR THE ILLINOIS ACADEMY OF SCIENCE

SENATOR ALAN J. DIXON

Thank you Doctor Pappelis for your kind introduction.

Ladies and Gentlemen of the Academy, I am honored to join you this morning. I must confess I suffered a touch of concern, if not outright foreboding, in this morning's panel discussion concerning a new role for the Illinois Academy of Science. Friends in the academic community have told me that scientists apply strict standards of academic excellence before permitting a student to join their ranks as a fully qualified member. You have heard that I am an attorney, legislator and a more or less successful practitioner of the political arts. But since politics and government have not risen to the level of exact sciences, I seriously questioned my qualifications for appearing before you today.

My scientific achievements are not found in any monograph or periodical. They can only be found in the notes and reports that passed in a flurry between my high school and college science instructors and parents. I am pained to admit to you that these messages and reports graphically traced a precipitous downward spiral in my scientific aptitudes. I was an early underachiever in the physical sciences.

I have been informed that it is somewhat unprecedented for a member of the Illinois State Senate to address the Academy. I am honored to be here to discuss with you areas of mutual concern and interest which have narrowed the gap between your professions and mine.

After some study and reflection, I am convinced the time is right for an exchange of views between you, as members of our state's scientific community, and me, as a representative of our state legislature. I hope that some of the comments I intend to make stimulate you to make further contacts with other legislators and agencies in Springfield. Only in this fashion will we be able to undertake a scientific dialogue.

I am not qualified to attempt this morning to enter your various fields of research and study. I will limit my remarks to a discussion of some needs that I have begun to perceive clearly during my twenty years in the state legislature. Needs that require your attention and consideration.

Since I was first elected to the general assembly at the age of 23 in 1950, the problems plaguing Illinois, problems that continue to demand some legislative resolution, have multiplied and expanded in geometric fashion. Decisions faced daily by the working legislator have become awesome in complexity. As a Committee Chairman in the House and more recently as Floor Leader of my Party in the Senate, I have participated actively in every major effort to come to grips with the problems that plague our state. During my years in the legislature, the state government in Springfield

has expanded tremendously. New agencies have been added and old ones expanded to meet continuing demands from the public for improved and expanded government services. Programs have been initiated to provide improved health care, better environmental protection, more recreational facilities and parks and more effective crime prevention and control.

But despite the progress that we have made, many of the problems that concerned me 20 years ago as a freshman legislator still plague our state today. And new ones have been added to the catalogue. Urban blight spreads despite our best efforts to push back its boundaries. Racial harmony and a solution to the nagging problems of urban and rural poverty are not in sight.

Ironically, our successes have produced problems that the legislature is required to cope with today. For example, improved public health care has enabled us to reduce Illinois' infant mortality rate and to lengthen the average life span of residents of the state. Our state's commercial and industrial development, our welfare standards and our large and flourishing economy have made it a magnet for citizens from poorer states. This enlarged population has created serious drains on our already overburdened resources.

Industrial growth has also been accompanied by the unwanted waste of environmental pollution and the serious problems of automation and structural unemployment. Industry through automation and mechanization has created a vast new requirement to train and retrain thousands upon thousands of our residents for jobs requiring higher and more technical skills.

Because of overcrowded conditions, our public elementary, secondary and higher education systems are faced with crisis after crisis. How will we allocate the state's resources for training new teachers? What will be the budget for our state's school systems? Are there new and better programs available to deal with the problems of meeting the educational needs of our adult citizens and taxpayers?

Each year, ladies and gentlemen, the legislature is called upon to deal with this depressing catalogue of nagging problems. The public demands action, action now, new solutions, and solutions now, not later. But the legislature is not composed of experts in these various fields. The legislature is a representative group. There are no ecologists there to offer their experience and advice on how to come to grips with the problems of environmental waste. Nor will you find highly qualified conservationists able to bring their expertise to bear on balancing the state's need for additional recreation space against its need for the preservation of plant and animal life.

The legislature is a representative body. Some of its members are labor union men. Others are shopkeepers. There are few doctors. Many are farmers. And most are members of the legal profession. None have a great deal of scientific or technical skill. These ordinary men and women, utilizing their limited training in an extraordinary way, are called upon to legislate and to resolve problems that often would require the concentrated concern of dozens of scientific experts. Make no mistake about it, there is a clear and urgent need for more and better qualified scientific advice if the legislature is to cope adequately with the pressing needs of our state.

In order to highlight the legislature's need for an improved system

for obtaining scientific and technical advice, allow me to discuss very briefly the present system utilized by the legislature in obtaining this type of information.

In most cases highly motivated and public spirited individuals, who happen to have special qualifications to comment on or offer advice concerning a proposed bill, request and are granted time to testify before legislative committees. At times committees will invite experts to testify when they are dealing with a particularly thorny issue. The information and advice we receive from these meetings often directly influence our views. But even when the testimony is the best available, it is often spotty and incomplete. There is no guarantee that all of the relevant data available on a particular subject has been presented. In a typical fifteen minute session of testimony, witnesses seldom move beyond the broadest generalities and recommendations.

Committee hearings also raise another type of problem in trying to find the best advice available. Many times the testimony offered, even when provided by one with the appropriate academic qualifications, represents the views of one or more special interest groups that have a particular stake in the legislation under consideration. Although these views have a right to be represented in committee, they rarely are completely reliable. This advocacy system, which at times becomes an adversary system, has been successful up to now. But the time has come when we must supplement these views with some additional source of scientific advice untainted by a particular special interest and more representative of larger public interests.

Another example of our present information gathering system are the commissions formed by the legislature from time to time to study a particular major issue. These commissions, composed of legislators and laymen, hire consultants, collect testimony, and assemble data prior to making recommendations to the Governor or General Assembly for specific action.

The commission system has been successful in reforming our judicial system. Important recommendations have been produced by the State Highway Commission for improving our state's system of roads. And more recently, I was a member of the commission which laid the groundwork for calling the constitutional convention. But the commission system is not appropriate for gathering and disseminating the amount of scientific information needed by the state government on a continuing basis.

There is one last technique by which the legislature obtains scientific advice. The executive branch of the state government, which makes legislative recommendations to the General Assembly, has a large staff of trained personnel who serve as advisor to our top policy makers. But these experts are not available directly to the legislature and often represent the views of their agency or political administration.

It has also been my experience that problems tend to outpace their recommendations for solutions. For some reason state agencies often lag behind other institutions such as our universities in projecting solutions for problems. They sometimes fail to even recognize let alone respond to a particular pressing problem until it bursts forth in public as a major controversial subject.

As you can see, the legislature's present system for accumulating technical and scientific advice is clearly unorganized and haphazard.

It does not provide for a continuous flow of the most timely data from our state's scientific community to the legislature. Clearly the individual legislator is not always able to make the kind of educated and informed judgment that is called for in response to a particular issue.

If the General Assembly is going to come to grips with the issues of the day that require clear and unbiased technical or scientific clarification, the best scientific advice available must be provided for every legislator on a continuing basis. Some agency in the state must undertake to coordinate and channel a continuous flow of the most recent scientific information, advice and recommendations to the state legislature. The time has long passed to be satisfied with our present archaic system.

It is clear, ladies and gentlemen, that the Academy is the only statewide organization qualified to coordinate all of the various scientific interests of the state in undertaking the task of becoming an official advisory body to the state on scientific and technical matters. I propose for your consideration that the Academy undertake this critically important function.

Since 1907 your organization has written a long and distinguished record of service to the people of the state of Illinois through the promotion of scientific research, and the diffusion of scientific knowledge throughout the state. In undertaking this new function you would again be undertaking a role of service.

The new advisory function for the Academy could be similar in broad outline if not in specific performance to that performed by the National Science Foundation for the Federal Government. Although the NSF does serve to channel new research and ideas to the Federal Government, this is not its sole function. As you also know, it plays a large role in fostering and directing pure research activities on a nationwide basis.

Although the Academy may consider a role of setting statewide science policy similar to that of the National Science Foundation at the national level, I am not qualified to recommend such a course of action. I am concerned, however, with the state government's need and especially the legislature's need for more and better scientific advice on a continuing and coordinated scale.

Other proposals have been made to form a statewide Illinois research commission or to create a new office of scientific research and technology in the Governor's office. Both of these proposals merit consideration. The concept of a research commission is not unprecedented since it is my understanding that the state of Connecticut is a leader in this area.

Perhaps the Academy could affiliate with such an organization to collect, analyze and summarize scientific and technological information helpful to all levels and agencies of state government. I understand that such an affiliation with the state is not unprecedented since the Academy is presently affiliated and works closely with the Illinois State Museum at Springfield.

In performing its new advisory role, the Academy could consider forming task forces in various problem areas to utilize specialists in our universities, industry and government agencies. Specialists from surrounding states and national and international experts could be called on for counsel and advice when needed.

Both Houses of the legislature, their Committees and the various state agencies in the Executive

Branch, should be permitted to request specific studies or information. In return, a grant or stipend would be provided by the state to underwrite the costs of the particular study. The work products of the task forces organized by the Academy would be made available to the legislative committee or the agency requesting information or to the appropriate agency when no specific request has been made.

Some topics that might be areas for concentration by early task forces could be agricultural and industrial research for statewide economic development. Second, new approaches to our public health needs could be sought. Third, there is a great need for a continual flow of technical information concerning air pollutants, their measurement and control. In time these studies could be expanded to include research and recommendations from eminently qualified experts in the behavioral sciences.

In conclusion, ladies and gentlemen, members of the General Assembly as well as other state officials need the advice which may most accurately be produced by the members of this Academy. The need for your skills are great. The problems are myriad. New channels of communication must be opened between this Academy and the members of the state legislature who must cope with the increasingly complex issues of our time. The job at hand is no small task. It cannot be undertaken without clear planning and solid groundwork.

But it is most practical for us, when in this age scientific knowledge is at a peak, to turn to you to share the value of your wisdom and skills. With your help perhaps we can move on to meet some of the old and new challenges that will face us during the final decades of this century.

I appreciate this opportunity to address you and hope that you will invite me to return to assist you if you choose to undertake this new task.

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BRYOPHYTES OF GOOSE LAKE PRAIRIE, ILLINOIS

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ABSTRACT.—Thirty species of bryophytes are reported new to Grundy County along with their ecology and a description of Goose Lake Prairie.

It is fortunate that the State of Illinois has set aside the last remaining fragment of undisturbed prairie for a prairie state park. Agricultural development during the early 19th century has led to rapid destruction of Illinois prairie lands, thus little detailed botanical information is available. The scarcity of this type of habitat, that once covered most of Illinois, makes Goose Lake Prairie a precious remnant of natural history. The following study presents the first list of bryophytes occurring in a natural Illinois prairie.

Goose Lake Prairie is located in Grundy County east of the town of Morris and south of the Illinois River in Sec. 34, T34N, R8E, and Sec. 3, 4, 9 & 10, T33N, R8E, and consists of approximately 1,800 acres. It lies on the valley train of the Old Lake Chicago Outlet which now contains the Illinois River. This area has a basement of Upper Ordovician Richmond Limestone. This is covered by less than 100 feet of Pennsylvania series "Coal Measures" of Carboniferous age; this contains sandstones, shales, clays, limestones, and coals in varying succession (Sauer, 1916). This is overlain by a thin surface deposit of Late Wisconsin outwash containing a few minor sand dunes and numerous glacial boulders. The slightly undulating land, reflecting major features of the bedrock surface, has developed into a luxuriant hydrophytic black soil grassland. Depressions, due to poor stream development, have very wet or submerged soil but small rises may at times be very dry.

The prairie climate is characterized by sharply contrasting seasons of long summer days with the sun nearly overhead, and short winter days with obliquely shining sun. The frost free season lasts for more than five months with the greatest rainfall in May and June, and the highest temperatures in July and August associated with strong and shifting winds (Sauer, 1916).

The vegetation is characterized by a large number of vascular plant species, 326 phanerogams according to a preliminary list compiled by Swink et al. (1970). These are abundant in local areas due to aspect dominance of one or several species during some period of the growing season.

Bryophytes do not form a major part of the prairie community. Accumulation of dead grass persists year after year and forms a thick dry humus layer; this excludes bryophytes from moisture and soil. Dense shading by grasses and forbs during favorable growing periods also impedes bryophyte colonization. Bryophytes do not occur as typical luxuriant mats covering extensive areas but as small shade-form patches of often attenuated plants. Three notable exceptions are *Polytrichum commune* and *Aulocomnium palustre* which have stems long enough to grow through the humus layer, and *Sphagnum compactum* which produces extensive hummocks in local areas. Thirteen of the thirty species collected are restricted to more exposed habitats on dry outwash sand dunes or along edges of stagnant pools. Of nine species collected among dense

vegetation of wet areas, *Amblystegium varium*, *Bryum caespiticium*, *Leptodictyum riparium*, *Cephalozia connivens*, and *Lophocolea heterophylla* are found later in summer and then only sporadically on sides of grass tussocks. Twenty two per cent of all the prairie species are ephemeral, growing for short periods early in spring or in late summer, thus avoiding flooding and competition with vascular plants. Fifty per cent were collected with sporophytes during some time of year. Of those collected with mature capsules, most were found in exposed habitats and only a few completed their life cycles in competition with the prairie dominants.

Climax vegetation of hydrophytic prairie soils does not favor most bryophyte species. Those that are present usually occupy the drier elevations or recently disturbed areas, or survive among the vascular dominants for short periods of time in spring or late summer.

Bruchia sullivantii and *Physcomitrium pyriforme* are endemic to North America; the rest are of widespread distribution in the northern hemisphere. All species are newly reported for Grundy County. Voucher specimens have been deposited in the bryophyte herbarium of Eastern Illinois University. All collection numbers cited are those of the author. Specimens bearing sporophytes are indicated by an asterisk (*). The nomenclature cited follows Crum et al. (1965) for mosses, and Schuster (1953) for hepaticae. I wish to thank Dr. W. B. Schofield for verifying the specimens and assistance with this paper, and the Illinois Nature Preserves Commission for granting permission to collect in Goose Lake Prairie.

List of Mosses

Amblystegium varium (Hedw.) Lindb.—prairie soil under tall grasses, 1017.

Atrichum angustatum (Brid.) B. S. G.—wet prairie soil, 976.

Aulacomnium palustre (Hedw.) Schwaegr.—very common in damper sites, 970*, 1019.

Bruchia sullivantii Aust.—ephemeral on exposed wet mud, 1041*.

Bryum caespiticium Hedw.—on wet sides of grass tussocks, 1036*.

Bryum pseudotriquetrum (Hedw.) Gaertn., Meyer & Scherb.—common in various microhabitats, 965*, 1047.

Ceratodon purpureus (Hedw.) Brid.—dry sandy hills and rocks, 998, 1024*.

Cratoneuron filicinum (Hedw.) Spruce—wet ditches, 1043*.

Desmatodon obtusifolius (Schwaegr.) Schimp.—exposed, wet, sandy soil, 967.

Ditrichum pallidum (Hedw.) Hampe.—exposed muddy soil, 1042*.

Drepanocladus aduncus (Hedw.) Warnst.—frequent in shallow standing water, 968, 1037. This is the form that resembles *D. fluitans* (Hedw.) Warnst. in habit.

Funaria hygrometrica Hedw.—dry cinders, 1032*.

Leptobryum pyriforme (Hedw.) Wils.—ephemeral on wet mud banks, 1018.

Leptodictyum riparium (Hedw.) Warnst.—frequent on shaded soil, 994, 1046. *Leptodictyum* is a questionable genus and this specimen will probably turn out to be a form of *Amblystegium*, "*A. riparium* fo. *Abbreviatum*", see Conard (1959) for a treatment of this problem.

Leptodictyum trichopodium (Schultz) Warnst.—wet soil, 1021*, 975*.

Leskea gracilescens Hedw.—soil at base of *Fraxinus*, 970*.

Mnium cuspidatum Hedw.—common in various microhabitats, 1045*.

Physcomitrium pyriforme (Hedw.) De Not.—exposed muddy soil, 1023*.

Pohlia nutans (Hedw.) Lindb.—shaded soil, 1025.

Polytrichum commune Hedw.—very common in both dry and wet sites, 977*.

Rhynchostegium serrulatum (Hedw.) Jaeg. & Sauerb.—wet prairie soil, 983.

Sphagnum compactum Lam. & DC—forming large hummocks, 963, 964, 1026.

Weissia controversa Hedw.—ephemeral on exposed clay soil, 1036*.

List of Hepatics

Cephalozia connivens (Dicks.) Spruce—ephemeral on rich humus, 986.

Cephaloziella hampeana (Nees) Schiffn.—shaded soil with *Aulacomnium palustre*, 984, 1020.

Fossombronia foveolata Lindb.—shaded or exposed soil, 966, 1044.

Lophocolea heterophylla (Schrad.) Dumort.—shaded humus, 978.

Phaeoceros laevis (L.) Proskauer—shaded or exposed wet mud, 1040*.
Riccia fluitans L.—floating, 1038.
Ricciocarpus natans (L.) Corda.—floating, 1039.

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FISHER AND PORCUPINE REMAINS FROM CAVE DEPOSITS IN MISSOURI

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ABSTRACT.—Remains of the fisher (*Martes pennanti*), a mustelid previously unknown from Missouri, are described from four cave and archaeological deposits in the Ozark Highland. A fourth prehistoric locality record for the porcupine (*Erethizon dorsatum*) in Missouri is presented.

Intensive exploration of Missouri caves, especially those located in the Ozark Highland region, by speleologists during the past decade has brought to light several significant bone deposits. The time span involved ranges from Late Pleistocene to present day, and a particular cave may contain remains of extinct forms such as peccary (*Platygonus* and *Mylohyus*), dire wolf (*Canis dirus*) and tapir (*Tapir*) as well as elements of a varied modern fauna. Thus far no completely stratified deposit has been found and, consequently, animal remains from caves are insufficient to illustrate a clear-cut sequential faunal change through time from the earliest deposition to the most recent. In spite of the heterogenous nature of such bone deposits — typically the result of some form of water action and/or animal activity, the actual presence of particular species often

FIGURE 1. County map of Missouri showing location of fisher (F) and porcupine (P) finds.

serves as an indicator of past environmental and species composition change in an area.

Recovery of bones of the fisher (*Martes pennanti*) from four cave deposits (Fig. 1) in the Ozark Highlands is especially noteworthy. This mustelid presently occurs throughout most of the Canadian provinces and, during early historic times, was locally common in northeastern United States with a range that extended southward in the Appalachians to North Carolina and Tennessee. Prehistoric archaeological records have shown that its range extended even farther west along the Illinois and Mississippi rivers in Illinois (Parmalee, 1958, 1960a) and south into northwestern Georgia (Parmalee, 1960b) and Alabama (Barkalow, 1961). Brown (1908) described two isolated teeth, four jaws and a skull section of the fisher that were recovered in the Pleistocene bone deposit of Conard Fissure in northern Arkansas. This mustelid had not been previously reported from Missouri.

Two of the four records to be described here are based on elements recovered from natural cave deposits, while the other two consist of bones obtained during archaeological investigations in Graham Cave, Montgomery County, in 1966 and Arnold-Research Cave, Callaway County, in 1956. Although all four are termed "cave," the latter two represent shallow, overhanging bluff rock shelters once inhabited by peoples of the Archaic and Woodland cultures. Whether all fishers represented in the archaeological deposits were taken by the human inhabitants or were present as the result of natural causes is, with one exception, a matter of speculation.

Arnold-Research Cave, located approximately two miles north of

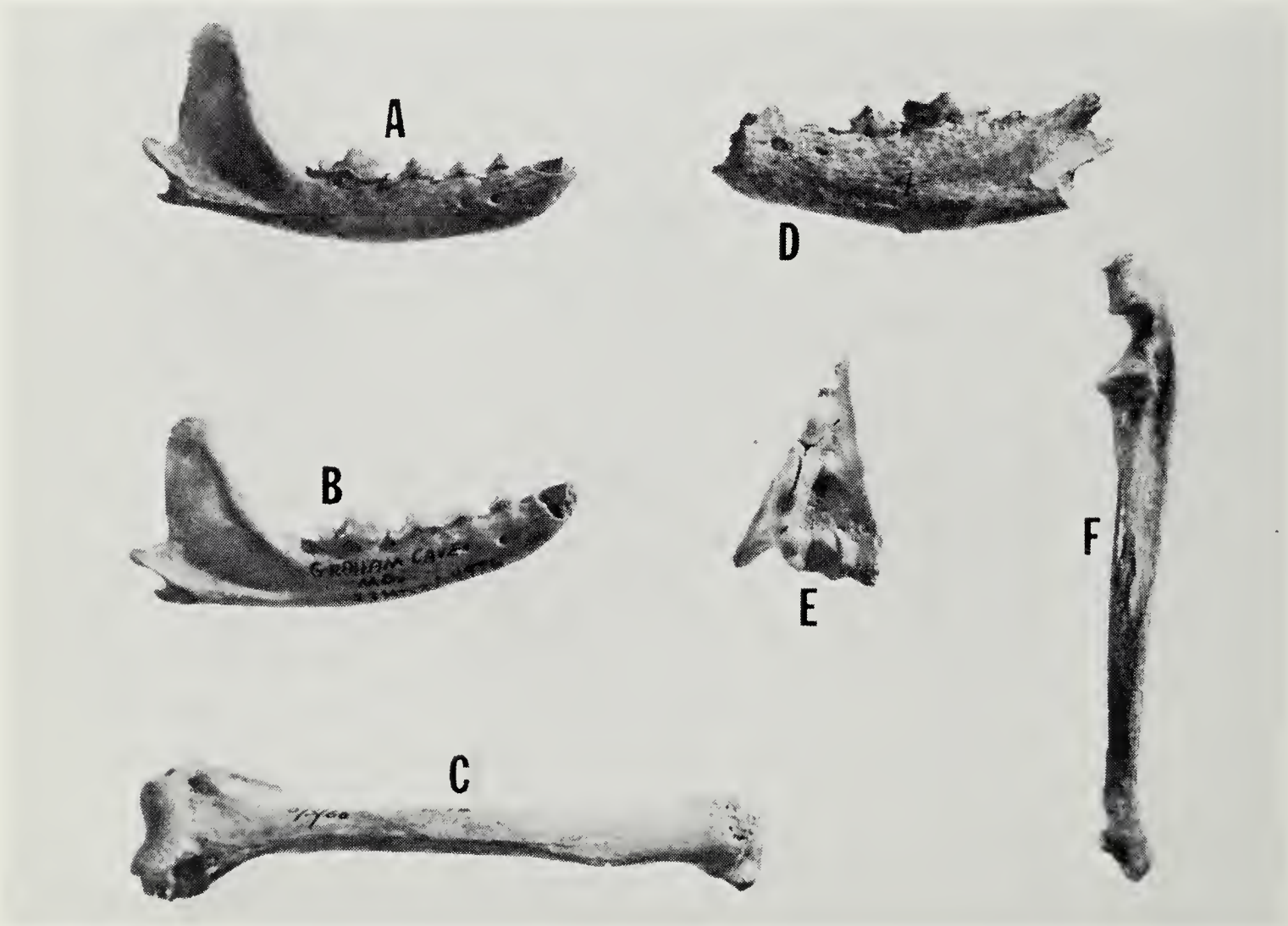

FIGURE 2. Fisher elements recovered from Graham (A-C), Brynjulfson (D), Arnold-Research (E) and Bat (F) caves, Missouri.

the Missouri River in southeastern Callaway County, was excavated under the direction of J. M. Shippee (Shippee, 1966). A total of approximately 36 species of vertebrates (all Recent forms) was identified by Mr. Carl R. Falk; and, in addition to the fisher, three other mustelids were represented (mink, badger, otter). The fisher element consisted of a section of the right maxillary containing $P^{3,4}$ and M^1 (Fig. 2). The animal was a mature adult, the teeth showing moderate wear; judging from the large size of the teeth, the individual was probably a male. Graham Cave, situated above Loutre Creek approximately 15 miles northeast of Arnold-Research Cave, was excavated under the direction of Dr. Walter E. Klippel; Carl R. Falk and Paul W. Parmalee identified the bone remains which included three elements of *M. pennanti*. These consisted of two small, lower right jaws, probably females, both of which contained $P_{2,3,4}$ and M_1's that showed only slight wear. One of these jaws (Fig. 2,A) exhibited deep cut marks between the condyle and angular process on the labial surface, plus faint skinning cuts below the alveoli of the canine and P_1, thus indicating utilization of the animal by the Indians. The third fisher element from Graham Cave was a nearly complete right humerus of a large adult, probably a male. There appears to be three individuals represented.

During the summer of 1962, the late Dr. M. G. Mehl and a field crew of students from the University of Missouri, Columbia, excavated a horizontal cave shaft located along Bonne Femme Creek, approximately seven miles south-southeast of Columbia, Boone County. The faunal complex of this deposit (Brynjulfson Cave) included remains of extinct forms (peccary, tapir, dire wolf, giant armadillo) as well as an abundance of Recent species. One fisher element, an incomplete lower left jaw containing $P_{2,4}$ and M_1, was recovered. Based on the large size of this jaw section and the considerable degree of wear on the teeth, it is judged that the animal was an old male (Fig. 2, D).

A large sample of vertebrate remains was removed from Bat Cave, located five miles northwest of Waynesville, Pulaski County, periodically from 1961 to 1968; most of the work was carried out under the direction of Mr. J. R. Reynolds, R. L. Foley and Dr. Oscar Hawksley. Like Brynjulfson Cave, this cave deposit contained a mixture of remains of modern forms and extinct species such as peccary and dire wolf. Fisher was represented in the faunal complex of Bat Cave by a complete left ulna of a large (male?) adult (Fig. 2, F).

The porcupine (*Erethizon dorsatum*) is another species, like the fisher, whose range has receded from its known extremities of prehistoric times. In pre-Columbian times this rodent occurred throughout the Appalachian Mountains south to northern Alabama (Barkalow, 1961), and, in the Midwest, along the Mississippi River in Illinois (Parmalee, 1967) and Missouri (Simpson, 1949). Today, east of the Mississippi River, the porcupine is not found south of central Wisconsin, Michigan and Pennsylvania. There have been three published records of the porcupine in Missouri, all based on elements recovered in prehistoric cave or archaeological deposits: (1) Two lower jaws from Cherokee Cave (Simpson, 1949), St. Louis County; (2) Sections of a femur, one lower and two upper incisors, and a left jaw from Crankshaft Cave (Parmalee, Oesch and Guilday, 1969), Jefferson County; (3) A lower left jaw section

from Tick Creek Cave (Parmalee, 1965), Phelps County.

The most recent recovery of porcupine remains in Missouri occurred in the two Brynjulfson Cave deposits. One of these two "twin" caves, located about 100 yards apart along Bonne Femme Creek, Boone County, has been previously discussed with reference to the recovery of a fisher jaw. The sample of bones from this cave (Brynjulfson Cave No. 1) included complete and/or sections of one skull, five jaws, seven incisors, one cheek tooth, one ulna, five femora and one tibia (Fig. 3,A) of the porcupine. At least four individuals, including both adults and juveniles, were represented. An extensive faunal sample recovered by Paul W. Parmalee and Ronald D. Oesch periodically during 1968 and 1969 at Brynjulfson Cave No. 2 contained an incomplete humerus, incisor and lower left jaw of the porcupine.

Approximately one-third of the state of Missouri — the central, southern and eastern parts — is classified as Ozark Highland. This region is an old, deeply dissected plateau which now appears as hills with steep intervening valleys cut by numerous streams arising in the higher elevations (Schwartz and Schwartz, 1959). There are many limestone outcrops along the stream banks, and the original mesophytic forest cover was apparently a western extension of the oak-hickory climax characteristic of the upland deciduous forests of eastern United States. Juniper and short-leafed pine also occurred throughout this hilly region.

Hagmeier (1956) presents a thor-

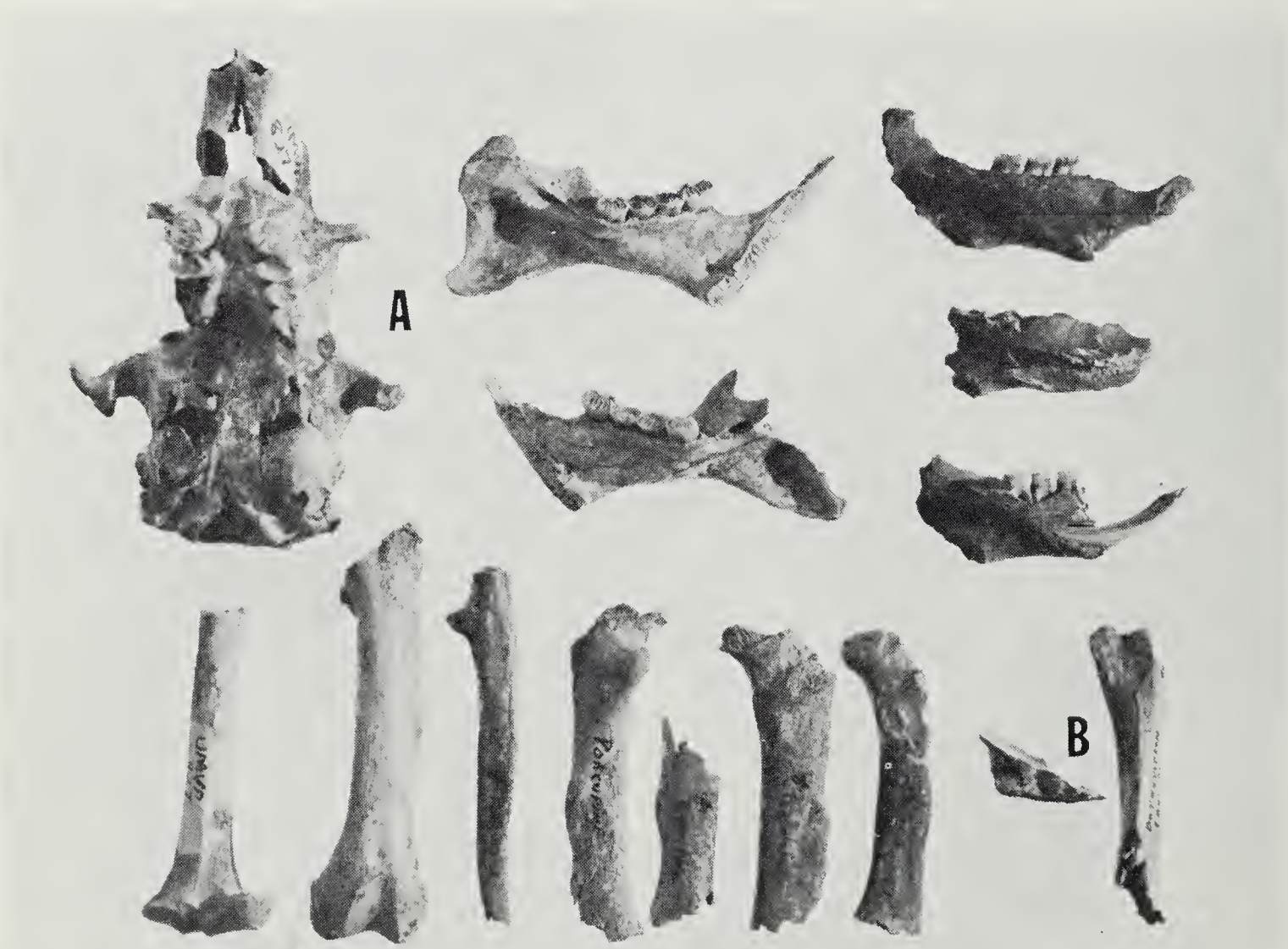

FIGURE 3. Porcupine elements recovered from the Brynjulfson Caves, Boone Co., Missouri.

ough discussion of the distribution and habitat requirements of the fisher in North America, and states that ". . . while they prefer heavy timber, they are frequently seen in open second-growth stands and occasionally in areas recently burnt over." Other authorities cited by Hagmeier (ibid.) indicate that fishers prefer low wet grounds and the banks of streams. This mustelid appears quite adaptable within a varied hilly-wooded-riverine environment; the Ozark Highland would have been well suited to its habitat requirements. This hilly, forested region of Missouri would have also provided an ideal habitat for the porcupine, a species that is dependent upon thick stands of mixed deciduous trees and conifers for food and massive rock outcrops for dens. Since the porcupine is known to be a favorite food of the fisher, there may well have been an interrelationship between the two animals, with the range extension and abundance of the fisher dependent to some extent upon the distribution and abundance of the porcupine. In any case, recovery of remains of these two species in cave and archaeological deposits has established the prehistoric occurrence of fisher and porcupine in the Ozark Highland region of north-central Missouri. Excavation of other deposits will probably bring to light new records of these species, especially in regions drained by the Missouri, Osage, Gasconade and Meramec rivers and their tributaries.

Acknowledgements

I would like to express my appreciation to the following individuals for permission to report on the fisher elements recovered during their field excavations, and for data pertinent to the specimens: Mr. Carl R. Falk, Midwest Archeological Center, Lincoln, Nebr.; Dr. Oscar Hawksley, Central Missouri State College, Warrensburg, Mo.; and Dr. Walter E. Klippel, Illinois State Museum, Springfield, Ill. Appreciation is extended to Charles W. Hodge and Marlin Roos, Illinois State Museum, for photographing the specimens in the Figures.

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CULTIVATION, LIFE HISTORY AND SALINITY TOLERANCE OF THE TIDEPOOL COPEPOD, *TIGRIOPUS CALIFORNICUS* BAKER 1912, IN ARTIFICIAL SEA WATER

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ABSTRACT.—A simple method of cultivating the marine harpacticoid copepod, *Tigriopus Californicus* Baker 1912, using artifically prepared sea water enriched with Purine laboratory rat chow is described. The copepod feeds upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter. The life history from egg through nauplius, copepodite and reproductive adult takes 18 to 21 days at 23 C. Life history stages are illustrated. The copepod is tolerant to a wide range of salinity (21.2 to 75.3 o/oo) and shows an optimum growth and reproduction in a salinity of 42.3 to 47.00 o/oo. Potential uses of this hardy and easily cultured organism in research and teaching are discussed.

The harpacticoid copepod, *Tigriopus californicus* Baker 1912, may be of interest to the invertebrate zoologist who is looking for a versatile marine organism to use in research and teaching. *T. californicus* lives in splash pools above the high tide zone along the rocky California coastline. It is a hardy organism that is able to withstand the wide fluctuations of temperature and salinity that are found in the unstable tidepool habitat (Monk, 1941).

The copepod is little-known, but potentially a valuable animal for use in various kinds of research. *Tigriopus* spp. have been used in ecological studies (Fraser, 1936), nutritional study (Provasoli, et al., 1959; Gilat, 1967), genetic study (Vacquier, 1962), radioactive tracer uptake (Chipman, 1958; Lear and Oppenheimer, 1962), as a food for marine killifish (Faye, personal communication) and as an experimental intermediate host for nematode parasites (Huizinga, 1966).

Most marine copepods are difficult to cultivate since they have exacting physiological requirements and must be fed a variety of specially cultured algae. However, *T. californicus* is a benthic filter-feeder and its nutritional needs are easily satisfied in laboratory culture with a diet of mixed unicellular organisms.

This paper describes a simple method for raising *T. californicus* in artificially prepared sea water culture. Observations are given on the life history, reproduction and salinity tolerance.

MATERIALS AND METHODS

Cultivation techniques. T. californicus can be obtained from Dr. Rimon Fay, Pacific Bio-Marine Supply Company, Box 536, Venice, California 90291. The animal is easily shipped via air mail in sealed polyethylene bags containing about one liter of sea water with an overlaying air space. Field collected copepods are placed into a standard 8 inch diameter finger bowl and left in the natural sea water for about one week to allow for equilibration to the laboratory conditions. They are fed Purina laboratory rat chow which is finely ground with a blender. A small amount of food (150 mg) is added at 4 to 7 day intervals to a bowl containing 1300 ml of sea water and approximately 500 adult copepods. The culture is covered and maintained at room temperature (20-25 C), without aeration. The initial water level is marked on the side of the bowl with a wax

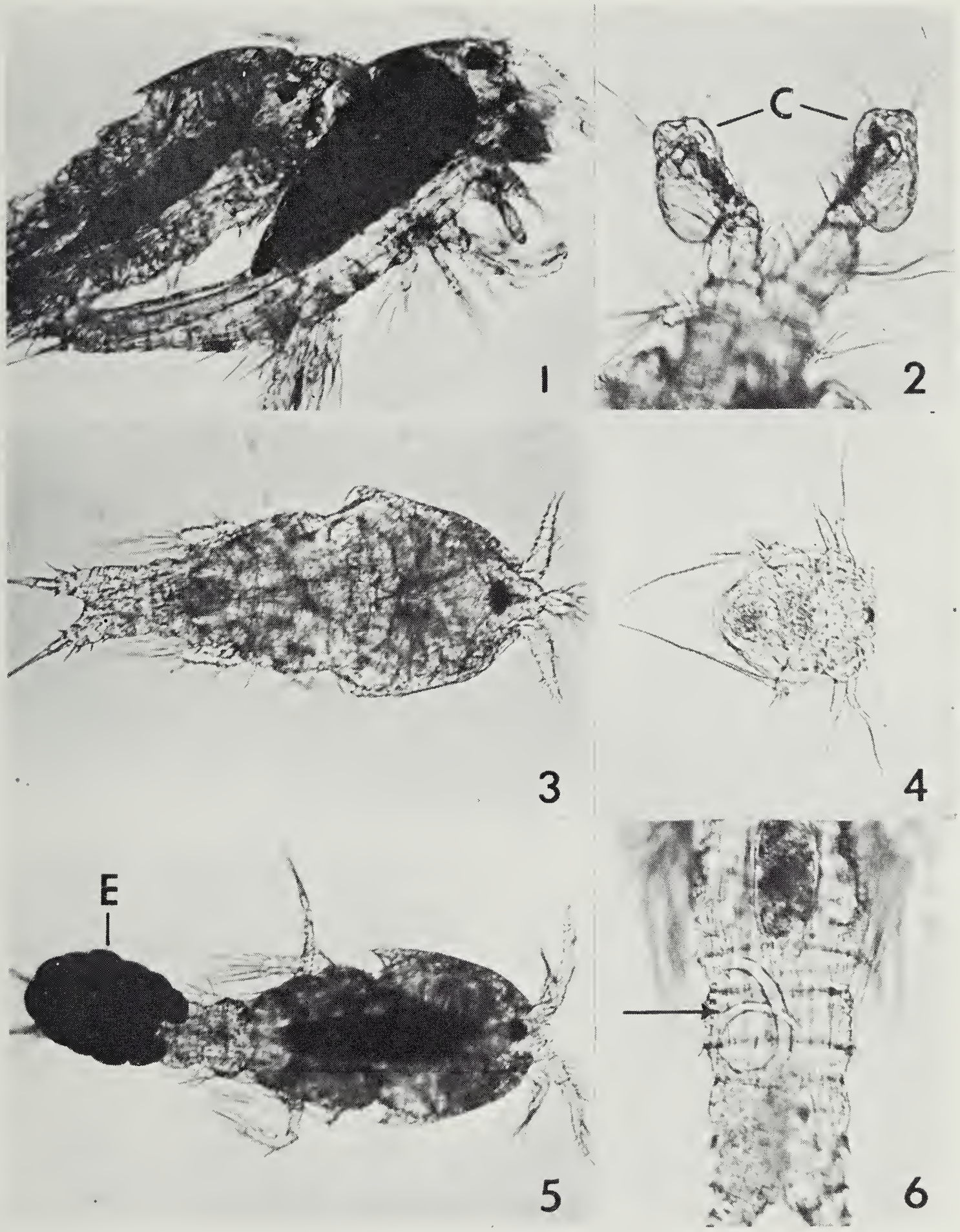

PLATE 1. Life history stages of the harpacticoid copepod, *Tigriopus californicus*.

FIGURE 1. Male (left) and female in copulation, magnification 58 X original.

FIGURE 2. Antennae of male, modified as clasping organs (c), 174 X.

FIGURE 3. Copepodite, 112 X.

FIGURE 4. Nauplius, 194 X.

FIGURE 5. Female with attached egg sac (E), 57 X.

FIGURE 6. Larval stage of the nematode *Contracaecum spiculigerum* (arrow) within the hemocoel of T. *californicus*, 220 X.

pencil, and water lost to evaporation is replaced with distilled water.

After the original culture begins to show multiplication of nauplii and copepodite stages (Figs. 3 and 4), subcultures are made at approximately two to three week intervals, depending upon the room temperature and growth characteristics of the culture. The frequency of subculturing is not critical, since a neglected culture will remain viable for several months with only a few copepods present. The animal can withstand extremely crowded conditions, but will attain a maximum population density in about 24 days, beyond which a decline of numbers will occur.

Subculturing is done by stirring the bottom debris and copepods into suspension with an artist's paintbrush and pouring one-half of the contents into a second bowl. Each bowl is then filled to the 1300 ml mark with artificially prepared sea water (42.6 gms of synthetic sea salts per liter of deionized tap water, salinity 35 parts per thousand, specific gravity 1.025, Dayno Sea Salts, Supreme Products, Lynn, Mass.). The resulting mixture is 50 parts original sea water and 50 parts artificial sea water. Trace elements were not added. Part of the original bottom debris is included in the subculture, since it contains the needed food organisms from the original field-collected sea water shipment (e.g. unicellular algae, bacteria and protozoa). The copepods will not thrive if they are placed into unconditioned artificially prepared sea water. A critical factor in the growth of *Tigriopus* is the quantity of microorganisms present. In my experience, a slightly cloudy medium indicates an optimum growth of microorganisms which are feeding upon the powdered food. If the culture becomes transparent and low in microorganisms, an immediate decline in the numbers of copepods will be observed. Overfeeding is not critical, since *Tigriopus* is tolerant of considerable putrifaction. The organism does equally well with or without aeration.

Adult copepods are harvested from cultures with a sieve that has a mesh of 500 microns. This allows the passage of smaller nauplii and copepodite stages and retains the larger adults which are transferred to other containers.

Attempts to raise *T. californicus* in a constant temperature plant incubator (25 C) with high intensity lighting (100-200 foot candles, 13 hours duration) failed. This light intensity stimulated excessive growth of undesirable red filamentous algae which caused an imbalance of the system and death of copepods. The fluorescent lighting of my office (20-40 foot candles, 8-10 hours duration) allowed for adequate growth of algae and produced good copepod cultures. A single-celled green alga (*Chlorococcum* sp.) was the predominant food item found in the intestinal contents of copepods along with lesser amounts of a blue-green alga (*Oscillatoria* sp.) bacteria, protozoa (*Oxyrrhis marina*, *Euplotes* spp.) and non-living particulate organic matter.

When a white fungus was observed to grow upon the bottom debris, subcultures were made and the fungal contaminant discarded. *Tigriopus* was resistant to infection, but the toxic effect of the fungus appeared to limit the growth of organisms serving as food for the copepod. Fungal contamination was minimized by autoclaving the powdered food and storing in a dry container.

The following method was used to study the life history of *T. californicus*. A gravid female (Fig. 5) was transferred from the stock cul-

ture with a capillary pipette into a 4 inch diameter petri dish containing 40 ml of filtered and conditioned sea water. Ten milligrams of powdered food (see above) was added. The dish was placed in a lighted room at a temperature of 23 C. Daily observations were made using a dissection microscope with bright illumination and a black background. A few drops of stock Harris hematoxylin stain, added to the dish at the termination of an experiment, caused copepods to adhere to organic matter on the bottom of the dish. The stained cope-

TABLE I.—Progeny from single egg sacs of isolated female *Tigriopus californicus*.

Isolated Female*	Day Killed	Numbers of Progeny		Adults	
		Nauplii	Copepodites	Male	Female
1	2	9	0	0	0
2	2	13	0	0	0
3	2	15	0	0	0
4	5	2	13	0	0
5	5	5	10	0	0
6	6	0	12	0	0
7	8	0	13	0	0
8	12	0	19	0	0
9	17	0	0	9	3
10	17	0	0	13	8

*Females were removed after one egg sac was laid.

TABLE II.—Salinity tolerance of *Tigriopus californicus* in artificial sea water.

Concentration Sea Salt, Gms per liter	Salinity o/oo	Maximum Survival in Days	Reproduction of Nauplii & Copepodites	Behavior	Overall Success of Culture
126.0	103.5	1	−	A,E**	4***
114.6	94.1	7	−	A,E	4
103.1	84.7	10	−	A,E,M	4
91.6	75.3	40*	+	A,E,M	3
80.9	65.9	40*	++	M,N	3
68.7	56.4	40*	+++	M,N	2
57.3	47.0	40*	++++++	M,N	1
51.6	42.3	40*	++++++	M,N	1
45.8	37.6	40*	++++	M,N	1
42.6	35.0	40*	++++	M,N	1
40.1	32.9	40*	+++	M,N	1
34.4	28.2	40*	+++	M,N	2
28.6	23.5	40*	+++	M,N	2
25.8	21.2	40*	+++	M,N	2
22.9	18.8	16	++	E,M,N	3
20.0	16.5	13	+	A,E,M	4
17.2	14.1	11	+	A,E,M	4
14.3	11.7	11	+	E,M	4
11.4	9.4	5	−	A,E	4
8.8	7.0	4	−	A,E,M	4
5.7	4.7	4	−	A,E	4
2.9	2.3	3	−	A,E	4
0.0	0.0	2	−	A,E	4

*terminated

**A = abnormal movement and feeding, E = epiphytes, M = mating, N = normal movement and feeding

***1. optimum, maximum numbers and survival, 2. average, moderate numbers and survival, 3. below average, limited reproduction and survival, 4. unsuccessful, poor to no reproduction, premature death.

pods continued to move in place for several minutes before death and were easily counted using a dissecting microscope and ruled petri dish.

Copepods were placed into various concentrations of artificial sea water to test their salinity tolerance (Table II). A stock concentration of sea water was prepared by adding 126 gms of synthetic sea salt per liter of deionized tap water and heating to boiling. Successive 10 per cent dilutions of the stock concentration were made with deionized water. Since the calcium sulfate, calcium manganate and calcium carbonate salts tended to precipitate from the supersaturated solution, they were stirred into uniform suspension before dilutions were made. The salinity and number of grams of sea salt per liter for each dilution were calculated based upon the manufacturer's specifications for oceanic sea water (Table II, Dayno Sea Salt, 42.6 gms per liter equals 35 o/oo).

Results

Life History. After the female copepod drops an egg sac, nauplii begin to hatch from the eggs within 24 hours (Fig. 4). These move along the bottom of the dish as they feed upon microorganisms. The first nauplius molts three times giving rise to three additional nauplii stages. The animal increases slightly in size after each molt. After 5 or 6 days, the final nauplius stage develops into the copepodite which resembles the miniature adult (Fig. 3). There is also a sequence of four copepodite stages over a period of 8 to 9 days. The final copepodite then develops into the adult copepod (Fig. 1). Development from the egg to adult takes about 15 to 18 days at 23 C.

Young male and female copepods begin to mate immediately. There is a striking sexual dimorphism and the male has specially modified antennae which are used to clasp the female during copulation (Figs. 1 and 2). The male pursues the female vigorously from behind and grasps her cephalothorax with a quick motion. He then rides the female for several minutes to hours during which the sperm are deposited in the seminal receptacle. The female goes about her usual feeding behavior, apparently unaffected by the male in tow. An inseminated female will begin to produce an egg sac in about 3 to 4 days. She will continue to produce egg sacs, in isolation from the male, by fertilizing the eggs with stored sperm from a single insemination. New egg sacs appear every 2 to 3 days. Isolated females were observed to lay three consecutive egg sacs. The average number of eggs per sac is 18. At room temperature, the entire life cycle from egg to egg takes 18 to 21 days. The numbers of progeny produced by isolated gravid female copepods (Fig. 5) at various time intervals are given in Table I.

Salinity Tolerance. T. californicus survived and reproduced for over 40 days in a wide range of salinity (21.2 to 75.3 o/oo, Table II). Above and below this range, the feeding, reproductive behavior and survival were abnormal. Epiphytic fungi grew upon the exoskeleton of abnormally sluggish copepods.

Copepods remained alive for one day in 84.7 o/oo which was nearly three times the concentration of normal sea water (35 o/oo), but they were sluggish and twitched convulsively. The copepod survived for two days in deionized water.

Moderate growth and reproduction of copepods was observed in the concentrations of 21.2 and 56.4 o/oo. Abundant cultures developed in the range of 32.9 to 47.0 o/oo. Based upon maximum reproduction

of nauplii and copepodites plus vigorous feeding and mating behavior, the optimum sea salt concentration was 42.3 to 47.0 o/oo.

Discussion

The simple method described here has been used in my laboratory for the continuous cultivation of *T. californicus* for over five years. The copepod is hardy and offers considerable potential for use in research and teaching, such as: an assay organism for environmental pollutants (Mileikovsky, 1970), model of population dynamics, study of nutritional requirements, and copepod reproductive behavior.

T. californicus is susceptible to experimental infection with *Contracaecum*, a larval nematode parasite (Huizinga, 1966, and Fig. 6 of this paper). The copepod is also capable of withstanding considerable salinity variation. It may therefore serve as an experimental intermediate host for various larval helminth parasites found in marine or estuarine environments.

The tolerance of the copepod to a wide range of salinity is not surprising in view of its natural habitat which is subject to evaporation and periodic dilution by rainwater. *Tigriopus* is also tolerant to considerable putrifaction and this is probably an adaptation to the tidepool which is normally exposed to fecal enrichment from marine birds.

T. californicus was observed to feed upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter. Provasoli et al. (1959) and Gilat (1967) showed similarly that *T. brevicornis* and *T. californicus* require a diet of mixed algae and bacteria for successful reproduction in laboratory culture.

Since *T. californicus* is easily cultivated, it can be used conveniently in the classroom to demonstrate the life history and reproduction of a marine copepod. The following student exercise is suggested to compare the theoretical and observed reproductive potential. Since a female copepod produces on average of 18 eggs per sac, and assuming a 50-50 sex ratio, the F1 generation will contain about nine females. Upon maturity in 21 days, each F1 female will then produce at least one egg sac giving rise to a total of about 162 F2 progeny. Continuing with a similar theoretical line of mathematics and optimum growth conditions, the F3 generation will contain about 1458 progeny in about 60 days. The actual number of offspring will be several times the above calculated number, since a single female copepod usually lays several consecutive egg sacs. Mortality must also be taken into account. The student can perform total or sample counts of the copepod stages present in the culture over a several week period (as Table I, Results). This study can be used to illustrate the high rate of biological productivity shown by the primary consumer copepod which serves as an important food organism for marine fish.

Summary

1. *Tigriopous californicus* was cultured in a simple system using artificially prepared sea water enriched with powdered Purine laboratory rat chow. The copepod fed upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter.
2. The life history of *T. californicus* from egg through four nauplii, four copepodites and the reproductive adult took 18 to 21 days at 23 C.
3. The copepod survived and reproduced in a wide range of salinities (21.2 to 75.3 o/oo). A salinity range of 42.3 to 47.0 o/oo

was optimum for growth and reproduction.

4. Potential uses of this hardy and adaptable organism in research and teaching are discussed.

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INCIDENCE OF MERCURY IN ILLINOIS PHEASANTS

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ABSTRACT.—Selected tissues from 20 pheasants collected in east-central Illinois during August 1970 were analyzed for elemental mercury by emission spectrography. Frequencies of occurrence and mean concentrations were found to be 35 per cent and <0.06 ppm in kidneys, 40 per cent and 0.03 ± 0.01 ppm in livers, 15 per cent and 0.32 ± 0.30 ppm in brains, 25 per cent and 0.02 ± 0.01 ppm in leg muscles, 15 per cent and 0.03 ± 0.02 ppm in sternal muscles. The high mean concentration in brains was due to 5.93 ppm found in one bird. The second highest individual concentration was 0.44 ppm and occurred in kidneys. Eight soil samples, collected from the fields in which the pheasants were taken, contained a mean of 0.02 ± 0.01 ppm of mercury. Thus, neither pheasants nor soils in east-central Illinois appear to be contaminated with potentially dangerous levels of mercury.

Mercury contamination became a subject of considerable concern in 1969 and 1970. Ring-necked pheasants (*Phasianus colchicus*) and Hungarian partridges (*Perdix perdix*) in Alberta, Canada, and in Montana were found to contain potentially dangerous levels of mercury (Wishart 1970, Dunkle 1969). Relatively high levels of mercury were also found in several species of North American waterfowl (National Wildlife Federation 1970), and mercury contamination prompted state governments to impose restrictions on fishing in many lakes and rivers (Sport Fishing Institute 1970). The pheasants and partridges in Alberta and Montana apparently became contaminated by eating seed grain treated with organic mercury fungicides.

The purpose of this study was to determine the incidence of mercury in selected tissues and organs of pheasants in east-central Illinois, the state's better pheasant range. This portion of Illinois is under intensive cultivation, with about 70 per cent of the total land area planted to corn and soybeans annually (calculated from Illinois Cooperative Crop Reporting Service 1970:64). According to M. C. Shurtleff, Professor of Plant Pathology, University of Illinois, Urbana (personal communication), mercury fungicides were used sparingly in Illinois to treat seed grain of small grains — wheat, oats, barley, and rye — through 1969. However, all recommendations for uses of mercury compounds in Illinois agriculture were discontinued in 1970. About 4 per cent of the total land area in east-central Illinois was planted to small grains in 1969 (calculated from Illinois Cooperative Crop Reporting Service 1970:68, 72).

METHODS

The pheasants used for this study were captured by nightlighting (Labisky 1968) during the nights of 24 and 25 August 1970. Ten of the pheasants were taken from four fields (three wheat stubble and one idle) located in southwestern Champaign County (townships T17N, R8E and T18N, R8E) and 10 were taken from four fields (one oat stubble, two brome, and one idle) located in southeastern Livingston County (townships T25N, R7E and T25N, R8E). Of the 20 pheasants collected, five were juvenile hens, five were juvenile cocks, nine were adult hens, and one was an adult cock. The juveniles were aged to the nearest week by examining advancement of molt of the primary flight feathers (Etter et al. 1970).

The pheasants were held over-

night in wooden crates, and were then weighed, decapitated, and dissected, one at a time, between 7:30 AM and 11:00 AM. The following tissue samples were excised from each bird and saved for analysis: both kidneys (3.2-5.6 g), entire liver (9.0-15.6 g), entire brain (1.9-3.8 g), 10-20 g of leg muscle (primarily the *femoris*), and 16-49 g of sternal muscle (*pectoralis thoracica*). The tissue samples were freed of extraneous material, weighed, and packaged in polyethylene bags or vials. The packaged samples were placed on dry ice within 15-20 minutes after the birds had been killed. Metal instruments (scissors, forceps, and scalpels) were used to dissect the pheasants.

A sample of soil was collected from each of the eight fields in which the pheasants were captured, and was saved for analysis. Each sample was obtained by collecting three subsamples at approximately the same location in the field — i.e., collected within 5 m of each other — and composited. The samples were taken from the upper 3 cm of soil. They were stored in polyethylene bags until they could be analyzed.

Analyses of the tissue samples and the soil samples for mercury were performed at Stewart Laboratories, Inc., Knoxville, Tennessee. After removing water from the tissues by freeze-drying (at < 1 mm Hg), the organic matter was destroyed in a manner that did not result in the loss of mercury. A number of investigators (Schöniger 1955 and 1956, Southworth et al. 1958, Gutenmann and Lisk 1960) have shown that the Schöniger method of combustion is applicable to the determination of mercury in organic compounds. By the use of this method, organic matter is destroyed in the combustion and the volatile elements are retained in a closed system. The combustion products are absorbed in a reagent (added to the system prior to combustion), which serves to fix the mercury and convert it to a form suitable for determination. Appropriate modifications were made to increase the specificity of these preparations for determination of mercury in nearly all organic matter. The soil samples were pretreated by a conventional dissolution procedure that resulted in a solution similar to that of the organic samples.

The mercury present in solution was withdrawn by an extractant that contained as its major component a highly volatile organic. By a process of successive evaporations (no heat), a concentrated solution was evaporated onto a buffer (carrier) compound packed in a carbon electrode. The carrier acts similar to a distilling column and allows for quick introduction of all the mercury from the sample into an electric arc. The light emission from the D.C. arc excitation was recorded by an emission spectrograph.

Although the absolute sensitivity of the spectrographic technique usually is not low for mercury, the ability to burn the extract of samples > 1 g in size in a single electrode results in adequately low detection limits for the technique. The lower limits of detection were 0.02 μg per g of wet liver, leg muscle, and sternal muscle; 0.06 μg per g of wet kidneys and brain; and 0.01 μg per g of dry soil. The absolute concentration ranges of the spectrochemical method employed were 0.01-10.0 μg of mercury. Spike recoveries averaged 82 per cent in the 0.01-0.10μ g range, 88 per cent in the 0.10-1.0μg range, and 95 per cent in the 1.0-10.0 μg range.

Concentrations of mercury in the pheasant tissues and in the soil samples are presented in ppm (μg per

g). When mean concentrations were calculated, values less than the lower limits of detection were considered to be 1/10 the lower limit.

Findings and Discussion

The pheasants from Champaign County and from Livingston County did not differ appreciably in body weight, advancement of molt of the primary flight feathers, or incidence of mercury in their bodies, when the possible influence of sex and age was taken into consideration. Thus, data for birds from the two counties were combined for statistical analysis and presentation.

Mean weights of the pheasants were 486 ± 40 g for juvenile hens, 719 ± 68 g for juvenile cocks, and 813 ± 12 g for adult hens. The adult cock weighed 1,043 g. Mean ages of the juvenile birds were 9.4 ± 0.4 weeks for hens and 10.6 ± 0.7 weeks for cocks. The adult hens had molted an average of 6.6 ± 0.3 primaries; the adult cock had molted 7 primaries. These physical characteristics are considered to be typical of pheasants in east-central Illinois during late August.

The frequencies of occurrence and the concentrations of elemental mercury in the pheasant tissues are summarized in Table 1. From the data in the table it appears that the incidence of mercury in juvenile birds and in adult birds was similar. When all 20 pheasants were considered, mercury was detected in 35 per cent of the kidneys, 40 per cent of the livers, 15 per cent of the brains, 25 per cent of the leg muscles, and 15 per cent of the sternal muscles. However, mercury was detected in at least one of the five tissues in 85 per cent of the pheasants included in this study.

Mean concentrations of mercury were low (0.07 ppm or less) in all tissue samples except brains of the juvenile pheasants. The high mean concentration in brains is attributable to a high value (5.93 ppm) found in one juvenile. This high value appears to be analytically correct. However, it is something of a paradox in that it occurred in a bird in which the other tissues had low values (0.08 ppm or less). We offer no explanation as to why mercury was abundant in the brain of this bird, except to point out that one of us (PLS) has observed similar phenomena with mercury in other biological materials. An

Table 1.—Incidence of mercury in pheasants in east-central Illinois, August 1970.

	Frequency of Occurrence	Concentrations (ppm)*		
		Mean	Median	Highest
Kidneys				
Juveniles	3/10	< 0.06	< 0.06	0.12
Adults	4/10	0.07 ± 0.04	< 0.06	0.44
Liver				
Juveniles	3/10	< 0.02	< 0.02	0.11
Adults	5/10	0.04 ± 0.02	< 0.02	0.20
Brain				
Juveniles	2/10	0.61 ± 0.59	< 0.06	5.93
Adults	1/10	< 0.06	< 0.06	0.28
Leg Muscle				
Juveniles	3/10	0.02 ± 0.01	< 0.02	0.10
Adults	2/10	< 0.02	< 0.02	0.08
Sternal Muscle				
Juveniles	1/10	< 0.02	< 0.02	0.08
Adults	2/10	0.05 ± 0.04	< 0.02	0.40

*μg per g of wet weight.

occasional high value appears to be the rule rather than the exception.

Because humans normally eat the muscular portions of pheasants, the incidences of mercury in leg muscle and in sternal muscle are of particular interest. Concentrations in the five samples of leg muscle that contained mercury above the limit of detection were 0.02, 0.06, 0.08, 0.08, and 0.10 ppm. Similarly, concentrations in the three samples of sternal muscle that contained detectable levels were 0.08, 0.10, and 0.40 ppm. Except for sternal muscles of adult birds, mean concentrations in muscular tissues were 0.02 ppm or less. The relatively high mean concentration in sternal muscle of adults (0.05 ppm) was due to 0.40 ppm that was found in one bird. For comparison, an average of 0.18 ± 0.03 ppm of mercury was found in flesh of pheasants and partridges collected in Montana (presumably in 1969); the frequency of occurrence was 100 per cent in a sample of 20 birds (Dunkle 1969:3). In Alberta, concentrations of mercury in muscular tissue of these game birds, collected in early summer 1969, averaged 0.45 ppm (Wishart 1970:5).

The U.S. Food and Drug Administration has established 0.50 ppm as a temporary guideline for mercury in fish, but the agency has not set tolerance levels for game birds, poultry, or other human foods. However, in Canada, the Food and Drug Directorate recently adopted 0.50 ppm as a temporary tolerance level for mercury in game birds. The World Health Organization suggests 0.05 ppm as the tolerance level in human foodstuffs. If 0.50 ppm is considered permissible for mercury levels in pheasants, all 20 of the birds examined during the present study would be safe for human consumption. On the other hand, if the permissible level is considered to be 0.05 ppm, four samples (20 per cent) of leg muscle and three samples (15 per cent) of sternal muscle might be considered unfit for human food. Mean concentrations of mercury in both leg muscle and sternal muscle of both juvenile pheasants and adult pheasants did not exceed 0.05 ppm (Table 1).

The samples of soil collected in the fields in which the pheasants were captured contained an average of 0.02 ± 0.01 ppm of mercury when expressed on a dry-weight basis. Mercury was detected in four of the eight samples, the highest individual concentration being 0.05 ppm. Because mercury occurs naturally in the environment (Bowen 1966:187), small amounts of this heavy metal might be expected to be present in the soil samples, as well as in the pheasants.

When interpreted in light of existing tolerance levels and available geochemical information, findings of this study indicate that neither pheasants nor soils in east-central Illinois are contaminated with potentially dangerous levels of mercury.

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LONGITUDINAL PATTERN OF NUCLEAR SIZE IN BULB SCALE EPIDERMIS OF *ALLIUM CEPA* AND CHANGES IN SIZE IN RESPONSE TO NECKROT

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ABSTRACT.—Nuclear size in onion bulb scale inner epidermis cells was found to vary, increasing in size from base toward the equator and decreasing in size toward the apex of the bulb. The mean nuclear area in basal cells was 3.4 x 10-6 cm2. The nuclear area increased to a maximum of 9.1 x 10-6 cm2 near the equator and decreased to 3.6 x 10-6 cm2 near the apex. Nuclear size may be related to meristematic activity near basal cells, to cell enlargement patterns during bulb development, and to cellular aging and death at the apex following harvest.

Epidermal nuclei adjacent to mycelium of neckrot pathogens responded to infection by decreasing in size. Such nuclei averaged 47 to 81 per cent of normal in area (X.S.), the amount of reduction depending apparently on their initial size.

Nuclear diameter and nuclear dry mass were studied in outer epidermis cells of the equatorial areas of the inner to outermost bulb scales of onion by Courtis (1964). He reported that average cell area, nuclear diameter, and nuclear dry mass increased from inner to outer bulb scales and that these characteristics were closely correlated. These increases were suggested to be age related since outer leaf bases are older than inner ones.

Changes in nuclear size have been reported in a number of studies, a nucleus frequently increasing and then decreasing in response to the same factor. Increases and decreases were reported during cell death by Ogilvie (1962), Cameron (1951), and Bessis (1964), in irradiated cells by Eckert and Cooper (1937), during autolysis by Kasten (1958), and in response to fungus infection by Goodman, *et al.* (1967). Increases have been reported during mitosis by Lyndon (1967) and with differentation of cells by Lorz (1947) and Courtis (1964). Decreases in nuclear size following irradiation have been reported by Schrek (1948). Nuclear size changes involve a gain or loss of water, of dry matter, or of some combination of the two.

This study was designed to determine the longitudinal distribution pattern of nuclear size in bulbs of onion based on a model of cell aging, the youngest cells being at the base of the bulb and the oldest cells at the apex. Since nuclear size and nuclear dry mass were highly correlated (Courtis, 1964), it was considered desirable to characterize longitudinal distribution of nuclear size in leaf bases in order to establish a basis for studies of cell senescence and death using quantitative interference microscopy.

In performing this study, we found that a number of bulbs exhibited fungal neckrots like those described by Walker (1968). In most cases, only the tip of the bulb apex was infected. *Botrytis allii* was easily isolated from many of these bulbs. The study reported herein includes a comparison of nuclear size in infected onions with nuclear size in non-infected ones. We assume the causal organism in the infected bulbs studied to be *B. allii* although no isolations were made from the tissue observed with the interference microscope.

MATERIALS AND METHODS

Inner (adaxial) epidermis of mature white onions, *Allium cepa* L., (approximately 10 cm in diameter) was used in a study of nuclear size

distribution. The outer dry papery scales were removed, and the first exposed turgid scale was used in each of six onions. Ten samples were taken from a 5 mm wide longitudinal strip extending from the stem at the base to the dead tissue at the apex of the bulb. Each sample was one-tenth of the longitudinal strip (approximately 1 cm long), and these were numbered 1 through 10 from base to apex. The inner epidermis was removed from each location, mounted in tap water, and microscopically studied. Ten to twenty nuclei were selected in each location and photographed using a Leitz Orthomat camera on a Leitz interference microscope at a magnification of 500x. No attempt was made to reduce variation among nuclei such as selecting all the nuclei from the same longitude or latitude within the sample area. Only those nuclei lying against tangential faces of cells were studied since nuclei at the radial faces were seen in edge view rather than face view. Nuclear sizes were determined by tracing nuclear images from prophotographic negatives and measuring the tracings with a planimeter. Nuclear size observed in face view will be called nuclear area (NA) throughout this paper.

Fungal infections were confined to apical locations. Since the nucleus and cytoplasm of cells in contact with mycelium were usually disintegrated, host nuclei in the intact cells just ahead of the mycelial front were studied as well as those well in advance of this front. Average NA of infected bulbs (minimum of five nuclei per replicate) were expressed as a percentage of average NA from comparable locations of non-infected bulbs.

TABLE 1.—Mean nuclear area[a/] in relation to location[b/] in the outermost live bulb scale (inner epidermis) of onion.

Location	Nuclear Area (10^{-6} cm^2)	Location	Nuclear Area (10^{-6} cm^2)
1	3.42	6	8.65
2	6.68	7	7.24
3	7.08	8	7.69
4	9.14	9	6.32
5	8.41	10	3.59

a. Six replicates, minimum of 10 nuclei per location per replicate.

b. Locations 1 through 10 are 10 equal distances from base to apex of the bulb scale.

RESULTS

The location means (Table 1) indicate that nuclear areas in different locations of the bulb scale are not uniform but are distributed according to a pattern. The mean nuclear area was least in location 1, generally increased to maximum in location 4, gradually declined to locations 7 and 8, and fell sharply from location 8 to 10. The nuclear areas of location 1 and 10 were nearly equal, and the greatest changes in nuclear areas were found between locations 1 and 2 and between 9 and 10.

An analysis of variance was performed which yielded F values of 4.54 and 16.28 for differences among replicate means and location means, respectively. Since the corresponding tabular values are 3.48 and 2.85 at the 1% level of probability (LeClerg, Leonard and Clark, 1962), then differences among both components are judged to be significant. Adjacent location means are compared using the Duncan mul-

TABLE 2.—Duncan multiple range analysis of differences between adjusted location means. Differences greater than 16.60 are significant at the 5% level and those greater than 22.19 are significant at the 1% level of probability. The line separates the significant differences above from the non-significant below at the 1% level. Those marked * are significant at the 5% level.

Location	Location								
	4	6	5	8	7	3	2	9	10
1	66.67	63.30	60.40	51.57	48.97	49.29	39.40	35.07	1.84
10	64.83	61.46	58.56	49.73	47.13	42.45	37.66	33.23	
9	31.60	28.23	25.33	16.50	13.90	9.22	4.33		
2	27.27	23.90	21.00*	12.17	9.57	4.89			
3	22.38	19.01*	16.11	7.28	4.68				
7	17.70*	14.33	11.43	2.60					
8	15.10	11.73	8.83						
5	6.27	2.90							
6	3.37								
4									

tiple range test (Table 2). The differences between locations 1 and 2, 3 and 4, and 9 and 10 are significant, whereas, the differences between all other adjacent locations are non-significant at the 1% level of probability. Although other comparisons can be made using Table 2, we would like to generalize as follows: no significant difference exists in the average nuclear sizes of locations 1 and 10; little difference exists among the location means in the equatorial region; and nuclear sizes at the polar regions are significantly smaller than those of the equatorial region.

In infected bulbs, the average NA in locations with mycelium and those adjacent to mycelium were found to be less than the NA averages in the same locations in non-infected bulbs (Table 3). However, for cells more distant from the infection, average NA increased. In the case of mycelium present in the location, the greatest reduction in NA occurred in location 7 and least in 10. These also were the locations initially largest and least in average NA (for the three locations studied in which the mycelial front was observed). The reduction in NA in locations adjacent to those with

TABLE 3.—Mean nuclear area (NA) of inner epidermal nuclei adjacent to and distant from the mycelial front in neck-rotted white onion bulbs. Percentages are comparisons of the mean NA of infected with the mean NA of non-infected bulbs. Locations 1 through 10 are equal distances from base to apex of the outermost turgid bulb scale. Infections were in apical locations.

Location	Number of Replications	NA x 10^{-6} cm^2 Infected	Percent of Non-Infected
Infected in location 10.			
10	4	2.9	81
9	4	6.1	97
Infected in locations 10 through 9.			
9	2	3.2	51
8	1	5.5	71
Infected in locations 10 through 7.			
7	2	3.4	47
6	2	5.6	64
Infected in 10, 10 through 9, and 10 through 7, above.			
6	8	9.7	111
1	8	3.6	106

mycelium was similar to that described above; the amount of reduction was related to the initial size. In all of the infected bulbs, the NA averages for locations 6 and 1 were greater than those in these locations in the non-infected bulbs.

Discussion

In the observed patterns of nuclear sizes in non-infected bulbs, two biological interpretations are possible: nuclear size increases with cell enlargement during development; and nuclear size increases with distance from the basal meristematic zone and then declines with senescence and death (at the apex) after bulb maturation and harvest.

In the first interpretation, it is possible that nuclear area and other nuclear properties vary with physiological condition of cells based on their location, *per se*, rather than on distance from the meristematic zone at the base. If a cell-nuclear size relationship exists (Giese, 1962), then significant differences between adjacent location means would be anticipated near the poles and only slight differences near the equator.

In the second interpretation, the youngest cells in the onion bulbs are the basal ones (Hoffman, 1933). Nuclear areas increase by approximately 2.7x from location 1 through 4. Some of the largest cells occur near the equator of the bulb, but it is not known if the increase in nuclear area is concommittant with cell enlargement. The bulb scales are leaf bases which either subtend or at one time subtended leaf blades. The drying and dying of blades at the top of a bulb begins in the field and ultimately results in dead bulb scale apices (Hoffman, 1933). It could be interpreted that the decrease in mean nuclear area from location 4 through 10 may represent a progressive deterioration during senescence in these locations after harvesting. Although the sharp difference in nuclear area between locations 9 and 10 may represent developmental differences, in all likelihood it represents progressive senescence and cell death since segment 10 borders the apical dead zone.

The nuclear sizes in Table 1, the analysis of nuclear size means in Table 2, and our observation of largest cells in the equatorial region and smallest at the poles support both interpretations. Studies of the cell-nuclear size relationship during bulb development, pre-harvest, and post-harvest phases of growth are required to resolve this question.

Although the NA is known to increase or decrease in pathological conditions (Bessis, 1964; Cameron, 1951; Eckert and Cooper, 1937; Goodman, *et al.*, 1967; Kasten, 1958; Kulfinski and Pappelis, 1969; Ogilvie, 1962), the pattern of NA observed in onion epidermal cells appears to be more related to normal growth and development processes. In this and other studies, we observed that NA of onion host cells decreased near the fungi and increased at some distance from the fungi (Kulfinski and Pappelis, 1969a, 1969b). In this study, the initial host NA varied due to location. We are assuming that the small nuclei in location 10 are less able to respond because they are in senescing cells. An additional test of this hypothesis would be to determine whether small non-senescing nuclei at the base of the bulb would be reduced in NA following infection in their vicinity.

From the study of infected bulbs, we conclude that neckrot fungi liberate chemical factors (enzymes and/or toxins) at the site of the infection, which diffuse through the tissue away from the infection site

and cause the nuclei of the host cells to decrease in size as a result of a combination of degradation of nuclear materials and changes in nuclear membrane character. The increase in NA at greater distances from the infection may be a response to metabolic changes in living host cells or to products from dead host cells nearer the infection. It appears that dying of host cells takes place very quickly in those cells in contact with the fungus and more slowly in cells more distant from the mycelium. It is also concluded, since host cell death in fungal parasitism and cellular autolysis (Kasten, 1958) have similar symptoms, that these degenerative changes and others as well may have common pathways, differing only in the specific form of initiation of the changes.

We conclude that the onion bulb epidermis represents a model in which to study nuclear control of cell development, senescence, death, and pathological changes. The fact that quantitative interferometric studies can be made on these living cells (Kulfinski and Pappelis, 1969a) without the obvious disruptions of morphology and composition which occur as a result of histological procedures contributes an important method to the study of the above-mentioned processes. Since NA correlates with nuclear dry mass (Courtis, 1964), and since there is a pattern of NA in the bulb scale, it may be that quantities of nuclear components vary with location and NA. Until the DNA content has been determined in these nuclei, an explanation of nuclear mass and NA changes can not preclude the possibility of endoploidy (Lorz, 1947). The determination of DNA, RNA, chromosomal histone, and total nuclear protein would be useful in the studies of development, senescence, cell death, and cellular changes in response to pathogens.

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VERSATILE APPARATUS FOR STUDYING REACTIONS INVOLVING GAS ADSORPTION OR EVOLUTION

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ABSTRACT.—Apparatus used for determining surface area by a dynamic sorption, or gas chromatography, method has great potential for other studies. Modifications and techniques can transform the apparatus into a powerful research tool for studying various types of gas-solid interactions, including chemical or thermal decomposition and chemisorption. Evolved gas analysis (EGA) is the basis for most of the methodology.

Nelsen and Eggertsen (1958) described a dynamic gas adsorption method involving relatively simple apparatus for determining the surface areas of particulate solids. The method was further evaluated and refined by Daeschner and Stross (1962), and a commercial version of the apparatus, called the "Sorptometer," was introduced shortly thereafter by the Perkin-Elmer Corporation (Norwalk, Conn.). Because of its similarity in operating principles to those used in gas chromatography, this method for determining surface area is commonly referred to as the chromatographic method. The surface area values determined with the classical BET (Brunauer, Emmett, and Teller, 1938) equation are in close agreement with those obtained by static methods in the more conventional pressure-volume apparatus.

With only slight modifications, the same apparatus is useful in considerably broader research studies. One of its major advantages, for example, is that the gaseous environment around a sample can be carefully controlled by adjusting the flow rates of gases or gas mixtures passing over the sample. With the addition of a furnace and temperature controller, thermal decomposition studies can be conducted at controlled temperatures and under controlled environments. A thermal conductivity detector is used for all gas measurements. The sensitivity and reliability of such a detector permit the use of small (<1 gram) samples, which is advantageous in decomposition studies.

The potential utility of this type of apparatus in physical research has not yet been fully realized or appreciated. This report, which is based on both published and unpublished work from our laboratory, calls attention to the versatility of the apparatus in studies involving gas-solid interrelationships.

APPARATUS

A schematic diagram of the apparatus is shown in Figure 1. The basic gas flow scheme is the same as that described by Nelsen and Eggertsen (1958).

A thermal conductivity cell assembly (Gow-Mac 9193-TE-II) is contained in an oil bath at a controlled temperature (± 0.1° C). The reference and measuring detectors form part of well-known bridge circuitry (Gow-Mac Bulletin TCTH-6-62-3M). A Sorensen QB12-2 d.c. power supply (Raytheon Company, South Norwalk, Conn.) provides a stable bridge current.

A Sargent Model MR (multirange) recorder equipped with a Disc integrator (Disc Instruments, Inc., Santa Ana, Calif.) is used for recording bridge signals and integrating peak areas.

Most gases are delivered at less than 10 psig. Matheson Series 70 low pressure regulators are used for pressure control. Hoke precision

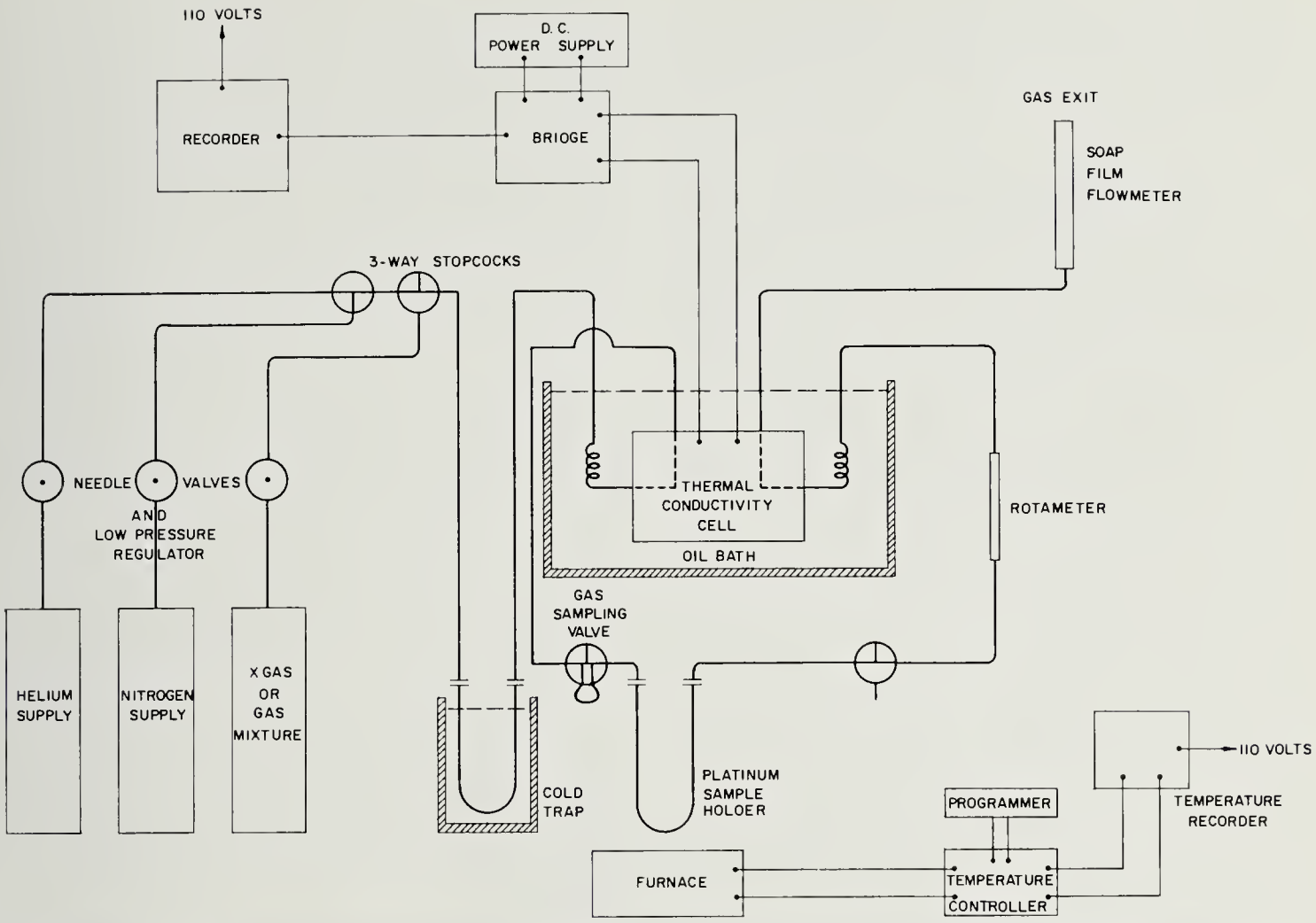

FIGURE 1. Schematic diagram of apparatus used for various gas-solid studies.

needle valves (Enpro, Inc., Villa Park, Ill.) are used in conjunction with the low pressure regulators for controlling the gas flow rates. Corrosive gases, if desired, can be introduced into the inert carrier gas stream via calibrated loops of a gas sampling valve located upstream from the sample. Gas mixtures containing, for example, a known concentration (dilute) of a corrosive gas in an inert gas also can be purchased. If sufficiently dilute (and usually dry) such a mixture can be passed through the whole system with little danger of corrosion of the regulators or of the TC cell. The gas flow at any time is monitored by a rotameter and determined with a soap film flowmeter.

Our U-shaped sample holder is is made from Pt-Rh (5%) tubing and is approximately 12 inches long by 5/16 inch in diameter. To connect it to the rest of the apparatus, glass ball joints affixed to graded seals are sealed to the sample tube. Swagelok "quick connect" fittings also have been used successfully.

The furnace constructed in our laboratory is about 5-1/2 inches high and 7 inches in diameter overall. The heated portion is an alumina cylinder 2-1/2 inches in diameter and closed at the bottom. The furnace, which has a handle attached, can be raised (or lowered) quickly into position around the sample holder. The temperature controller, also of our design, controls the furnace temperature to ± 0.5°C. Temperature is recorded directly by a Brown Electronik recorder, Model X-42A.

A Data-Trak, Model 5300, programmer (Research, Inc., Minneapolis, Minn.) has been found useful for temperature programming. With this unit a temperature pro-

gram incorporating particular pauses at given temperatures or temperature intervals can be easily set up.

Uses of the Apparatus

Surface Area. The significance of this parameter should not be underestimated. The surface of a solid differs from the rest of the substance in that the atoms or molecules at the surface are coordinatively unsaturated compared with the atoms or molecules below the surface. If surface area is increased by fine grinding, or if the surface area is large because of particle porosity, then the reactivity of the substance is usually greatly enhanced. For example, increased surface area generally increases the rate of solubility, ion exchange, catalysis, corrosion, oxidation-reduction and thermal decomposition.

There is little need to discuss here either the operation of the apparatus or the calculations involved in surface area determinations. These details were given by Nelsen and Eggertsen (1958). Suffice it to say that the adsorbate (usually nitrogen) is adsorbed near its boiling point at three relative pressures established by changing the flow rate of the adsorbate gas in the mixed gas stream. Helium acts as the carrier gas. Adsorption, or desorption, of the adsorbate gas by the sample produces a peak on the recorder chart. The gas volume adsorbed (or desorbed) is determined from the peak area and from calibration curves relating peak area to known volumes of the adsorbate gas. Since detector response differs for the same volume of different gases, calibration is necessary for any gas that is introduced into or removed from a given gas stream.

Some of the more interesting surface area studies for which the apparatus is particularly well suited involve the generation of surface within solid particles by thermal decomposition (Thomas, Hieftje, and Orlopp 1965). The rate of thermal decomposition, which also can be followed with the apparatus, is discussed in the next section. Substances such as carbonates, nitrites, nitrates, hydroxides, carbonyls, and oxalates decompose thermally, evolving one or more gases and producing new solid phases that may be quite porous with consequent large internal surface area when decomposition is conducted under proper conditions. From naturally occurring carbonates, for example, calcium oxide and magnesium oxide can be produced that have surface areas of about 60 m^2/g and 500 m^2/g, respectively. Because of the large available surface, such substances are often referred to as "active."

A controlled environment is necessary for the study of "active" substances. Changes in the surface area of the "active" phase can be studied as a function of the sample environment (temperature or atmosphere) either during the course of decomposition or after decomposition is complete. The differences that do occur involve crystal growth and sintering mechanisms. With the apparatus described, a sample can be decomposed, either wholly or to a specific partial extent, the surface area determined, and further experimentation conducted to affect surface changes, all without moving the sample or exposing it to a deleterious laboratory atmosphere.

Decomposition. "Active" solids with large surface areas are produced readily, under some conditions, from solid-state thermal decomposition reactions of the type A(solid) —— B(solid) + C(gas or gases). Most of these reactions continue to completion under nonequilibrium conditions if the gas or

gases are continually removed during a run, either by vacuum or by sweeping the sample with an inert gas such as helium. The rates of such reactions and the variables that influence the rates are of general interest in solid-state chemistry. This type of study with the apparatus basically is evolved gas analysis (EGA).

In our apparatus a controlled constant flow of helium removes the gaseous decomposition product as it evolves and carries it through the thermal conductivity cell for measurement. The decomposition is easily followed to completion on the recorder chart (Figure 2). The four different decomposition curves shown are for different sieve-size fractions of Iceland spar (calcite). All runs were made under the same experimental conditions. From the furnace-on position (850°C. initially), about one minute elapses before gas detection occurs.

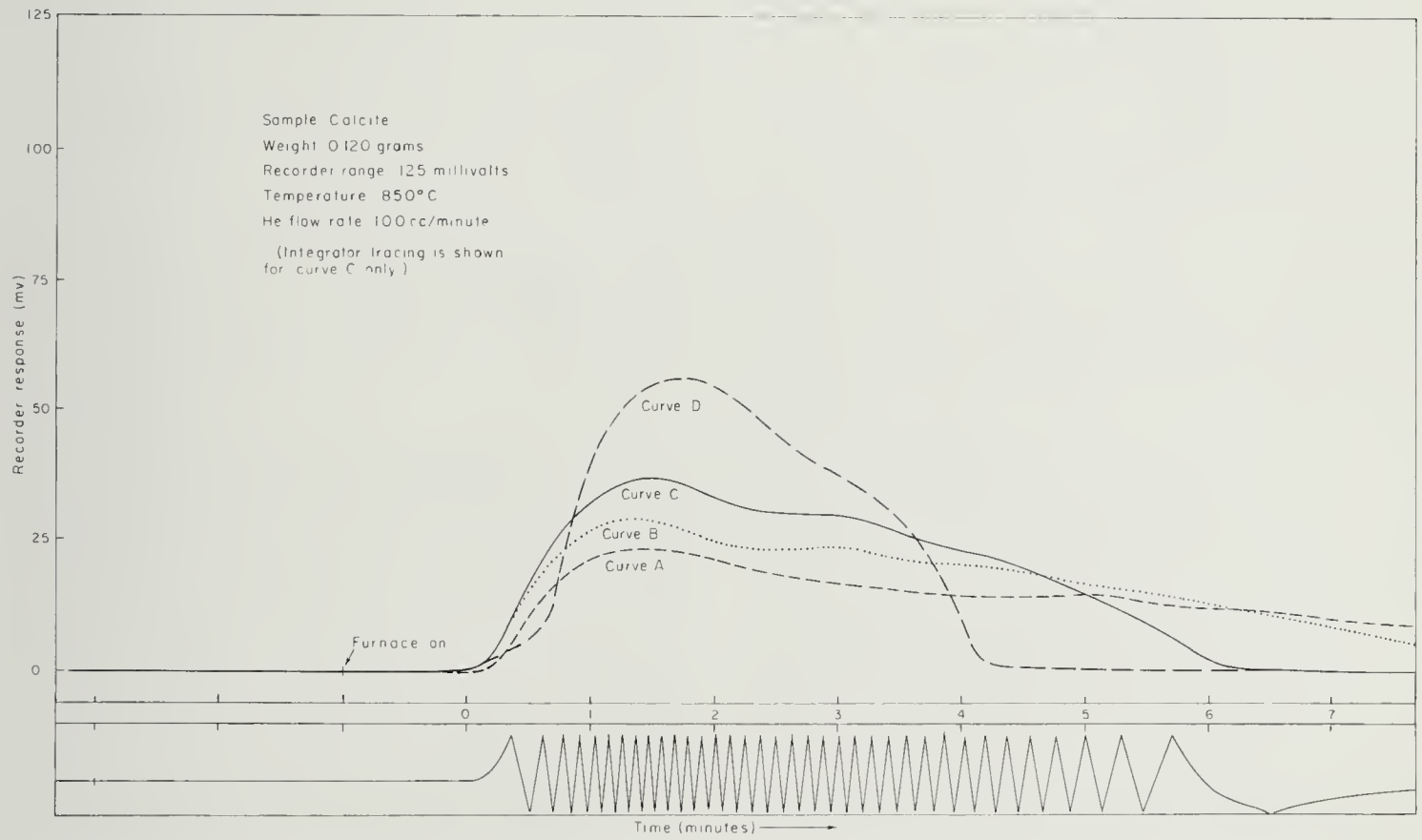

FIGURE 2. Thermal decomposition of calcite cleavage pieces. Size fraction: A = 833-991μ, B = 295-417u, C = 74-89μ, D = $<10\mu$.

Recorder data also can be presented in the more common plot of α(fraction decomposed) versus time (Figure 3). The fraction decomposed (α) is determined from the integrator counts, as a fraction of the total integrator counts representing complete decomposition, at time (t), which is determined from the recorder chart speed.

It is evident from these data that with decreasing crystallite size (not necessarily particle size) the rate of decomposition increases. This is well recognized for solids and is explained by the fact that, in general, the more perfect a crystal is, the smaller its reactivity. The more reactive centers are found at places where defects or faults occur in the crystal order. As mentioned earlier, the surface of a crystal is one such place, as the outer-most, or surficial, atoms are not bounded in three dimensions as are their neighbors immediately below the surface. Therefore, increasing the surface by subdividing the solid increases its reactivity, as is shown by the increased rate of thermal decompo-

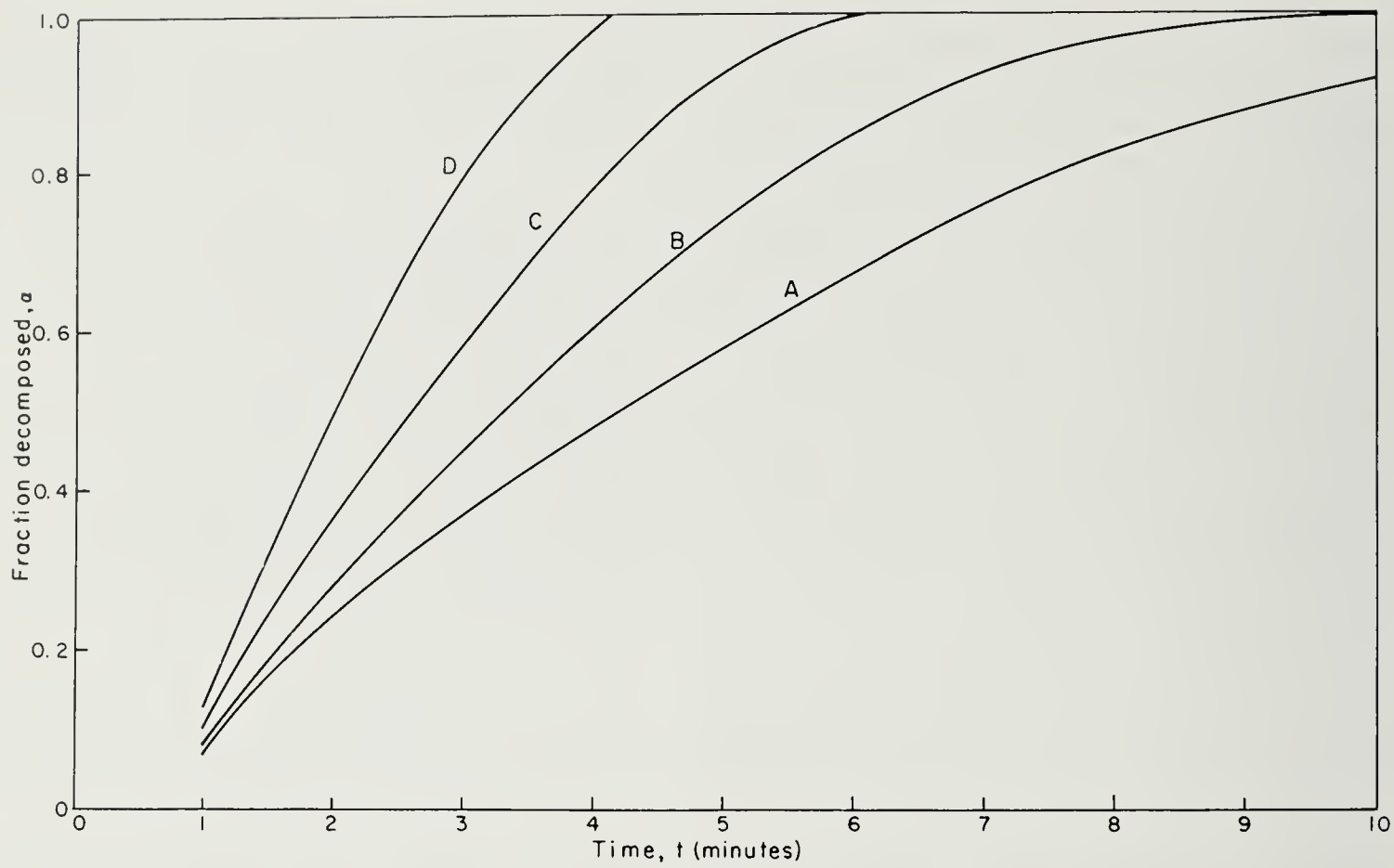

Figure 3. Fraction decomposed (ζ) versus time plots for decomposition of calcite (data from Figure 2).

sition. Defects within crystals may be caused by impurities, and they also can be introduced by irradiation.

The rate of thermal decomposition also depends upon the temperature and upon the rate of removal of the gaseous products. Our apparatus readily lends itself to the study of the influence of these variables on decomposition. For the past 10 years much of this type of research has been conducted with TGA (thermogravimetric analysis) apparatus, with which weight loss is directly recorded as a function of time at a given temperature. TGA is an excellent technique in this respect, but such apparatus is not well suited for low-temperature surface area measurements.

The modified dynamic sorption apparatus also can be used for studies of chemical decomposition. Thomas and Hieftje (1966) described the use of the apparatus in the analytical determination of carbon dioxide from carbonate-containing samples. The method is rapid and precise. It can be used advantageously with small samples containing low ($< 1\%$) percentages of carbonate impurities. A sample tube with a side arm and rubber septum is used for injecting hydrochloric acid onto the sample from a hypodermic syringe. The carbonate portion of the sample decomposes, and helium carrier gas transports the released carbon dioxide through the apparatus and into the thermal conductivity cell for measurement. Absorption tubes containing magnesium perchlorate and anhydrous copper sulfate are attached to the apparatus between the sample tube and detector to remove water and undesirable acidic gases from the helium-carbon dioxide stream.

Chemical Reactivity of Solids. A logical extension of the studies of decomposition and surface area is the study of chemisorption and chemical reactivity of solids. The sample tube functions as a reaction

tube for the study of gas-solid reactions at elevated temperatures on the "active" oxides formed. Carbon dioxide, for example, will recombine with "active" oxides of calcium and magnesium if equilibrium is shifted in favor of the carbonates. A study of the rate of recombination at various temperatures or at various partial pressures of carbon dioxide is possible with the apparatus.

Studies now in progress in our laboratory are concerned with the uptake of sulfur dioxide by "active" oxides of calcium and magnesium. A practical application of these studies is the removal of sulfur dioxide, an air pollutant, from stack gases. One of the less expensive processes now being tested for removing sulfur dioxide involves the injection of limestones into a particular portion of the hot zone of the stack.

In our studies a known volume of sulfur dioxide is introduced into the helium carrier gas by means of a gas sampling valve located upstream from the sample. From the amount (by calibration) of sulfur dioxide that is measured at the detector, the relative efficiency of a particular oxide in removing sulfur dioxide from the gas stream and reacting with it to form a new solid phase can be determined. X-ray diffraction is used to determine the nature of the new phase, or phases. The decomposition of the limestones, the surface area measurements, and the measurements on the efficiency of sulfur dioxide removal as a function of surface area and other variables, are all conducted on the sample in situ. To be able to control sample history as closely as is possible with this apparatus gives considerable confidence in the interpretation of results.

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CATALOG OF PALEOZOIC PALEOZOOLOGICAL TYPE AND FIGURED SPECIMENS AT THE ILLINOIS STATE MUSEUM

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ABSTRACT.—The paleontology collections of the Illinois State Museum contained numerous type and figured specimens. Over the years, many of these specimens have been transferred elsewhere resulting in much confusion as to the location of these important specimens. The following is a list of those now known to be in the Museum collections, exclusive of Pleistocene material.

The paleontology collections of the Illinois State Museum have, at one time or another, contained many type specimens and others figured in the literature. Dr. A. R. Crook (1911), Museum Director 1906 to 1930, wrote that the Museum paleontology collection contained 4700 described and 3500 figured specimens in 1910.

The most noteworthy single collection was that accumulated by A. H. Worthen (1813-1888), State Geologist of Illinois from 1858 until his death in 1888. Another significant collection is one built by George Langford, Sr. and his son, George Langford, Jr., which the Museum acquired in the late 1930's. This collection is composed primarily of plant fossils although many invertebrate forms are also present.

Worthen, in collaboration with several paleontologists, published descriptions of many new species and figured other fossils in the *Geological Survey of Illinois* (Volumes I-VIII) between 1866 and 1890. Other new species were first described in the *Proceedings of the Academy of Natural Sciences, Philadelphia* and later redescribed and figured in the *Geological Survey of Illinois*.

Later, portions of the collections were studied and the results published by various workers, among them Janssen (1939, 1940), Carpenter (1943), Raymond (1945), Petrunkevitch (1946), Kjellesvig-Waering (1948), Lowenstam (1948) and Langford (1958, 1963).

Many of the type and figured specimens formerly housed at the Illinois State Museum have over the years been transferred elsewhere. For example, many types were taken to the Illinois State Geological Survey in Urbana, Illinois, during the 1930's. Because of the resulting confusion regarding the locations of these important specimens, it was decided to locate and list as many as possible of those remaining in the Museum's collections and to make this information available. Since their transfer into more accessible storage in the Museum's new building (1963), the collections have been searched for types and figured specimens.

Hanson and Scott (1967) published a catalog of Worthen types now located at the University of Illinois. Their catalog has been of great assistance to workers searching for these important materials.

In this catalog both "type" and figured specimens are listed under the name used in the publication cited. In those instances in which a new species was figured in a publication later than the original description, this reference is also given.

In the Worthen collection, in particular, specimens designated as "type" may be holotype, syntype, paratype or, in a few cases, merely hypotypes. In those instances where it was possible to determine the

correct designation, it has been used. In other cases the original designation, "type," has been retained.

No attempt has been made to modernize the original author's stratigraphic or locality information. In nearly all cases this information is too generalized to permit improvement.

No effort was made to standardize references to formation, group or lithology. The same strata may be referred to as "group" in one description and "limestone" in another. Locations may be given by county, the nearest city, an area such as "Mazon Creek area," or in more general terms as, in the case of some figured specimens, "common in southern Illinois." The original terminology has been retained and, in the case of both stratigraphy and locality, must be understood as such.

The 66 specimens, representing 54 species, are distributed as follows: Invertebrates: 48 species (27 types, 33 figured specimens); Ordovician-2, Silurian-13, Devonian-2, Mississippian-8, Pennsylvanian-35. Vertebrates: 6 species (5 types 1, figured specimen); Devonian-1, Mississippian-5.

These specimens are available for examination at the Illinois State Museum; in some cases, loans may be arranged. Study of these and other specimens in the Museum's collections is not only welcomed but encouraged.

ABBREVIATIONS USED IN THE CATALOG ARE:

Co	County
Coal Meas	Coal Measures
Dev	Devonian
f	figure(s)
gr	group
Ia	Iowa
GSI	Geological Survey of Illinois
Ill	Illinois
Ind	Indiana
ISMNHB	Illinois State Museum of Natural History Bulletin
ISM Sci Pap	Illinois State Museum Scientific Papers
ls	limestone
Miss	Mississippian
Mo	Missouri
M&W	Meek and Worthen
N&W	Newberry and Worthen
no	number
Ord	Ordovician
p	page(s)
PA	Paleontographica Americana
pl	Plate
PPA	Proceedings, Philadelphia Academy of Natural Science
pt	part
Sil	Silurian
ss	Sandstone
STJ&W	St. John and Worthen
U&E	Ulrich and Everett
v	volume
W&M	Worthen and Meek

THE CATALOG

INVERTEBRATES

PHYLUM ARTHROPODA

Class Arachnoidea

Curculioides gracilis Petrunkevitch. Holotype, ISM 14862. Petrunkevitch, 1946, ISM Sci Pap III, no 2, p 68-70, textfig 34, pl 2, f 8-10. Penn. Mazon Creek, Ill.

Discotarbus deplanatus Petrunkevitch. Figured specimen, ISM 14869. Petrunkevitch, 1913, Trans Conn Acad, v 18, p 121-122, textfig 75, 76, pl 12, f 10. ——, 1946, ISM Sci Pap III no 2, p 23, pl 1, f 7. Penn. Mazon Creek, Ill.

Euproops danae M&W. Figured specimen, ISM 14808. Raymond, 1945, ISM Sci Pap III no 3, p 4-6, pl 2, f 1. Francis Creek sh, Penn. Grundy Co., Ill.

E. danae M&W. Figured specimen, ISM 14812. Raymond, 1945, ISM Sci Pap III no 3, p 4-6, pl 2, f 1. Francis Creek sh, Penn. Grundy Co., Ill.

E. thompsoni Raymond. Figured specimen, ISM 14807. Raymond, 1945, ISM Sci Pap III no 3, p 4-6. Francis Creek sh, Penn. Grundy Co., Ill.

Lepidoderma mazonense M&W. Figured specimen, ISM 14813. Kjellesvig-Waering, 1948, ISM Sci Pap III no 4, p 17-24, pl 2, f 3, 4. Francis Creek sh, Penn. Will Co., Ill.

L. mazonense M&W. Figured specimen, ISM 14814. Kjellesvig-Waering, 1948, ISM Sci Pap III no 4, p 17-24, pl 3, f 1. Francis Creek sh, Penn. Will Co., Ill.

L. mazonense M&W. Figured specimen, ISM 14815. Kjellesvig-Waering, 1948, ISM Sci Pap III no 4, p 17-24, pl 3, f 2. Francis Creek sh, Penn. Will Co., Ill.

L. mazonense M&W. Figured specimen, ISM 14816. Kjellesvig-Waering, 1948, ISM Sci Pap III no 4, p 17-24, pl 2, f 1, 2. Francis Creek sh, Penn. Will Co., Ill.

Ootarbus ovatus Petrunkevitch. Holotype, ISM 14866. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 41, 42, textfig 23, pl 1, f 5, 6. Francis Creek sh, Penn. Mazon Creek, Ill.

O. pulcher Petrunkevitch. Type, ISM 14861. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 36-40, textfig 15-20, pl 3, f 12, 13. Francis Creek sh, Penn. Mazon Creek, Ill.

O. pulcher Petrunkevitch. Paratype, ISM 14870. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 40, 41, textfig 21, 22, pl 3, f 17. Francis Creek sh, Penn. Mazon Creek, Ill.

O. pulcher Petrunkevitch. Figured specimen, ISM 14868. Petrunkevitch. 1946, ISM Sci Pap III no 2, p 39, 40, pl 4, f 22. Francis Creek sh, Penn. Mazon Creek, Ill.

Orthotarbus robustus Petrunkevitch. Type, ISM 14863. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 29-32, textfig 9-11, pl 3, f 14, 15. Francis Creek sh, Penn. Mazon Creek, Ill.

O. robustus Petrunkevitch. Paratype, ISM 14865. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 32, 33, pl 3, f 16. Francis Creek sh, Penn. Mazon Creek, Ill.

O. robustus Petrunkevitch. Paratype, ISM 14867. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 32, 33, textfig 12, 13, pl 4, f 20, 21. Francis Creek sh, Penn. Mazon Creek, Ill.

O. robustus Petrunkevitch. Figured specimen, ISM 14874. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 34-, pl 4, f 18, 19. Francis Creek sh, Penn. Mazon Creek, Ill.

O. robustus Petrunkevitch, Figured specimen, ISM 14878. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 34-36, textfig 14, pl 4, f 23. Francis Creek sh, Penn. Mazon Creek, Ill.

Paratarbus carbonarius Petrunkevitch. Holotype, ISM 14864. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 25-27, textfig 5, 6, pl 2, f 11. Francis Creek sh, Penn. Mazon Creek, Ill.

Pleophrynus ensifer Petrunkevitch, Holotype, ISM 14873. Petrunkevitch, 1946, ISM Sci Pap III no 2, p 52-58, textfig 25-27, pl 1, f 1-4. Francis Creek sh, Penn. Mazon Creek, Ill.

Class Insecta

Architarbus rotundatus Scudder. Figured specimen, ISM 14871. Scudder, 1868, GSI, v 3, p 568, textfig 4. Petrunkevitch, 1913, Trans Conn Acad, v 18, p 125-128, textfig 79, 80, pl 12, f 72-75, pl 13, f 76-78. ——, 1946, ISM Sci Pap III, no 2, p 20, 21, textfig 1. Coal Meas, Penn. Mazon Creek, Grundy Co., Ill.

Heterologus langfordorum Carpenter. Holotype, ISM 14879. Carpenter, 1943, ISM Sci Pap III no 1, p 15, 16, textfig 4, pl 3. Francis Creek sh, Penn. Will Co., Ill.

Lithoneura mirifica Carpenter. Holotype, ISM 14880. Carpenter, 1943, ISM Sci Pap III no 1, p 13, 14, textfig 3, pl 2. Francis Creek sh, Penn. Will Co., Ill.

Syntonoptera schucherti Handlirsch. Figured specimen, ISM 14881. Carpenter, 1943, ISM Sci Pap III no 1, p 12, textfig 2. Francis Creek sh, Penn. Will Co., Ill.

Teneopteron mirabile Carpenter. Holotype, ISM 14887. Carpenter, 1943, ISM Sci Pap III no 1, p 17-20, textfig 5, pl 4. Francis Creek sh, Penn. Will Co., Ill.

Thesoneura americana Carpenter. Holotype, ISM 14876. Carpenter, 1943, ISM Sci Pap III no 1, p 10, 11, pl 1, f 1, Francis Creek sh, Penn. Will Co., Ill.

Class Trilobita

Acidaspis hamata Conrad. Figured specimen, ISM 2202, M&W, 1868, GSI, v 3, p 390, pl 7, f 15. Lower Helderberg, Dev. Perry Co., Mo.

Illaenus crassicauda Wahlenberg. Figured specimen, ISM 12031. M&W, 1868, GSI, v 3, p 322, pl 3, f 1. Galena gr., Ord. Galena, Ill.

PHYLUM BRACHIOPODA

Athyris subtilita Hall. Figured specimen, ISM 2939. M&W, 1873, GSI, v 5, p 570, pl 25, f 14. Coal Meas., Penn. La Salle Co., Ill.

Orthis carbonaria Swallow. Figured specimen, ISM 2939. Worthen, 1873, GSI v 5, p 571, pl 25, f 4. Upper Coal Meas., Penn. LaSalle, Ill.

Productus longispinus Sowerby. Figured specimen, ISM 2927. M&W, 1873, GSI v 5, p 569, pl 25, f 10. Coal Meas., Penn., Ill.

Spirifer cameratus Morton. Figured specimen, ISM 8491. M&W, 1873, GSI, v 5, p 573, pl 25, f 6. Coal Meas., Penn.

PHYLUM BRYOZOA (POLYZOA)

Archimedes grandis Ulrich. Type, ISM 2861. Ulrich, 1890, GSI, v 8, p 569, pl 63, f 10. Keokuk gr., Miss. Jersey Co., Ill.

PHYLUM COELENTERATA

Zaphrentis centralis Edwards & Haime. Figured specimen, ISM 2569. Worthen, 1890, GSI, v 8, p 72, pl 9, f 2. Burlington ls, Miss. Henderson Co., Ill.

PHYLUM ECHINODERMA

Alisocrinus? heterodactylus Brower. Paratype, ISM 1565. Brower, 1970, PA, in press. Girardeau ls, Ord. Orchard Creek, Alexander Co., Ill.

Barycrinus hoveyi Hall. Figured specimen, ISM 1617. M&W, 1873, GSI, v 5, p 486, pl 13, f 1. Keokuk gr. Miss. Crawfordsville, Ind.

B. hoveyi var *herculeus* M&W. Type, ISM 1805. M&W, 1868, PPA 1868, p 341. ——, 1873, GSI, v 5, p 485, pl 13, f 2a, 2b, Keokuk, gr, Miss. Crawfordsville, Ind.

Eucalyptocrinus lindahli Wachsmuth & Springer. Type, ISM 10467. W&S, 1892, Amer Geol v 10, p 139. ——, 1897, Mem Mus Com Zoo Harvard, v 20, p 347, pl 82. *E. wortheni;* Miller & Gurley, 1893, ISMNHB no 3, p 53, pl 4, f 2. Niagaran, Sil. Wayne Co., Tenn.

Eucalyptocrinus sp. Figured specimen, ISM 15976. Lowenstam, 1948, ISM Sci Pap IV, pl 6, f 3, Blue Island Zone, Sil., Ill.

Eupachycrinus boydi M&W. Holotype, ISM 2443. M&W, 1870, PPA, p 30. ——, 1873, GSI v 5, p 554, *E. Asperatus* Worthen. Worthen 1882, ISMNHB 1, p 34, ——, 1883, GSI v 7, p 311, 312, pl 29, f 4. Chester gp. Miss. Monroe Co., Ill.

Myelodactylus sp. Figured specimen, ISM 15977. Lowenstam, 1948, ISM Sci Pap IV, pl 6, f 7, Blue Island Zone, Sil. Blue Island, Ill.

Pisocrinus benedicti S. A. Miller. Figured specimen, ISM 15972. Lowenstam, 1948, ISM Sci Pap IV. pl 5, f 4, 5. Liston Creek fm, Sil. Wabash, Ind.

P. campana S. A. Miller. Figured specimen, ISM 15978. Lowenstam, 1948, ISM Sci Pap IV, pl 6, f 8. Racine-Guelph, Sil. Thornton, Ill.

P. quinquelobus Bather. Figured specimen. ISM 15973. Lowenstam, 1948, ISM Sci Pap IV, pl 5, f 7. Racine-Guelph, Sil. Thornton, Ill.

Scaphiocrinus carbonarius M&W. Figured specimen, ISM 1908. M&W, 1873, GSI, v 5, p 562, pl 24, f 2. Coal Meas., Penn. Springfield, Ill.

Zeacrinus (Hydreionocrinus?) acanthophorus M&W. Type, ISM 1906. M&W, 1870, PPA, p 28. ——, 1873, GSI, v 5, p 563-565, pl 24, f 11. roof of coal no 1, Penn. Seaville, Fulton Co., Ill.

PHYLUM MOLLUSCA

Class Gastropoda

Loxonema cf. *leda* Hall. Figured specimen, ISM 15979. Lowenstam, 1948, ISM Sci Pap IV, pl 7, f 6-8. Dalmanites Zone, Sil. Blue Island, Ill.

Class Pelecypoda

Allorisma costata M&W. Type, ISM 2975. M&W 1869, PPA, p 171. ——, 1873, GSI, v 5, p 585, 586, pl 26, f 15. Lower Coal Meas., Penn. Warren Co., Ill.

A. elongata Worthen. Type, ISM 2542. Worthen, 1884, ISMNHB no 2, p 12. ——, 1890, GSI, v 8, p 133, pl 19, f 10. Keokuk ls, Miss. Warsaw, Ill.

A. illinoiensis Worthen. Type, ISM 2540. Worthen, 1884, ISMNHB no 2, p 11. ——, 1890, GSI, v 8, p 132, pl 18, f 1, 1a. Keokuk ls, Miss. Warsaw, Ill.

Bakevellia illinoiensis Worthen. Type, ISM 2525. Worthen, 1884, ISMNHB no 2, p 14. ——, 1890, GSI, v 8, p 126, pl 18, f 4, 4a. Upper Coal Meas. Penn. LaSalle Co., Ill.

Grammysia rhomboidalis M&W. Type, ISM 4603. M&W, 1865, PPA, 1865, p 248. ——, 1868, GSI, v 3, p 439, pl 11, f 3a, 3b. Hamilton gr, Dev. Jackson Co., Ill.

Macrodon sangamonensis Worthen. Type, ISM 2585. Worthen, 1890, GSI, v 8, p 123, pl 21, f 3. Coal Meas., Penn. Ralls Ford, Sangamon Co., Ill.

Schizodus varsoviensis Worthen. Type, ISM 2500. Worthen, 1884, ISMNHB no 2, p 10. ——, 1890, GSI, v 8, p 107, pl 19, f 7. Keokuk gr., Miss. Warsaw, Ill.

PHYLUM PORIFERA

Anthaspidella scutula U&E. Type, ISM 2639. U&E, 1890, GSI, v 8, p 261, pl 3, f 1, 1a. Trenton ls, Sil. Dixon, Ill.

Astraeospongia meniscus Roemer. Figured specimen, ISM 15967. Lowenstam, 1948, ISM Sci Pap IV, pl 1, f 11, 12. Liston Creek fm, Sil. Wabash, Ind.

A. meniscus Roemer. Figured specimen, ISM 15968. Lowenstam, 1948, ISM Sci Pap IV, pl 2, f 5, 6. Blue Island Zone, Sil. Blue Island, Ill.

A. meniscus Roemer. Figured specimen, ISM 15969. Lowenstam, 1948, ISM Sci Pap IV, pl 3, f 7-9. Blue Island Zone, Sil. Blue Island, Ill.

A. mensicus Roemer. Figured specimen, ISM 15971. Lowenstam, 1948, ISM Sci Pap IV, pl 5, f 1, 2. Blue Island Zone, Sil. Blue Island, Ill.

Astylospongidae sp. indet. Figured specimen, ISM 15970. Lowenstam, 1948, ISM Sci Pap IV, pl 4, f 6. Waukesha fm, Sil. Elmhurst, Ill.

VERTEBRATES

PHYLUM CHORDATA
Subphylum Vertebrata

Class Pices

Cochliodus Leidyi StJ&W. Type?, ISM 12824. StJ&W, 1883, GSI, v 7, p 127-130. Chester ls, Evansville, Ill.

Dinichthys (Eastmanosteus) pustulosus Eastman. Type, ISM 416238. Eastman, 1902, Am Nat, v 36, n 428, p 653-657, f 1. Hamilton ls, Dev. Andulusia, Rock Island, Co., Ill.

Physonemus falcatus StJ&W. Type, ISM 8720. StJ&W, 1883, GSI, v 7, p 252, pl 24, f 6. St. Louis ls, Miss., St. Louis, Mo.

Pnigeacanthus trigonalis StJ&W, Type?, ISM 7179, StJ&W, GSI, v 7, p 259, 260. St. Louis ls, Miss., Alton, Ill.

Psammodus crassidens StJ&W. Type, ISM 7083. StJ&W, 1883, GSI, v 7, p 218, pl 18, f 6. St. Louis ls, Miss. St. Louis, Mo.

P. Plenus StJ&W. Type, ISM 7154. StJ&W, 1883, GSI, v 7, p 213, pl 16, f 4. St. Louis ls, Miss. St. Louis, Mo.

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THE CYPERACEAE OF ILLINOIS. XII. CAREX, PART 1

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ABSTRACT.—This is the initial article in a series on the systematics of the genus *Carex* in Illinois. Treated in this paper are the sections Divisae, Chordorrhizeae, and Arenariae. A new variety is named. Keys and descriptions are provided.

This is the first in a series of publications dealing with the genus *Carex* in Illinois. The other eleven genera of Cyperaceae in Illinois have been treated in prior issues of The American Midland Naturalist and The Transactions of the Illinois State Academy of Science. The illustrations are by Fredda Burton. One or more sections of *Carex* will be the subject of each of the papers. Keys to various groups will be provided when necessary. A key to all taxa of *Carex* in Illinois will follow the systematic account of the taxa.

Species of *Carex* are perennials with grass-like leaves. Their stems are frequently triangular and bear 3-ranked leaves. The leaves are composed of a blade, a sheath, and a ligule.

The flowers are borne in spikes or heads in the axils of bracts (scales). The flowers are unisexual and borne either in different parts of the spikes or in separate spikes on the same culm. Rarely are the plants dioecious.

Neither the staminate nor the pistillate flower has a perianth. The staminate flower merely consists of three stamens in the axil of a scale. The pistillate flower consists of one pistil which is enclosed in a sac (perigynium) in the axil of a scale. The style is either 2- or 3-cleft.

The fruit is an achene enclosed in the perigynium. It may be lenticular, plano-convex, or trigonous.

Carex in Illinois is generally considered to be divided into two subgenera. Subgenus Vignea has mostly uniform and sessile spikes, two styles, and a lenticular or plano-convex achene. Subgenus Carex has at least some all-pistillate spikes, usually three styles, and a trigonous achene.

The Illinois species of *Carex* fall into eleven sections of subgenus Vignea and twenty-seven sections of subgenus Carex.

This paper is concerned with sections Divisae, Chordorrhizeae, and Arenariae of subgenus Vignea.

In addition to having the usual characters of the subgenus, the taxa of these three sections all possess slender, well-developed rhizomes, have some or all spikes androgynous, and are monoecious.

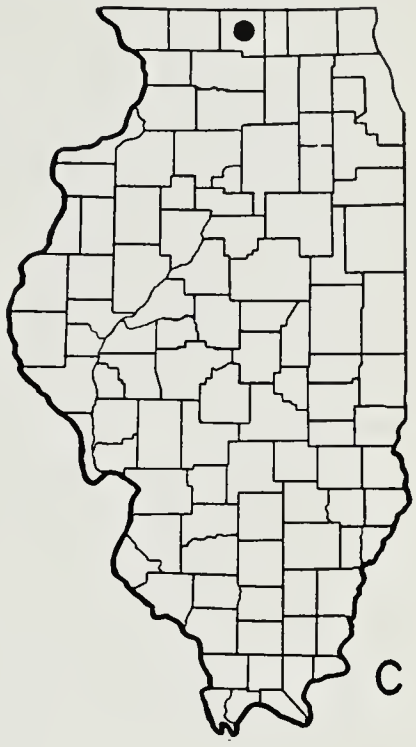
SYSTEMATIC TREATMENT

§Divisae

Rhizomatous perennials; culms mostly solitary; spikes androgynous, distinct or more often in interrupted or subcontinuous heads, the lowest bracts awned, awn-tipped, or bearing a short blade; styles 2; perigynia coriaceous, usually with a narrow margin, with an entire or serrulate short beak; achenes lenticular or plano-convex.

Key to the Taxa of § Divisae in Illinois

1. Rootstock wiry, with profuse fibrous roots; culms to 15 cm tall; leaves stiffly erect, to 12 cm long, with sharply triangular tips; inflorescence head-like, 0.8-1.3 cm long; perigynia 3-7 per spikelet, with wrinkled striations ventrally . . . . 1. *C. stenophylla* var. *enervis*
1. Rootstock stout, with few fibrous roots; culms to 65 cm tall; leaves ascending to spreading, to 35 cm long, flattened throughout; inflorescence spike-like, 2-4 cm long; perigynia 8-12 per spike-



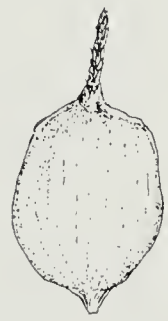


FIGURE 1. *Carex stenophylla* var. *enervis*. a. Habit, x 1/2. b. Inflorescence, x 1-1/2. c. Map. d. Inflorescence, x 1-1/2. e. Staminate scale, x 7-1/2. f. Pistillate scale, x 7-1/2. g. Perigynia, dorsal view, x 7-1/2. h. Perigynia, ventral view, x 7-1/2. i. Achene, x 7-1/2.

let, without ventral nerves and wrinkles.....2. *C. praegracilis*

1. *Carex stenophylla* Wahlenb. var. *enervis* (C.A.Mey.) Kükenth. in Engl. Das Pflanzenreich 4(20):122. 1909. Fig. 1.

Carex enervis C.A.Mey. in Ledeb. Fl. Altaica 4:29. 1833.

Carex eleocharis Bailey, Mem. Torrey Club 1:6. 1889.

Plants cespitose, from fibrous roots with scaly, wire-like rootstocks; culms slender, smooth, canaliculate, (3-) 6-15 cm tall, slightly exceeding the leaves with the base fibrillose; leaves 2-4, (2-) 6-12 cm long, 0.75-1.50 mm wide, arising near the base, canaliculate, ascending, becoming triangular at the tip with the margins subscabrous; sheath apex truncate, the old sheaths persistent; inflorescence androgynous, 8-13 mm long, 3-5 mm wide; spikelets 4-7, scarcely distinguishable; pistillate scales ovate, 2.5-3.5 mm long, 1.5-2.0 mm wide, acuminate, reddish-brown, margins hyaline, completely concealing the perigynia; staminate scales narrow-lanceolate, 3-4 mm long, 1.0-1.5 mm wide, acuminate, reddish-brown in upper half, hyaline in the lower half; bracts encircling the culm with the lowest awn-tipped; perigynia 3-7 per spikelet, 2.5-3.0 mm long, 1.25-1.50 mm wide, plano-convex, ovate-acuminate, the margins elevated ventrally with the upper half serrate, obscurely striate ventrally and dorsally, greenish-brown, substipitate, finely reticulate throughout with the bidentate beak 1 mm long, serrate, hyaline-tipped and obliquely cleft dorsally; achene 1.50-1.75 mm long, 1.25-1.50 mm wide, lenticular, yellow-green, finely puncticulate, jointed to a short style; stigmas 2.

Habitat: Gravel-bluff prairie.

Range: Manitoba northwest to Yukon, south to eastern Oregon, Utah, New Mexico, Iowa, and northern Illinois.

Both Eurasian and American material were originally called *Carex stenophylla* Wahlenberg. However, Eurasian material tends to have perigynia 3.0-3.5 mm long and nerved, while American material has perigynia 2.5-3.0 mm long and essentially nerveless. American material was first segregated as *Carex enervis* C.A. Mey., then as *Carex stenophylla* var. *enervis* (C.A. Mey.) Kükenth., and finally as *Carex eleocharis* Bailey.

Carex stenophylla var. *enervis* is best distinguished by its leaf shape. The leaf tip becomes triangular, stiff, and sharp-pointed, accounting for its common name, the Needleleaf Sedge. Its short, erect stature, fibrous roots with wiry rootstock, and nerveless perigynia provide good identifying characters as well.

Although Fernald (1950) remarks that the spikelets appear in a definitely interrupted head, the Illinois material examined had spikelets that were scarcely distinguishable from each other.

Carex stenophylla var. *enervis* is primarily a western and northern sedge and can be found in abundance in the mixed prairie states. Hanson and Churchill (1961) remark that *C. eleocharis* is considered a dominant in the blue gramagrass-needlegrass-sedge communities on the upland plateaus of western North Dakota. Weaver and Albertson (1956) report that this taxon ranks high among the species of the mixed prairie where it occurs in dense patches and provides early grazing.

This taxon is rare in Illinois, the easternmost limit of its range, and may be limited to Winnebago County where Fell (1958) reports the only known location.

Specimens Examined: WINNEBAGO: Camp Grant, S. of Rockford, gravel-bluff prairie, April 29, 1957, *Fell 57-9* (ISM, ILL); Camp Grant, S. of Rockford, gravel-bluff prairie, May 6, 1957, *Fell 57-68* (ISM, ILL); Camp Grant, S. of Rockford, May 18, 1957, *Fell 57-157* (ISM, ILL); Camp Grant, S. of Rockford, gravel-bluff prairie, May 26, 1957, *Fell 57-248* (ISM, ILL).

2. *Carex praegracilis* W. Boott in Coult. Bot. Gaz. 9:87. 1864. Fig. 2.

Plants from stout, horizontal, brown to black, scaly and fibrillose rootstocks; culms slender, sharply angled and scabrous above, less angled and smooth below, (15-) 30-65 cm tall, usually equalling or slightly exceeding the upper leaves, the base scaly; leaves 2-4, 5-35 cm long, flattened, canaliculate, 2-3 mm broad, ascending to spreading with the margins scabrous; sheath apex truncate, the old sheaths persistent; inflorescence androgynous, narrowly cylindric to slightly spreading, 2-4 cm long, 3-5 mm broad; spikelets 5-12, the upper ones crowded, interrupted in the lower half; pistillate scales ovate, 3.5-4.0 mm long, 1.5-2.0 mm broad, acuminate, pale to brown with a darker keel, margins hyaline, concealing the perigynia; staminate scales narrowly lanceolate, inconspicuous, 3-4 mm long, 1-2 mm broad, acuminate, pale, margins hyaline; bracts lanceolate, the lowest enlarged and scabrous-awned, becoming smaller and less awned toward the apex, often encircling the culm with a dark ring at the base; perigynia 8-12 per spikelet, 2.8-3.75 mm long, 1.25-1.50 mm broad, plano-convex, stipitate, ovate-lanceolate, sparsely nerved dorsally, nerveless ventrally, thin-winged and serrulate above, brownish-black with the 1 mm long serru-

FIGURE 2. *Carex praegracilis*. a. Habit, x 1. b. Inflorescence, x 1. c. Map. d. Staminate scale, x 7-1/2. e. Pistillate scale, x 7-1/2. f. Perigynia, dorsal view, x 7-1/2. g. Perigynia, ventral view, x 7-1/2. h. Achene, x 7-1/2.

late beak cleft dorsally; achene 1.5-2.0 mm long, 0.75-1.25 mm broad, lenticular, dark brown, polished, finely puncticulate, jointed to a short style; stigmas 2.

Habitat: Low prairies, roadsides, and dry sterile soil.

Range: Manitoba northwest to Yukon, south to Mexico, Missouri, Iowa, northern Illinois, and northern Michigan.

Carex praegracilis W. Boott is a most variable species and without striking characteristics. The inflorescence may be linear-cylindric with appressed spikelets or with a short-spreading head. The color of the inflorescence varies from pale to dark brown. The species, though usually tall, slender, and sometimes lax, may also be short and stiffly erect. *Spongberg 61/38* and *61/38A* collected from Bell Bowl Prairie show evidence of burning and therefore the short, erect stature of that material may be anomalous.

Carex praegracilis, especially immature material, may be confused with *C. sartwellii* Dew. or *C. foenea* Willd. However, the combination of brownish-black perigynia without ventral nerves and the often tall, triangular and scabrous culms provides identifying characters.

Fernald (1950) equates *Carex marcida* Boott with *C. praegracilis*; however, Boott's illustration of *C. marcida* shows a thicker inflorescence and a markedly winged perigynia. Such characters are not present in Illinois material.

Carex praegracilis, found commonly in the western states and Canada, is reported from only two areas east of the Mississippi River, northern Illinois and northern Michigan. In Illinois this very rare sedge is limited to Winnebago, Kane, and DeKalb counties.

Specimens Examined: DEKALB: Near Kingston, June 8, 1953, *Fell 53371* (ISM); W. edge of Kingston, June 2, 1956, *Fell 56-91* (ILL); W. edge of Kingston, May 13, 1955, *Fell 55-144* (ILL.) KANE: Elgin, April 27, 1919, *Benke 3591* (F) [previously determined as *C. siccata* Dew.]. WINNEBAGO: Camp Grant, June 14, 1955, *Fell & Fuller 17200* (ILL, ISM, SIU); Camp Grant, August 2, 1953, *Fell 53856* (ISM) [originally determined *C. sartwellii* Dew.]; S. of Rockford, May 21, 1952, *Fell 52105* (ISM); Bell Bowl Prairie, May 10, 1961, *Spongberg 61/38, 61/38A, 61/41, 61/41A* (ISM); Perryville Road, June 6, 1955, *Fell 55-440* (ILL); Camp Grant, May 29, 1955, *Fell 55-318* (ILL); Camp Grant, June 5, 1956, *Fell 56-113* (ILL); Perryville Road, May 29, 1956, *Fell 56-73* (ILL); S. of Cherry Valley, June 6, 1951, *Fell & Fell 51-149* (ILL).

§ Chordorrhizeae

Rhizomatous perennials with cord-like roots; culms mostly solitary, with secondary culms axillary and decumbent at the base; spikes androgynous, head-like, the lowest bracts awn-tipped; styles 2; perigynia spongy, without wings, distinctly nerved, short-beaked; achenes plano-convex.

Only the following species occurs in Illinois.

3. *Carex chordorrhiza* Ehrh. in L. f. Suppl. Pl. Syst. Veg. 414. 1781. Fig. 3.

Culms slender, subscabrous, canaliculate, 10-35 cm tall, exceeding the leaves, base reclining with the nodes bearing fertile and sterile culms; leaves 1-4, 2-20 cm long, 1-2 mm broad, canaliculate, ascending to appressed with scabrous margins becoming smooth near the base; sheath apex concave, the old sheaths persistent; inflorescence androgynous, 1.00-1.75 cm long, 0.5-1.0 cm wide; spikelets 3-5, crowded into a head with staminate flowers conspicuous; pistillate scales ovate, 3-4 mm long, 1.5-2.5 mm wide, acute to acuminate, reddish-brown with hyaline margins; staminate scales narrowly lanceolate, 3-4 mm long, 1.0-1.5 mm wide, reddish-brown to hyaline; bracts (at least the lowest) awn-tipped; perigynia 2-6 per spikelet, 2.5-3.5 (-4.0) mm long, 1.5-2.0 mm wide, plano-convex, margins smooth and thickened, strongly nerved on both sides, glossy, yellow-brown, stipitate,coriaceous and spongy throughout with the beak 0.5 mm long, hyaline-tipped and slightly emarginate dorsally; achene 1.75-2.00 mm long, 1.25-1.75 mm wide, plano-convex, yellow-brown, puncticulate (except margins), continuous with the short style; stigmas 2.

Habitat: Sphagnous swamps.

Range: Newfoundland and eastern Quebec to Alaska, south to Saskatchewan, northern Iowa, northern Illinois, northern Indiana, central New York, southwestern Vermont, and central Maine.

Carex chordorrhiza Ehrh. is best distinguished by the reclining base that gives rise to secondary culms in the axils of the previous year's leaves. The strongly nerved and otherwise distinctive perigynia provide good identifying characters as well.

Although Fernald (1950) remarks that the perigynia have serrulate beaks, and Mackenzie (1940) illustrates serrations, no Illinois specimens examined exhibited this character.

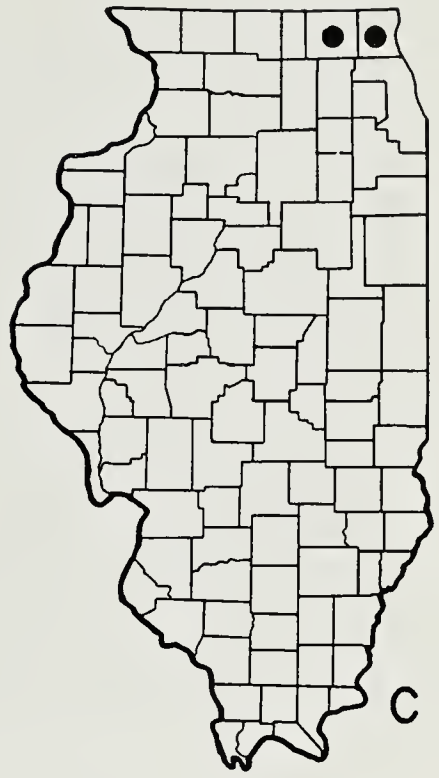


FIGURE 3. *Carex chordorrhiza*. a. Habit, x 1/4. b. Inflorescence, x 1-1/2. c. Map. d. Staminate scale, x 7-1/2. e. Pistillate scale, x 7-1/2. f. Perigynia, dorsal view, x 7-1/2. g. Perigynia, ventral view, x 7-1/2. h. Achene, x 7-1/2.

The roots, lacking in most Illinois collections, appear wiry and cord-like and can be found at the nodes on some decumbent bases.

This species is rare in Illinois and may be limited to the boggy areas of the northeastern part of the state as indicated by the four Vasey collections. It apparently has not been collected in Illinois since the last half of the nineteenth century.

Specimens Examined: LAKE: Without locality, 1862, *Vasey s.n.* (*F 25933*); without locality, 1862, *Vasey 29371* (ILL); sphagnous swamps, no date, *Vasey s.n.* (*F 211538*); Wauconda, May 30, 1905, *Hill s.n.* (ILL). MCHENRY: Ringwood, no date, *Vasey s.n.* (*F 25937*); Ringwood, no date, *Vasey s.n.* (ILL). NORTHERN ILLINOIS: No specific location, no date, *Vasey 18408* (ILL).

§ Arenariae

Rhizomatous perennials; culms mostly solitary; spikes androgynous, distinct or continuous, elongate, the lowest bract scabrous-awned; styles 2; perigynia nerveless ventrally to distinctly nerved on both faces, with a narrow margin or wing, with a serrulate, sharply bidentate beak; achenes lenticular or plano-convex.

Key to the Species of § Arenariae in Illinois

1. Rootstock black, fibrillose; culms 50-80 cm tall; inflorescence 2.5-6.5 cm long; pistillate scales 2.5-3.6 mm long; perigynia (3-) 10-25 per spikelet, 2.0-4.1 mm long, narrowly winged to the base; beak 0.75 mm long; achene 1.30-1.75 mm long, without striations. . . . . 4. *C. sartwellii*
1. Rootstock brown, scaly; culms 6-30 cm tall; inflorescence 1-4 cm long; pistillate scales 3.5-4.75 mm long; perigynia 3-12 per spikelet, 4.50-5.25 mm long, the upper two-thirds narrowly winged; beak 2-3 mm long; achene 1.90-2.25 mm long, finely striate. . . . 5. *C. foenea*

4. *Carex sartwellii* Dew. Am. Jour. Sci. 43:90. 1842. Fig. 4.

Plants from thick, fibrous-scaly, horizontal, black rootstocks; culms subcanaliculate, sharply triangular, scabrous, 5-8 dm tall with the inflorescence well-exceeding the leaves; leaves 3-5, 9-23 cm long, 2.30-4.25 mm broad, arising from the lower half of the culm, ascending to spreading, with the margins scabrous; sheaths often striate ventrally, apex truncate to concave, some old sheaths persisting; inflorescence androgynous, 2.5-6.5 cm long, 0.5-1.2 cm wide; spikelets 10-35, the upper often entirely staminate and crowded, the lower mostly pistillate and often interrupted; pistillate scales ovate, 2.5-3.6 mm long, 1.20-1.75 mm wide, acute to acuminate, reddish-brown, margins hyaline, not concealing the perigynia; staminate scales narrowly lanceolate, 2.5-4.0 mm long, 0.5-1.2 mm wide, acuminate, mostly hyaline throughout; bracts lanceolate, the lowest scabrous-awned; perigynia (3-) 10-25 per spikelet, 2.0-4.1 mm long, 1.2-1.6 mm wide, plano-convex, substipitate, ovate-lanceolate, distinctly nerved dorsally and ventrally, narrowly winged to the base with the upper 1/3 serrate, light brown, with the bidentate beak 0.75 mm long, serrate and hyaline-tipped; achene 1.30-1.75 mm long, 0.9-1.0 mm wide, plano-convex, reddish-brown, puncticulate, margins single-nerved, jointed to a short style; stigmas 2.

Habitat: Low wet prairies and meadows, creek and river bottoms, marshes, dunes, peaty swamps, and open cold bogs.

Range: Southwest Quebec to northern British Columbia, south to Colorado, Nebraska, Missouri, Illinois, Indiana, Ohio, and western New York.

Boott (1865), apparently seeing no characters to distinguish *Carex sartwellii* Dew. from European *Carex disticha* Huds., illustrates both as *C. disticha*. However, Boott's figures 2a,b of perigynia and achenes from French material are considerably different from those of *C. sartwellii*. We feel the difference is enough to consider both as distinct species.

Carex disticha Huds. is not accurately reported from Illinois. The Illinois collections reported to be *C. disticha* are actually *C. sartwellii*. These are noted in the list of specimens examined for this species.

Carex sartwellii has an extremely variable inflorescence. The spikelets of the linear to ellipsoid-cylindric head vary from very crowded to much interrupted. The upper half of the inflorescence is often entirely staminate (*Bebb s.n.* [*F 32311*]) with the spikelets much smaller and more sharply pointed and hyaline than the lower, subglobose, pistillate ones. In other material, pistillate spikelets occur throughout the inflorescence with a few staminate scales occurring in the tips of the spikelets. Further still, some specimens (*Wolf s.n.* [*F 211662*] and *Fell 533098* [ISM]) have spikelets aggregated into a short, compact head.

Standley and Steyermark have determined their collection *28138* (F) as *C. sartwellii* Dew. var. *stenorrhynca* Hermann. However, the perigynia length of

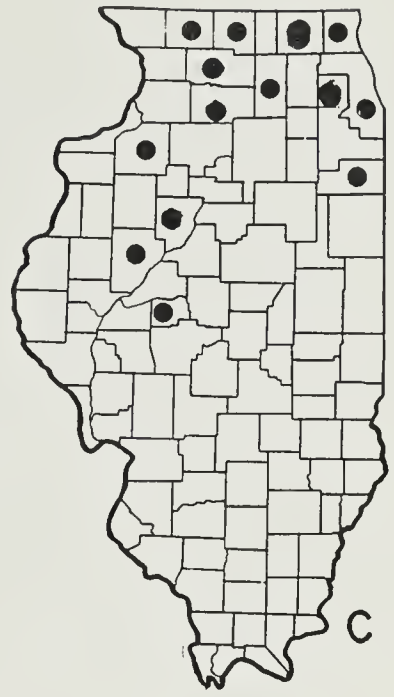


FIGURE 4. *Carex sartwellii*. a. Habit, x 1/4. b. Inflorescence, x 1. c. Map. d. Inflorescence, x 1. e. Staminate scale, x 7-1/2. f. Pistillate scale, x 7-1/2. g. Perigynia, ventral view, x 7-1/2. h. Perigynia, dorsal view, x 7-1/2. i. Achene, x 7-1/2.

this material falls within the variable limits of typical *C. sartwellii*, not 4.0-4.5 mm long as Hermann (1938) describes in the variety *stenorrhynca*. Thus this variety should be excluded from Illinois.

The continuous black rootstock combined with scabrous culms and distinctly nerved perigynia provide good identifying characters.

Although Fernald (1950) remarks that the pistillate scales are obtuse or mucronate, all Illinois material examined and Mackenzie's illustrations show the scales to be acute to acuminate. Also, the green-striate sheath mentioned by Fernald and Mackenzie, although occurring often, is not present in all Illinois material (*Dobbs s.n.* [ILLS 18511, 10030]).

Carex sartwellii, a common sedge in the northern United States and southern Canada, is found only occasionally in the bottoms, swamps, and bogs of the northern third of the state. However, it does occur as far south as Fulton and Menard counties. The Jones and Fuller (1950) report of its occurrence in Will County is not verified by this study.

Specimens Examined: COOK: Ashburn, May 26, 1906, *Cowles 8172-0* (ILLS); northeast of Bartlett, May 21, 1956, *Bennett s.n.* (*ILLS 69232*); east side of Calumet Lake, June 2, 1948, *Steyermark 68221* (F, ILL); Chicago, no date, *Monroe s.n.* (*F 68997*) [originally determined *C. disticha* Huds.]; Chicago, June, 1891, *McDonald s.n.* (*F 398474* [originally determined *C. disticha* Huds.]; near Colehour, April 29, 1876, *Hill 122* (ILL); near Chicago, June 5, 1890, *Moffatt 156* (ILL); near Chicago, June 7, 1890, *McDonald s.n.* (ILL). DEKALB: W. of Kirkland, June 7, 1961, *Evers 69180* (ILLS); north of Fairdale, June 1, 1953, *Fell 53276* (ISM, SIU); near Kirkland, June 10, 1953, *Fell 53396* (ISM), *53398* (ISM). DUPAGE: N. of Wheaton, May 22, 1894, *Moffatt 94* (ILL); N. of Wheaton, May 2, 1894, *Moffatt 142* (ILL). FULTON: Canton, no date, *Wolf s.n.* (*F 211662*), (*F 211663*); without location, no date, *Brendel s.n.* (ILL) [originally determined *C. disticha* Huds.]. HENRY: E. of Geneseo, May 30, 1937, *Dobbs s.n.* (*ILLS 10030*). KANKAKEE: Kankakee, May 17, 1870, *Hill s.n.* (*F 206465*) [originally determined *C. disticha Huds.*]; Kankakee, May 29, 1870, *Hill s.n.* (ILL) [originally determined *C. disticha* Huds.]. LAKE: Waukegan dunes, June 13, 1940, *Standley & Steyermark 28138* (F); Waukegan, June 5, 1916, *Benke 1512* (F) [originally determined *C. siccata* Dew.]. LEE: Near Amboy, June 8, 1959, *Long 939* (ILL). MACON: Decatur, May 22, 1899, *Clokey 31235* (ILL). MENARD: Athens, 1861, *Hall s.n.* (*F 32212*), (*F 45050*). MCHENRY: Ringwood, no date, *Vasey s.n.* (ILL). OGLE: N. of Davis Junction, May 24, 1953, *Fell 53396* (ISM), *53398* (ISM). PEORIA: Peoria, no date, *Brendel s.n.* (ILL). STEPHENSON: Northeast of Ridott, June 28, 1953, *Fell 53202* (ISM), *53-203* (SIU). WINNEBAGO: Near Rockford, May 28, 1952, *Fell 52-126* (ILLS), *52-136* (ISM); Fountaindale, 1870, *Bebb s.n.* (*F 32331*) [originally determined *C. disticha* Huds.]; Rockton Township, June 10, 1952, *Fell 52306* (ISM); N. of Shirland, June 8, 1952, *Fell 52268* (ISM); Kent Creek, July 1, 1951, *Fell 51251* (ISM); North Central Avenue, Rockford, June 14, 1952, *Fell 52356* (ISM, SIU). WITHOUT PRECISE LOCALITY: No date, *Vasey s.n.* (*F 32741*, *32742*, *32743*) [*F 32742* apparently cited in Boott as *C. disticha* Huds.]; no date, *Vasey 18425* (ILL); June, 1860, *Hall s.n.* (*F 206219*) [originally determined *C. disticha* Huds.].

5. *Carex foenea* Willd. Enum. Hort. Berol. 2:957. 1809. Fig. 5.

Carex siccata Dew. Am. Journ. Sci. 10:278. 1826.

Roots from brown, scaly, slender, horizontal rootstock; culms slender, mostly fertile, 15-75 cm tall, sharply angled and scabrous at the apex, becoming rounded, canaliculate and smooth near the base, equalling or exceeding the leaves; leaves 3-5, 6-30 cm long, 2-3 mm broad, flattened, becoming triangular at the tip, ascending to spreading with the margins scabrous; sheath apex truncate to concave; inflorescence androgynous, 1-4 cm long, 0.5-1.0 cm broad; spikelets 4-8, slightly interrupted to widely spaced at the base, becoming indistinguishable at the apex, the lower 1-3 spikelets usually pistillate, the middle spikelets staminate and the terminal gynecandrous and larger; pistillate scales lanceolate, 3.50-4.75 mm long, 1.5-2.0 mm broad, acute to mostly acuminate, light reddish-brown with hyaline margins, shorter than the perigynia; staminate scales acuminate, 3.5-4.2 mm long, 1.0-1.5 mm broad, light brown to hyaline; bracts lanceolate, the lowest encircling the culm with scabrous awns; perigynia 3-12 per spikelet, 4.50-5.25 mm long, 1.5-1.8 mm broad, plano-convex, cuneate, substipitate, ovate-lanceolate, light reddish-brown, coriaceous, distinctly nerved on both faces, rarely nerveless ventrally, the upper 2/3 narrowly winged and serrulate with the beak 2-3 mm long, serrulate, sharply bidentate and obliquely cleft dorsally; achene 1.90-2.25 mm long, 1.25-1.50 mm broad, lenticular, substipitate, yellow-brown, finely striate, puncticulate, jointed to the style; stigmas 2.

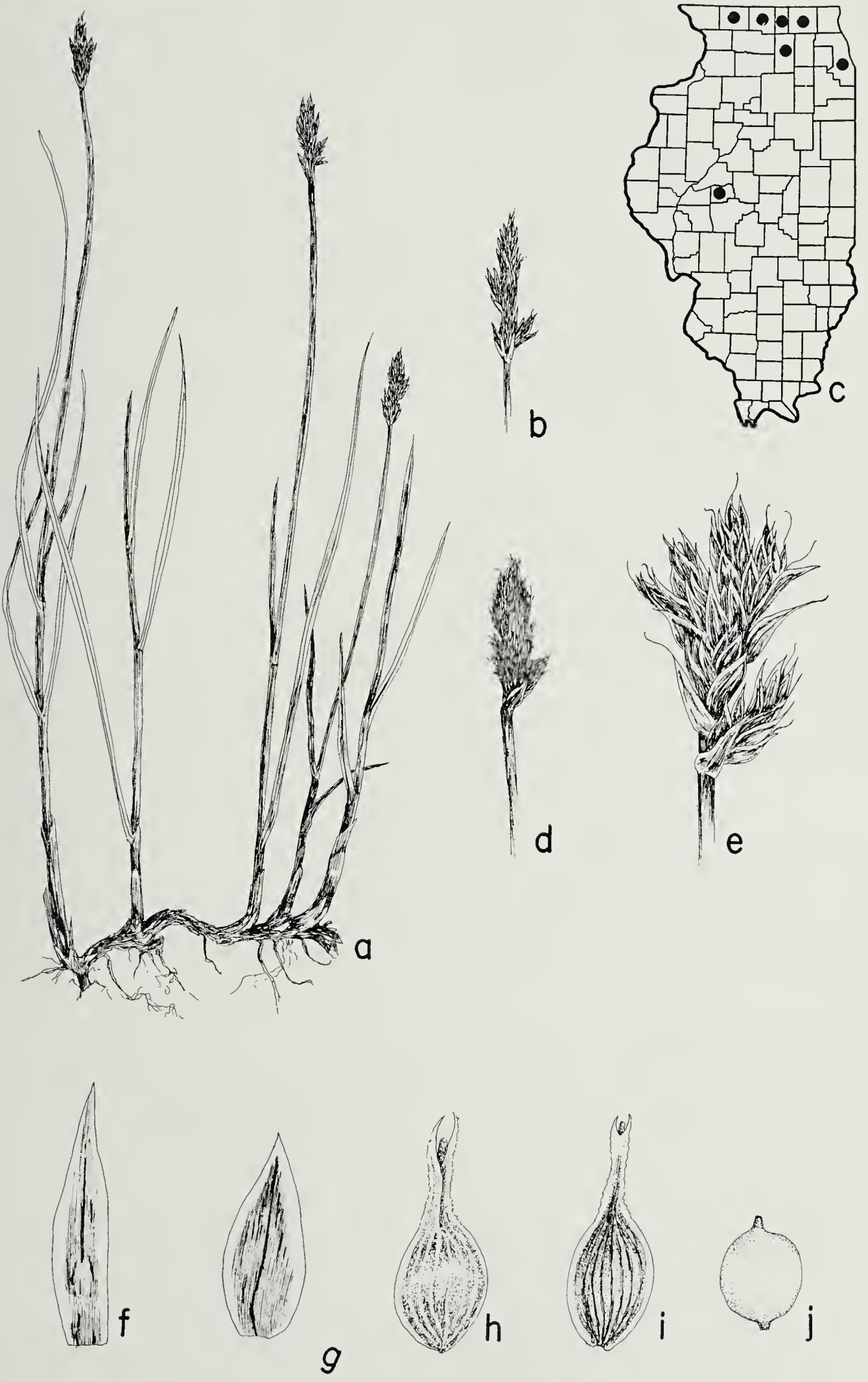


FIGURE 5. *Carex foenea* var. *foenea*. a. Habit, x 1/2. b. Inflorescence, x 1. c. Map. d. Inflorescence, x 1. e. Inflorescence, x 1-1/2. f. Staminate scale, x 7-1/2. g. Pistillate scale, x 7-1/2. h. Perigynia, dorsal view, x 7-1/2. i. Perigynia, ventral view, x 7-1/2. j. Achene, x 7-1/2.

Habitat: Mostly prairie or sandy soil, sometimes sandy woods and roadsides.

Range: Southwest Quebec to Mackenzie, south to Arizona, New Mexico, Illinois, northern Indiana, Ohio, New Jersey, and New York.

Although this species has been called *Carex siccata*, Svenson (1938) has given reasons for accepting *C. foenea* as the proper binomial.

Two varieties may be recognized.

1. Perigynia nerved on both faces, tapering gradually to the beak. . . . . 5a. *C. foenea* var. *foenea*
1. Perigynia nerveless on the ventral face, tapering abruptly to the beak. . . . . 5b. *C. foenea* var. *enervis*

5a. *Carex foenea* Willd. var. *foenea*

Perigynia nerved on both faces, taper ing gradually to the beak.

Carex foenea Willd. var. *foenea* strongly resembles *Carex praegracilis* Dew. because of the more slender inflorescence. However, *C. foenea* is distinguished by its perigynia which are many-nerved on both faces and are larger with a proportionately longer beak, by its rhizomes brown and scaly, and by its middle spikelets usually wholly staminate.

Some variation can be found in this taxon. *Fell 211* (SIU) contains one culm bearing perigynia with widely divergent teeth while the other characters are typical.

A few collections have been erroneously identified as this taxon. These are *Benke 3591* (F) from Elgin in Kane County, previously determined as *C. siccata* Dew., which actually is *C. praegracilis* Dew., and *Cowles 8007-0* (ISM) from South Chicago, Cook County, determined as *C. siccata* Dew., which actually is *C. bebbii* Olney.

Specimens Examined: BOONE: Northeast of Belvidere, May 10, 1954, *Fell 54214* (ISM). COOK: Lakeview, May 15, 1884, *Ohlendorf s.n.* (*F 1358087*). DEKALB: Near Fairdale, May 20, 1953, *Fell 53112* (ISM). MENARD: Without specific locality, 1876, *Hall s.n.* (*F 206244*); Athens, 1861, *Hall s.n.* (*F 32213*). MCHENRY: Ringwood, no date, *Vasey s.n.* (*F 32745, F 325742*). STEPHENSON: Northeast of Ridott, May 28, 1953, *Fell 53211* (ISM, SIU). WINNEBAGO: E. of Rockford, June 5, 1952, *Fell 52-228* (ILLS), *52-227* (ISM); S. of Rockford, May 20, 1951, *Fell 5153* (ISM); S. of Rock Cut, June 23, 1952, *Fell 52473* (ISM); River Road S. of Cherry Valley, May 31, 1952, *Fell 52176* (ISM); Sugar Creek Forest Preserve, May 18, 1952, *Fell 5286* (ISM); Sugar Creek Forest Preserve, May 19, 1951, *Fell 5140* (ISM). WITHOUT PRECISE LOCALITY: 1896, *Dewey s.n.* (*F 467223*).

5b. *Carex foenea* Willd. var. *enervis* Evans & Mohlenbr., var. nov. Fig. 6.

Perigynium enerve ventrali superficie, rostro abrupte decrescenti.

Type: *French s.n.* (SIU), from Illinois.

The type collection was made in 1869 by G. H. French in "sandy plains Ill." Although Irvington, Illinois [Washington County] is written on the label, the collection is probably not from there since Washington County seemingly is a little too far south. French, who lived for a while in Irvington, is known to have written Irvington on the labels of other specimens which were actually collected elsewhere in Illinois.

The lack of nerves on the ventral face of the perigynia and the more abruptly tapering beak differentiate this taxon from *C. foenea* var. *foenea*.

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Vasey, G. s.n. (3, 4, 5a), 18408 (3), 18425 (4), 29311 (3).
Wolf, J. s.n. (4).

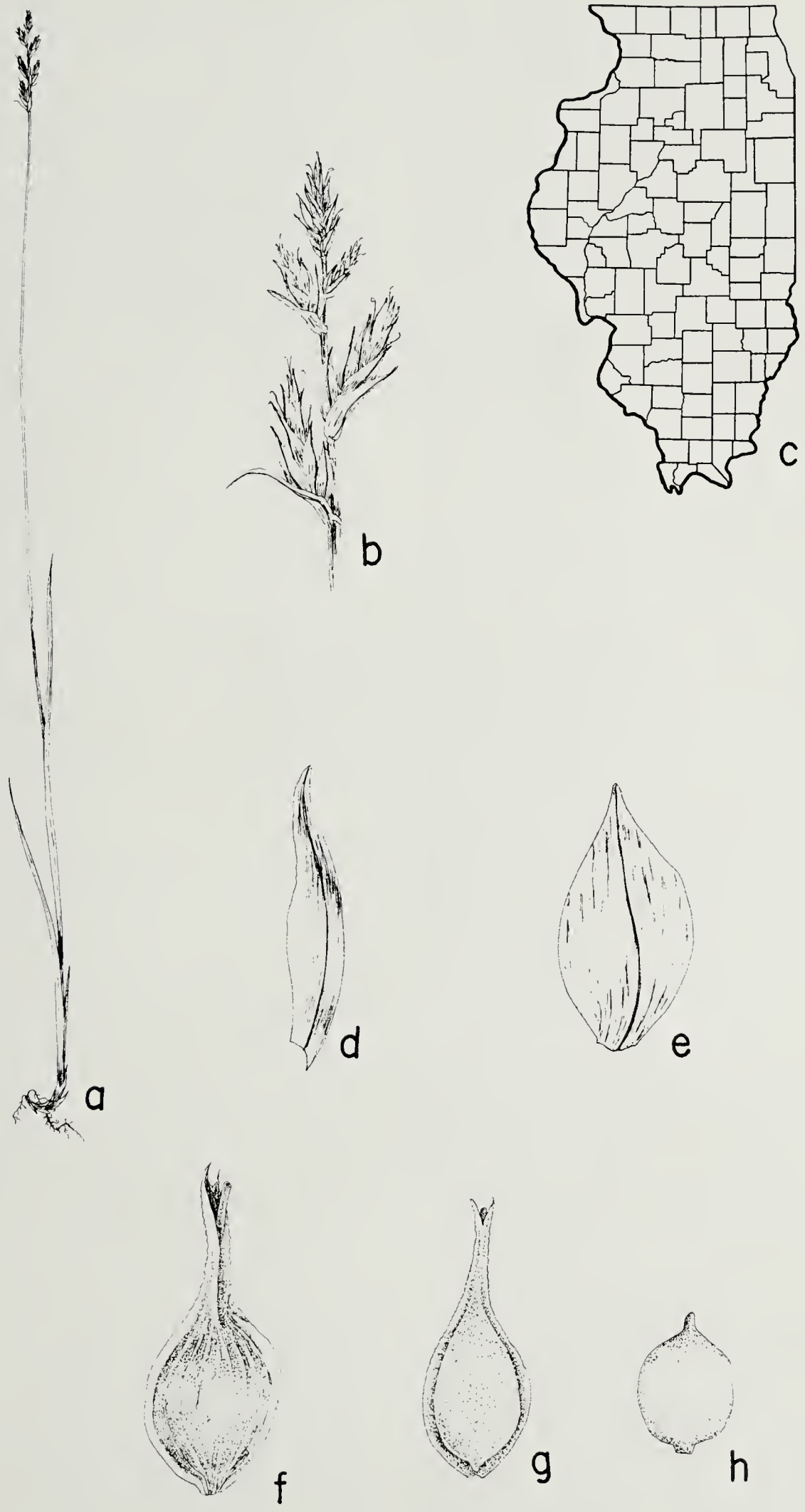

FIGURE 6. *Carex foenea* var. *enervis*. a. Habit, x 1/2. b. Inflorescence, x 2. c. Map. d. Staminate scale, x 7-1/2. e. Pistillate scale, x 7-1/2. f. Perigynia, dorsal view, x 7-1/2. g. Perigynia, ventral view, x 7. h. Achene, x 15.

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Manuscript received June 24, 1970

GASTRIC MORPHOLOGY IN SELECTED MORMOOPID AND GLOSSOPHAGINE BATS AS RELATED TO SYSTEMATIC PROBLEMS

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ABSTRACT.—Stomachs of selected species of two groups of North American bats [Mormoopidae and Glossophaginae (Phyllostomatidae)] were studied grossly, histologically, and histochemically. The stomachs of two mormoopids, *Pteronotus parnellii* and *Mormoops megalophylla* are most similar in gross and histological features to those of fish-eating bats of the family Noctilionidae. Species of the predominantly nectar-feeding Glossophaginae have stomachs of two basic morphological forms, which are considered to probably reflect early divergence in food habits. Additionally, studies of the histochemistry of gastrointestinal mucins suggest a similar dichotomy in glossophagines. The above results are reviewed in light of several recent studies of the systematics of the Mormoopidae and Glossophaginae. It is concluded that a comprehensive study of the food habits of glossophagines should be undertaken to test some proposed relationships between gastric morphology and feeding characteristics in these bats.

Recent investigations of certain genera and subfamilies of the North American leaf-nosed bats, family Phyllostomatidae, have proposed noteworthy changes in the traditional systematic arrangements, and explanations of phylogeny of these groups. Insect-feeding bats of the subfamily Chilonycterinae have been reviewed critically by Smith (1971) who regards them as distinctive enough to be relegated to a separate family, Mormoopidae. Vaughn and Bateman (1970) support this proposal with evidence from studies of the functional morphology of flight. Phillips (1971) examined the deciduous and adult dentitions of selected nectar-feeding species of the subfamily Glossophaginae. He concluded that this group is either diphylletic in origin and not a natural assemblage, or that the subfamily contains a dichotomy of types that is the product of an early evolutionary divergence. Based on karyotypic evidence, Baker (1967) previously had reported a dichotomy in the glossophagines, but the two species groups devised by him do not correspond precisely with those of Phillips.

In an effort to further examine these three systematic proposals, bats of species representing the groups were selected and their stomachs were examined morphologically and compared with those of closely related taxa. The relationship between the Mormoopidae and the fish-eating bats of the family Noctilionidae also was studied and the results are discussed herein. The systematic value of such studies of gastrointestinal morpholopy has been discussed previously by Forman (1971) and Schultz (1970).

MATERIALS AND METHODS

Preserved stomachs of one species of mormoopid and four species of glossophagines were examined grossly, histologically, and histochemically. The kinds and numbers examined were as follows: Mormoopidae; *Mormoops megalophylla* (2), Glossophaginae; *Glossophaga commissarisi* (2), *Anoura geoffroy* (1), *Choeroniscus godmani* (1) and *Lichonycteris obscura* (2). Previous accounts of gastric morphology and histochemistry in the mormoopid *Pteronotus parnellii* (Forman, 1971), and the glossophagines *Glossophaga soricina* (Forman, *op. cit.*) and *Leptonycteris sanborni* (Rouk and Glass, 1970) are employed in discussions to follow.

All specimens from which stomachs were removed are stored in the Museum of Natural History, The University of Kansas.

Standard histological and histochemical procedures employing Harris' haematoxylin and eosin, and periodic acid Schiff-Alcian blue for gastric mucus were used (see Forman, 1971). Hale's colloidal iron reaction was used in addition to Alcian blue as a test for acid mucopolysaccharides (procedure after Lillie, 1965). All stomachs were sectioned at five to seven microns. Drawings of stomachs were produced by projecting midlongitudinal sections onto paper, by means of a photographic enlarger.

Results

Gastric Morphology in the Mormoopidae. The stomach of *Mormoops* (Fig. 1) is simple in overall configuration, and is nearly identical in topography to that of *Pteronotus parnellii* (Forman, 1971). The stomach is symmetrical (gastroesophageal junction midway along lesser curvature) with a short, pointed fundic caecum, and an equally short and unusually broad pyloric endpiece. The cardiac vestibule is minute, as it is in nearly all other insectivorous bats examined thus far (see Forman, 1971).

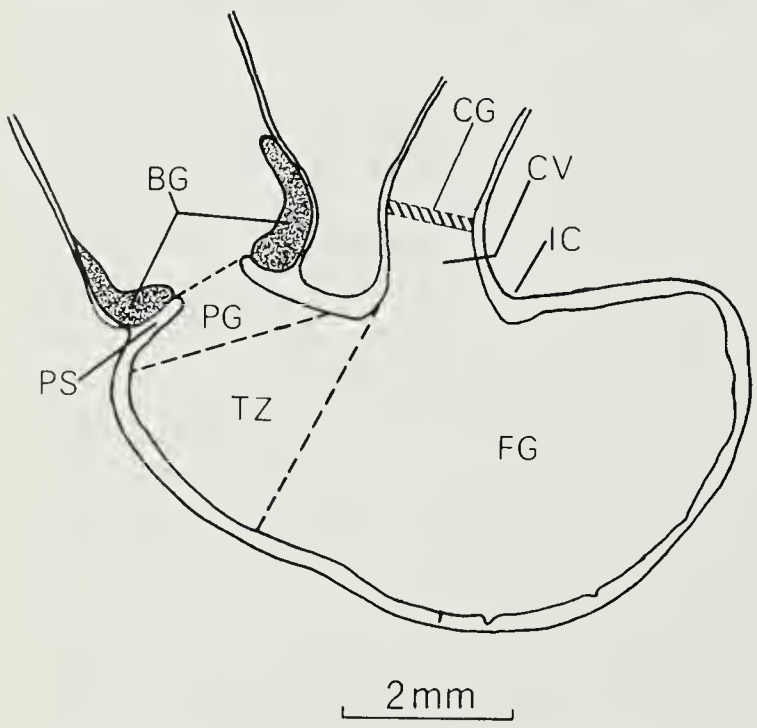

FIGURE 1. Mid-longitudinal representation of the stomach of *Mormoops megalophylla*. Explanation of symbols: CG, cardiac glands; BG, Brunner's glands; PG, pyloric glands; TZ, transitional zone; FG, fundic glands; IC, incisura cardiaca; CV, cardiac vestibule; PS, pyloric sphincter.

The pyloric sphincter is asymmetrical, as in *Pteronotus*, the valve on the greater curvature being longer and narrower than that of the lesser curvature. However, the marked reduction in mass of the lesser valve seen in *Pteronotus* is lacking in *Mormoops*. The circular muscle layer of the stomach wall is extremely thick, as in *Pteronotus*.

The glands of Brunner at the gastroesophageal junction of *Mormoops* are indistinguishable in cellular morphology from those of *Pteronotus parnellii*, as well as *Noctilio leporinus* and *N. Labialis* (Noctilionidae). The tubules are narrow and the cells are small with extremely small, flattened nuclei not juxtaposed to the basement membrane. This condition is in contrast to the broader tubules with larger, circular juxtaposed nuclei usually found in Brunner's glands in bats of the families Vespertilionidae, Phyllostomatidae, Emballonuridae and Molossidae. Brunner's glands are unusually abundant in *Mormoops* (Fig. 1) and *Pteronotus*, a condition shared with *Noctilio*. The extreme breadth of the duodenum at the gastroesophageal junction of *Mormoops*, *Pteronotus*, and *Noctilio* is, in part, due to the unusually large complement of Brunner's glands in these genera.

The distribution of gastric mucosa in *Mormoops* closely resembles that of *Pteronotus*, and thus is distinctive among bats studied to date (except for *Noctilio*) by virtue of having an extremely narrow zone of mucus-producing pyloric glands (Fig. 1). The proportion of gastric mucosa that may be considered

transitional (between fundic and pyloric glands) is extremely high in mormoopids, although a similar condition is found to occur in some phyllostomatids and in *Noctilio*. The relative frequency of mucosal folds is low in *Mormoops* and *Pteronotus*, considerably lower than in any other kinds thus far examined.

The fundic mucosa of *Mormoops*, which produces hydrochloric acid and pepsin, has a number of features found to occur additionally only in *Pteronotus* and in the *Noctilionidae*. The fundic tubules are long, highly convoluted, and extremely narrow (Fig. 2) in contrast to the broader, often shorter glands in members of other families of North American bats (Fig. 3). All cellular elements of the fundic glands (parietal, mucous neck, chief, and argentaffin cells) are extremely small in comparison to other bats, and correspondingly are relatively abundant. In comparison with other species, nuclei of these cells are of moderate size, suggesting a decrease in cytoplasmic mass. Zymogenic (chief) cells are abundant within the bases of fundic glands only in the cardiac vestibule and lesser curvature of *Mormoops* and *Pteronotus*.

Gastric Morphology in the Glossophaginae. Careful examination of

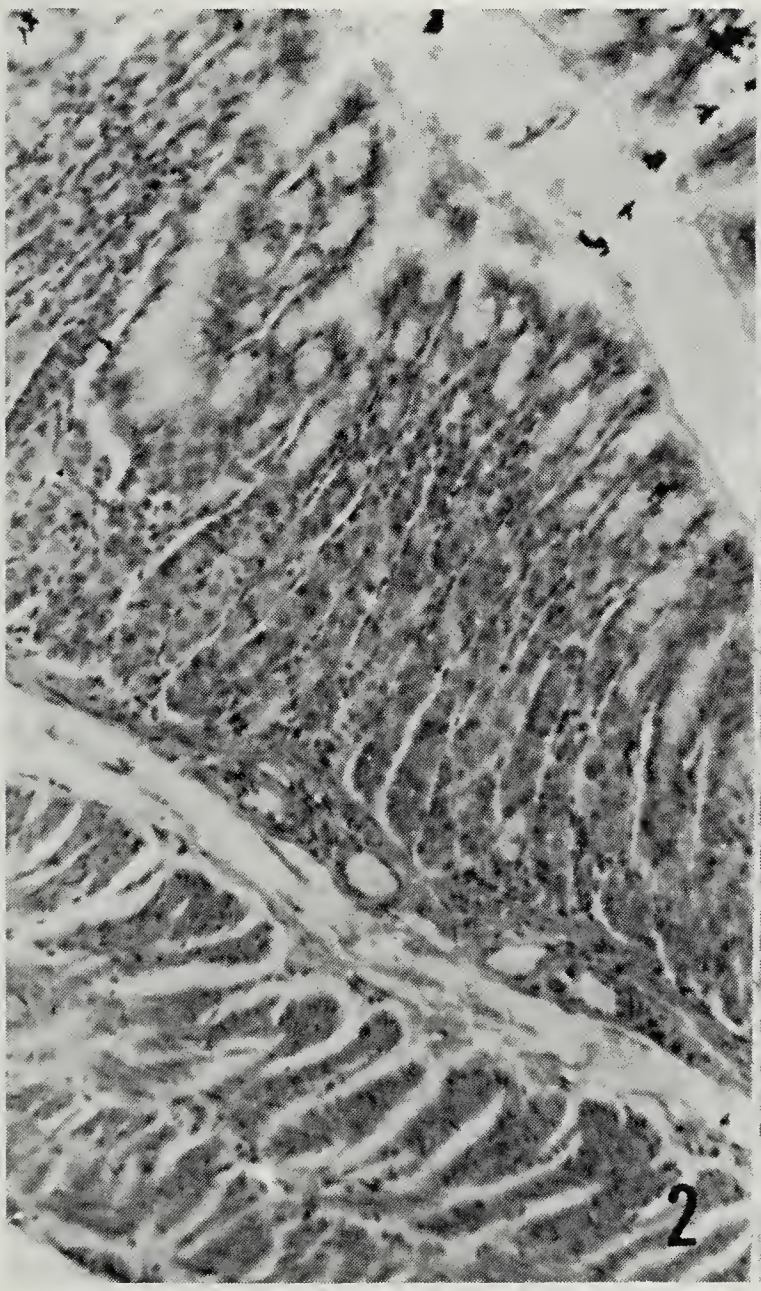

FIGURE 2. Fundic glands in the stomach of *Mormoops megalophylla*. Note the great length and narrowness of these glands (X200).

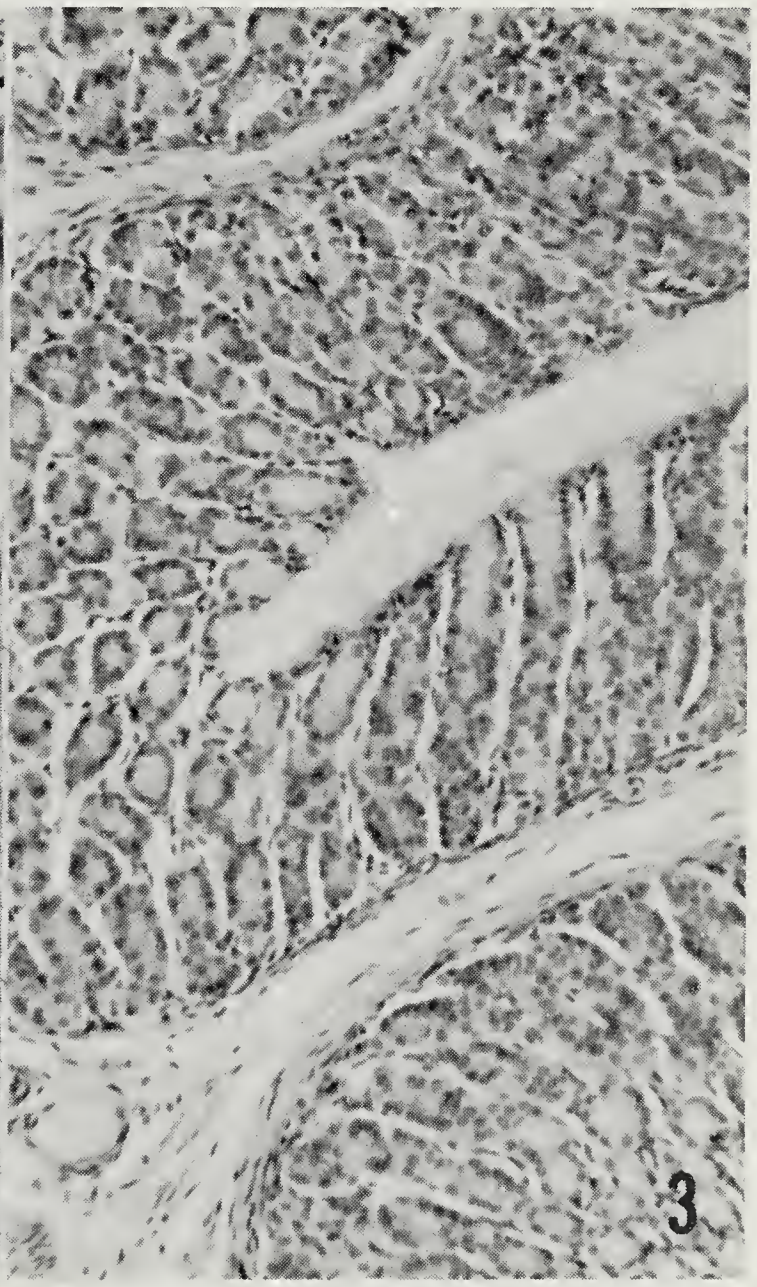

FIGURE 3. Fundic glands in the stomach of *Glossophaga soricina* (family Phyllostomatidae). Note the greater breadth of these glands as compared to those in Fig. 2 (X200).

Figures 4 to 7 reveals the extensive variation in gross morphology of stomachs within the nectar-feeding Glossophaginae. Stomachs of glossophagines thus far examined can

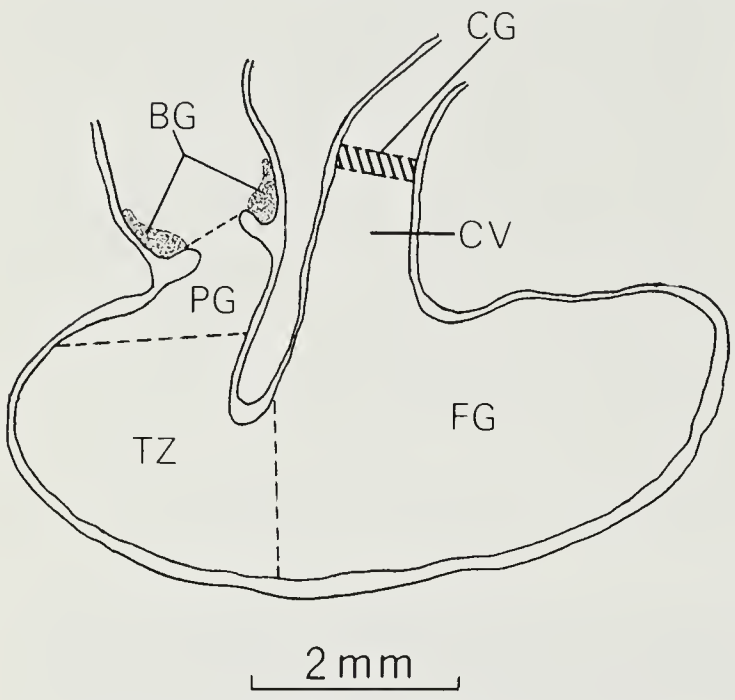

FIGURE 4. Mid-longitudinal representation of the stomach of *Glossophaga commissarisi*. For explanation of symbols, see Fig. 1.

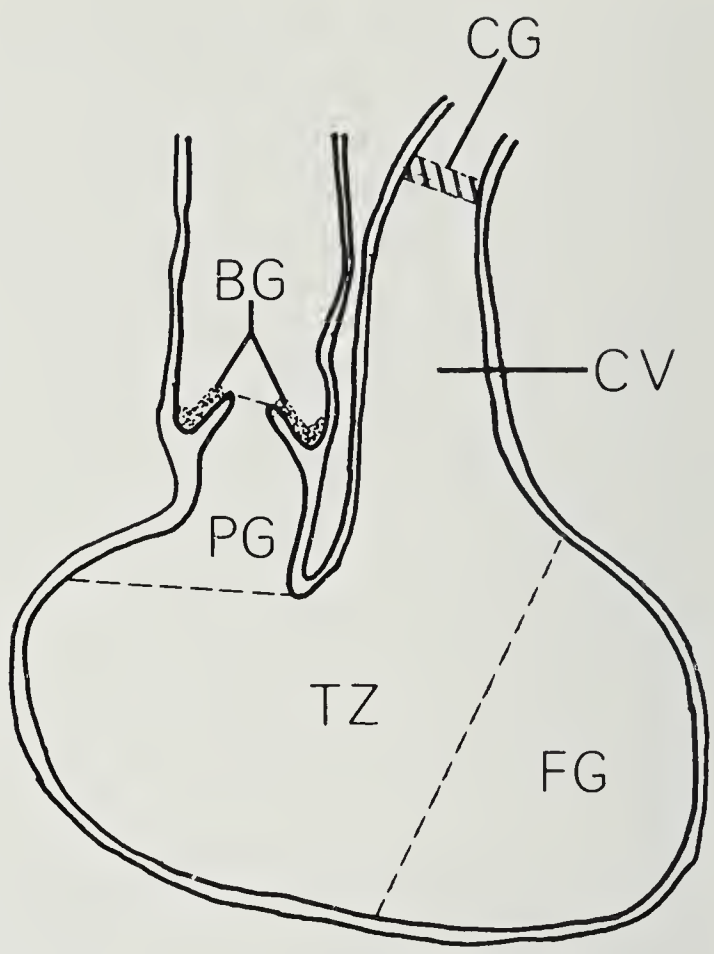

FIGURE 6. Mid-longitudinal representation of the stomach of *Choeroniscus godmani*. For explanation of symbols, see Fig. 1.

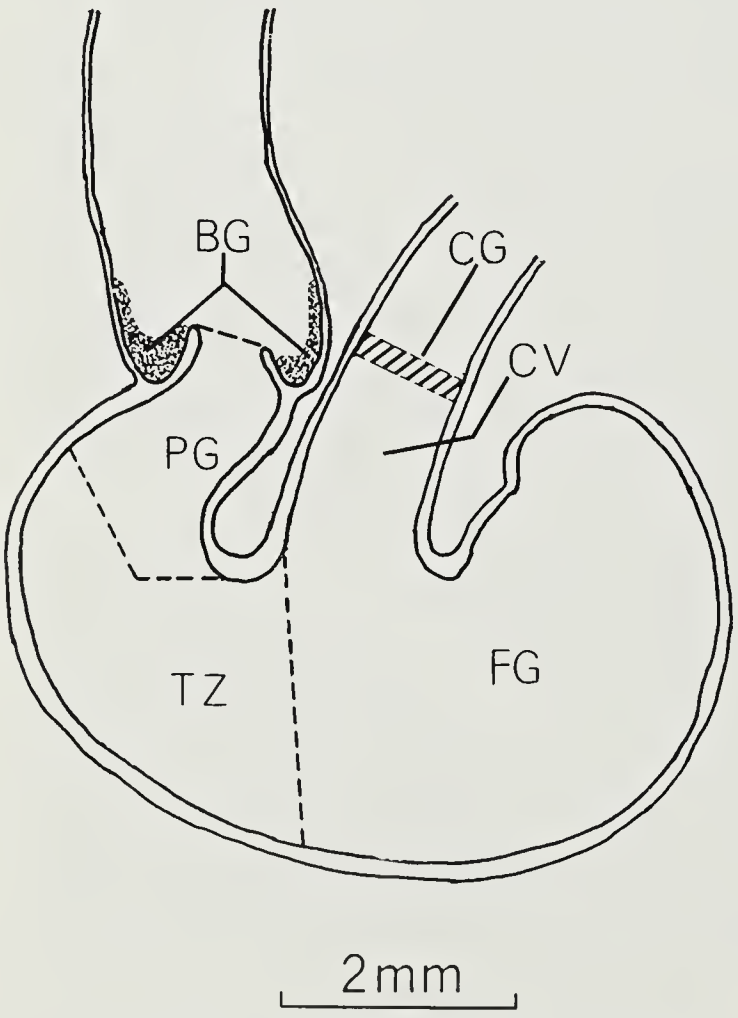

FIGURE 5. Mid-longitudinal representation of the stomach of *Anoura geoffroy*. For explanation of symbols, see Fig. 1.

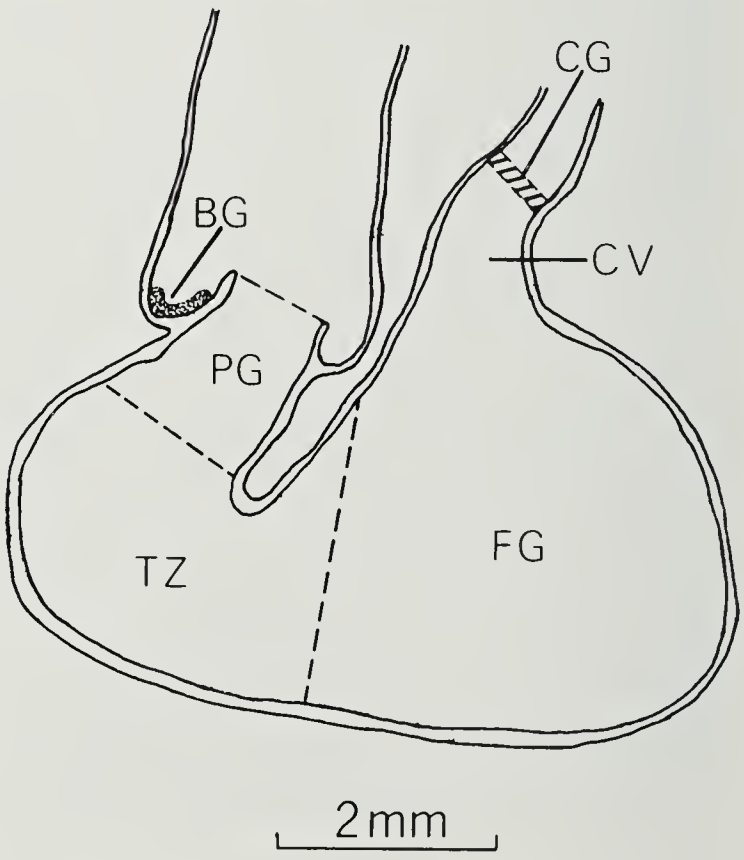

FIGURE 7. Mid-longitudinal representation of the stomach of *Lichonycteris obscura*. For explanation of symbols, see Fig. 1.

be divided into two groups on the basis of topographic features and various features of the gastric mucosa. Group A includes *Glossophaga soricina* (examined by Forman, 1971), *G. commissarisi* (Fig. 4), *Leptonycteris sanborni* (examined by Rouk and Glass, 1970), and *Anoura geoffroy* (Fig. 5). Group B includes *Choeroniscus godmani* (Fig. 6) and *Lichonycteris obscura* (Fig. 7).

The stomachs of Group A are characterized by a relatively long, narrow fundic caecum, and a comparatively small or no cardiac vestibule (tubular portion of stomach between gastroesophageal junction and lesser curvature). The incisura cardiaca (fold in the stomach wall at the junction of the cardiac vestibule and fundic caecum) is moderately to extensively developed. The terminal portion of the stomach (tubular segment between the gastroesophageal junction and pyloric sphincter) is always moderately well developed. The pyloric sphincter possesses comparatively short valves, with well developed musculature, and is always assymmetrical, with the valve of the greater curvature being the most massive.

In regard to gross morphology of the stomach, *Glossophaga soricina* is most deviant from the general plan within Group A. A cardiac vestibule is completely lacking in this species, and the apparent relationship between this condition and insect-feeding in this bat previously has been discussed (Forman, 1971).

The stomachs of Group B are distinguished from those of Group A on the basis of morphology of the fundic caecum and pyloric sphincter. The fundic caeca of *Choeroniscus* and *Lichonycteris* are short, rounded, and slightly dilated on the dorsal or "back" surface. An incisura cardiaca is completely lacking. The cardiac vestibule is generally better developed than those of Group A, and more closely approximates the type found to occur in fruit-eating leaf-nosed bats. The terminal portion of the stomach is relatively elongate as in Group A, however, the pyloric sphincter differs by having narrower valves of greater length. This latter condition more closely resembles the pattern found in frugivorous phyllostomatids, than that of Group A.

The mucus-producing glands of Brunner reveal distributional and structural differences between the two groups. These submucosal glands within the duodenal wall at the pyloric sphincter are relatively more abundant in both species of *Glossophaga* and in *Anoura* than in *Choeroniscus* and *Lichonycteris*. They are limited to a narrow band on only the lateral one-half of the pyloric sphincter in *Lichonycteris* (Fig. 7). The tubules of Brunner's glands are more highly coiled and considerably broader in Group A than in the second group, suggesting greater production of mucus from Brunner's glands in *Glossophaga* and *Anoura*. Of the three species examined in Group A, *Anoura* has Brunner's glands which most closely approach those of Group B in morphology of the tubules.

The entire inner lining of the stomach of all glossphagines is occupied by typical tubular gastric glands. Differences in the distribution of various portions of the gastric mucosa within the Glossophaginae are not striking, except for the extremely limited distribution of true fundic glands in *Choeroniscus godmani* (Fig. 6). The zone of transitional mucosa between fundic and pyloric glands is characteristically broad in glossophagines, being maximal in *Choeroniscus* with the absence of zymogenic cells

throughout the cardiac vestibule (Fig. 6).

Although distinctive differences in distributions of glandular types are not apparent among the glossophagines, two conditions of the frequency of zymogenic cells are found to occur within the fundic glands. Fundic glands of *Glossophaga* and *Anoura* contain relatively large numbers of zymogenic cells within the basal one-fourth to one-third of each tubule. These cells are abundant throughout that portion of the stomach in which they occur, being most abundant within the fundic caecum (*Anoura*) and lesser curvature (*Glossophaga*). In contrast, chief cells are considerably less frequent in both *Lichonycteris* and *Choeroniscus*, being least abundant in the latter, and are generally restricted to the lower one-seventh to one-tenth of each tubule in both. Parietal cells, which occupy only the midregion of fundic tubules in *Glossophaga* and *Anoura*, are usually found among chief cells in *Lichonycteris*, and are always together with these cells in *Choeroniscus*. The most abundant concentration of chief cells in both species of Group B is within the extreme apex of the fundic caecum.

Histochemistry of the Gastric Mucosa. Staining of stomachs with Alcian blue, Hale's collidal iron stain, and the periodic acid Schiff reaction (paS) revealed few differences in the quality of gastric mucus among mormoopid or glossophagine bats. Alcian blue and Hale's iron, both of which are considered to color acid mucopolysaccharides, stained mucus only of the cardiac glands in *Mormoops* and *Pteronotus*. Mucus of the surface epithelium of these genera stained strongly with paS (for neutral mucins), as did cells of the gastric pits within the cardiac and pyloric glands. Mucous neck cells interspersed among parietal cells in the fundic and transitional glands stained moderately strong with paS, as did cells within the basalmost one-fifth of the pyloric glands. Brunner's glands gave a strong reaction to paS in both species of mormoopids. With the exception of somewhat less reactivity in the mucous neck cells of *Mormoops*, there was little difference between mormoopids in staining with paS.

The surface epithelium of all glossophagine stomachs gave a strong reaction to paS, but none with Alcian blue or Hale's colloidal iron. Results also were consistent for the cardiac glands, with the upper three-fourths of each tubule staining strongly with paS and not at all with either acid mucopolysaccharide technique. The basal one-fourth of the cardiac gland tubules stained only moderately with paS, and moderate to heavily with both Alcian blue and Hale's colloidal iron.

The pyloric glands and mucous neck cells of glossophagines did not stain with Hale's iron, but revealed interspecific variation in staining with Alcian blue. Mucous neck cells within the lower portions of the fundic glands stained strongly in *Glossophaga*, moderately in *Anoura*, and not at all in *Choeroniscus* and *Lichonycteris*.

The pyloric glands stained in a similar fashion. Those of *Glossophaga* (both species examined) colored intensely throughout the length of the tubule, whereas, those of *Anoura* stained with equal intensity, but only within the basal arc of each tubule. The pyloric glands of *Choeroniscus* and *Lichonycteris* did not stain with Alcian blue.

Discussion

Review of Food Habits. The food of mormoopid bats is fairly well established in the literature as consisting mostly of flying insects. In

contrast, however, the food habits of many glossophagine species, which previously were assumed to be obligate nectar feeders, are inadequately or inaccurately documented. *Glossophaga soricina* now may be considered an omnivore (see Goodwin and Greenhall, 1961; Arata *et al.*, 1967), that consumes insects as well as nectar. The food of *Anoura geoffroy* is said by Goodwin and Greenhall (*op cit.*) to consist of nectar and soft fruit pulp. There is little evidence, as yet, that this bat takes insects. *Choeroniscus godmani* is assumed to be an obligate nectar feeder, but it is of interest to note that *C. intermedius* on Trinidad has been reported to include numerous insects in its diet (based on a single specimen examined by Goodwin and Greenhall, *op cit.*). The teeth of *Choeroniscus* are greatly reduced in breadth and height in comparison to most other glossophagines. Thorough masceration of insect material, which is characteristic of insect-feeding bats, seems unlikely in *Choeroniscus*, and thus insect feeding by this bat is probably rare. Stomach contents of *Leptonycteris sanborni*, as examined by Hoffmeister and Goodpaster (1954), consisted of 92 per cent pollen and eight per cent insect remains. Although Barbour and Davis (1969) reported that this species will eat a variety of fruits in captivity, insects possibly are not uncommon in the diet of this species. *Leptonycteris nivalis* is not formally known to take insects, however, it seems likely that general accounts of *Leptonycteris sanborni* apply to this species. Little is known, directly, of the food habits of *Choeronycteris mexicana*. Indirect evidence (heads and faces of specimens covered with pollen, as reported by Barbour and Davis, *op cit.*) would suggest that this bat probably is an obligate nectivore. Little information is available concerning the food habits of *Lichonycteris obscura*, but it is assumed that this species feeds on pollen and nectar.

Gastric Morphology in Relation to Feeding. Numerous morphological features of the stomachs of *Pteronotus* and *Mormoops* (simple overall configuration, thick and assymmetrical pyloric sphincter, small fundic caecum, and an extensive complement of zymogenic cells) are strikingly similar to conditions found in insect-feeding bats of the large North American family Vespertilionidae. Elongation of the terminal portion of the stomach, found to occur in North American insectivorous bats of the families Molossidae, Natalidae, and Emballonuridae, as well as in frugivorous and nectivorous phyllostomatids, is lacking in mormoopids. Mormoopids thus appear to parallel only vespertilionids in maintenance of a generalized gastric form. The glands of Brunner at the gastroesophageal junction are extremely abundant in both *Pteronotus* and *Mormoops*, resembling the condition found in other insectivorous, as well as fish-eating kinds. Non-carnivorous bats generally possess relatively fewer and less well developed Brunner's glands than carnivorous kinds.

Upon a review of the morphological features of stomachs of the glossophagines examined, it can be seen that there are two general structural forms. Of genera examined, *Glossophaga* and *Anoura* have stomachs of one form; *Choeroniscus* and *Lichonycteris*, the other. The somewhat more saccular stomach with expanded cardiac vestibule found in *Choeroniscus* and *Lichonycteris* more closely approximates the form found in frugivorous phyllostomatids, than does the form found in the other group of glossophagines. The stomachs of *Glossophaga* and *Anoura* are more tubular, with

less well developed cardiac ampullae, more closely resembling gastric configurations of insectivorous or carnivorous species. In the absence of adequate studies of food habits of most glossophagine genera, it would be premature to make an unqualified statement about gastric morphology as it relates to feeding in the Glossophaginae. However, there is sufficient evidence to suggest that there are two morphological forms of stomachs, and that an increase in stomach complexity is probably related to a decrease in insect- or flesh-eating. The descriptive account of gastric morphology, as well as an included diagram, of the stomach of *Leptonycteris sanborni* by Rouk and Glass (1970) closely parallels that of *Glossophaga soricina* (Forman, 1971), *G. Commissarisi*, and *Anoura geoffroy*. For this reason, the stomach of *Leptonycteris sanborni* here is considered to be most like those of Group A.

Several microanatomical features, which are found in two conditions or states in glossophagines, apparently are related to food habits, and reflect trends found to occur in other groups of bats. For example, there are two patterns of distribution of the glands of Brunner in the Glossophaginae that tend to parallel the two conditions observed in frugivorous and insectivorous or carnivorous species. Infrequent Brunner's glands, as found in *Choeroniscus* and *Lichonycteris*, is common, although not universal among frugivores. On the other hand, relatively abundant glands (*Glossophaga* and *Anoura*) is a characteristic of carnivorous bats. Additionally, zymogen or "chief" cells (those cells that presumably secrete proteolytic enzymes) are abundant in the fundic mucosa only in *Glossophaga* and *Anoura*. This probably is a further indication of the carnivorous habits of these bats.

Studies of gastrointestinal histochemistry revealed no striking differences between insect- and nectar-feeding bats. The significance of acid mucopolysaccharide in the fundic and pyloric glands of *Glossophaga* and *Anoura*, and the apparent absence of this material, except for universal staining in the cardiac glands, in either of the other two glossophagines or in the insect-feeding mormoopids remains unexplained.

Systematic Relationships as Revealed by Gastric Morphology. Systematic relationships among various closely related families of North American bats (Phyllostomatidae, Mormoopidae, Noctilionidae and Emballonuridae) recently have been reviewed by Smith (1971). He stated, on the basis of several features of external and internal morphology, that the Mormoopidae, Noctilionidae and Phyllostomatidae resemble one another more than they resemble any other New World group. He further suggested, on the basis of morphology of the male phallus and position of wing attachment, that mormoopids and noctilionids may be more closely related than previously thought. An examination of gastric structure in these two groups provides support for Smith's proposal.

Forman (1971) studied gastric morphology in the insect-feeding *Noctilio labialis*, and reported the presence of several features, which are noted here as being found together also only in *Mormoops* and *Pteronotus*. A large complement of Brunner's glands occurs at the gastroesophageal junction of *N. labialis*. These glands are indistinguishable in cellular morphology from those of the mormoopids examined, and differ from most other bats in possessing small cells with extremely small nuclei. Stomachs of *Noctilio* and the Mormoopidae

also resemble one another in having extremely limited distributions of pyloric glands, extremely long, narrow, and slightly coiled, gastric glands, short and pointed fundic caeca, and a relatively short terminal portion (that portion between the gastroesophageal junction and pyloric sphincter). None of the afore-mentioned features has yet been found in species of the Phyllostomatidae. A suggestion that similarity in gastric morphology is entirely attributable to consumption of similar foods by noctilionids and mormoopids, and therefore of little or no taxonomic value, would not be in order. Most of the morphological features discussed above have not been found to occur in other families of bats that characteristically consume insects or flesh (*e.g.* Natalidae, Vespertilionidae and Molossidae).

Baker (1967) studied several species of Glossophagines and found three species-groups based on chromosome number and morphology. One group included *Choeronycteris mexicana* and *Choeroniscus godmani*. The second group was composed of *Leptonycteris sanborni* and three species of *Glossophaga* (plus three genera of phyllostomatids not assigned to the subfamily Glossophaginae). The third group included only *Anoura geoffroyi*. Baker suggested that *Leptonycteris* and the species of *Glossophaga* form a natural assemblage because their karyotypes are quite different from those of other phyllostomatids in that karyotypic grouping. Differences between the first and second groups included differences in both fundamental number and morphology of individual chromosomes. Additional support for the distinctiveness of karyotypic groups in the Glossophaginae is provided in the observation by Baker that similarities between karyotypes of *Phyllostomus* (Phyllostomatinae) and *Leptonycteris* are greater than those between the *Choeroniscus* group and *Leptonycteris*. The karyotype of *Anoura* was said to be somewhat intermediate in having a fundamental number and X and Y elements resembling those of the *Glossophaga* group, but yet showed similarities with the *Choeroniscus* group in morphology of some autosomes.

Similar results are found in the studies of Phillips (1971) on dentitions (adult and deciduous) in glossophagines. He recognized 1) a *Glossophaga* group of 10 genera, within which were included *Glossophaga*, *Leptonycteris* and *Anoura*, and 2) the *Choeronycteris* group including *Choeroniscis*.

The results of studies of gastric morphology and histochemistry presented here indicate that there are two groups of glossophagines. The features of gastric morphology used as criteria for determining composition of the two groups appears elsewhere in this discussion. The groups recognized are 1) *Glossophaga*, *Anoura*, and *Leptonycteris* and 2) *Choeroniscus* and *Lichonycteris*.

Although the groups presented here do not correspond precisely with those of Baker or Phillips, they most closely approximate those of the former author. Baker indicated that *Anoura* possessed chromosomes with features of the other two groups of glossophagines. The stomach of *Anoura* tends, in several respects, to be intermediate between those of the other two groups recognized here. Examples of distinguishing features include a larger and more elongate cardiac vestibule than in either *Glossophaga* or *Leptonycteris*, and a tendency toward intermediate staining of acid mucopolysaccharide within the fundic and pyloric glands. As do Baker and Phillips, I consider the sub-

family Glossophaginae to be composed of at least two groups of species that probably represent an unnatural assemblage. Differences in gastric morphology are tentatively considered to be the result of early divergence in feeding habits. Stomachs of the *Glossophaga* group more closely resemble those of obligate insect feeders than those of the *Choeroniscus* group. Stomachs of the latter assemblage possess many features found to occur otherwise only in fruit-eating species. This divergence probably involves unknown differences in quality or quantity of food ingested, or a combination of these factors. Thorough examination of feeding ecology and ethology should be undertaken to test this proposal.

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STUDIES ON THE PHENOL-SOLUBLE LIPOPOLYSACCHARIDES FROM *SERRATIA MARCESCENS* BIZIO

I. ISOLATION AND CHEMICAL CHARACTERIZATION

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ABSTRACT.—By means of ethanol precipitation and Sephadex G-200 Gel filtration, a lipopolysaccharide and a lipoglycoprotein were isolated from the phenol phase after the endotoxin complex of *Serratia marcescens* Bizio was submitted to 45% hot aqueous phenol extraction. The results of the chemical analyses of these two fractions indicated that the protein moiety may be covalently linked to the endotoxin molecule.

In the course of studying the isolation and fractionation of endotoxin (lipopolysaccharide-protein complex) from *Serratia marcescens* Bizio (Tsang and Rilett, 1970), we submitted the purified endotoxin to hot aqueous phenol extraction (Westphal *et al.*, 1952) with the purpose of removing the protein moiety from the endotoxin complex. However, our observation indicated that the effect of phenol treatment was more degradative than simple deproteinization. Most of the fatty acids and significant amounts of carbohydrates of the original endotoxin were removed along with the protein moiety in the phenol phase. Since several phenol-soluble lipopolysaccharides have been reported to occur in other Gram-negative bacteria (Raff and Wheat, 1968), it might be of interest to isolate and characterize the phenol-soluble materials from the endotoxin of *S. marcescens* Bizio. This report represents the study of isolation, fractionation, and chemical characterization of the phenol-soluble material. Electrophoretic, immunochemical, and biological studies of the various fractions will be reported subsequently.

MATERIAL AND METHODS

Serratia marcescens Bizio, grown on an inorganic medium, was supplied by General Biochemicals, Chagrin Falls, Ohio. The endotoxin was isolated and purified according to the procedures reported previously (Tsang and Rilett, 1970). Phenol extraction of the endotoxin was performed according to the procedure of Westphal with 45% aqueous phenol at 65-70° C for 30 minutes (Westphal, *et al.*, 1952). After the phenol phase was separated from the aqueous phase, it was washed three more times with equal volumes of water. The aqueous phases were combined, dialysed exhaustively against water, and lyophilized (Fr. LPS-A). Ethanol was added to the phenol phase in the ratio of 9.5:1 v/v (ethanol: phenol phase), and the mixture was allowed to stand overnight at 4° C. The precipitate (Fr. GP) was collected by centrifugation (3,000 rpm for 15 minutes) and washed consecutively with acetone, acetone: ether (1:1 v/v), and finally ether.

Total neutral sugars, uronic acids, hexosamines, protein, and fatty acids were determined according to procedures reported previously (Tsang and Rilett, 1970; Tsang and Kallvy, 1971). Heptose was determined by the method of Dische as modified by Osborn (Osborn, 1963). Phosphorus was determined by the method of Bartlett (Bartlett, 1959). The presence of 2-keto-3-deoxy-octonic acid was demonstrated qualitatively by the thiobarbiturate

method of Weissbach and Hurwitz (Weissbach and Hurwitz, 1959).

Paper chromatographic analysis of the hydrolyzed samples (2 N HC1 at 110° C for 4 hours) was performed by the descending method on Whatman No. 1 filter paper with the following solvent systems:

A. Ethyl acetate:pyridine:water (3.6:1:1.5 v/v)
B. Butanol:pyridine:water (11:3.6:5.5 v/v)

Reducing sugars were detected by spraying with ammoniacal silver nitrate and p-anisidine hydrochloride, while amino sugars and other amino compounds were detected by spraying with ninhydrin (in 0.2% butanol).

Column chromatography of fraction GP was performed on Sephadex G-200 in 0.05 M pyridine-acetate buffer, pH 7.8. Fractions were collected every 3 ml. Aliquots of 0.2 ml were taken for protein and total carbohydrate analysis (anthrone method).

Results and Discussion

The phenol-soluble material (Fr. GP) isolated by ethanol precipitation from the phenol phase was a white amorphous solid. It was soluble in most of the aqueous buffers at slight alkaline pH, such as pyridine/acetate, pH 7.8, and borate buffer, pH 8.4. Paper chromatographic analysis after acid hydrolysis revealed that there was no qualitative difference in the sugar components between fraction GP and the corresponding lipopolysaccharide fraction LPS-A or the starting endotoxin fraction. Glucose, mannose, galactose rhamnose, glucosamine, uronic acid, and heptose were identified. Heptose and 2-keto-3-deoxy-octonic acid were also detected by colorimetric methods. With the exception of uronic acids, the other sugars represented the sugar components of the basal core of bacterial lipopolysaccharide (Alaupovic, *et al.*, 1966). Table 1 shows the chemical composition of

Table 1.—Chemical Composition of the Endotoxin Complex, the Lipopolysaccharide (Fraction LPS-A), and the Phenol-Soluble Material (Fraction GP) of *Serratia marcescens* Bizio.

	Endotoxin Complex %	Lipopolysaccharide (Fr. LPS-A) %	Phenol-Soluble Material (Fr. GP) %
Protein	8.0	10.0	30.5
Fatty Acids	22.6	13.5	22.8
Anthrone-Positive Carbohydrates	18.7	30.2	40.0
Glucosamine	2.4	0.9	3.6
Uronic Acids	6.9	11.2	10.6
Heptose	5.3	6.5	6.3
KDO	+	+	+
Phosphorus	1.19	1.35	2.25

the starting endotoxin, the phenol-treated lipopolysaccharide (Fr. LPS-A), and the phenol-soluble fraction (Fr. GP). The results of the chemical analyses clearly indicated that fraction GP is a complex molecule consisting of lipid, polysaccharide, and protein.

In order to study the homogeneity of fraction GP and to demonstrate that this fraction was not a mixture of degraded components of lipids and carbohydrates or proteins, it was submitted to column fractionation on Sephadex G-200. Two distinct components were obtained (Figure 1). The major component was recovered in 90% yield from the exclusion volume. Rechromatography of the major compo-

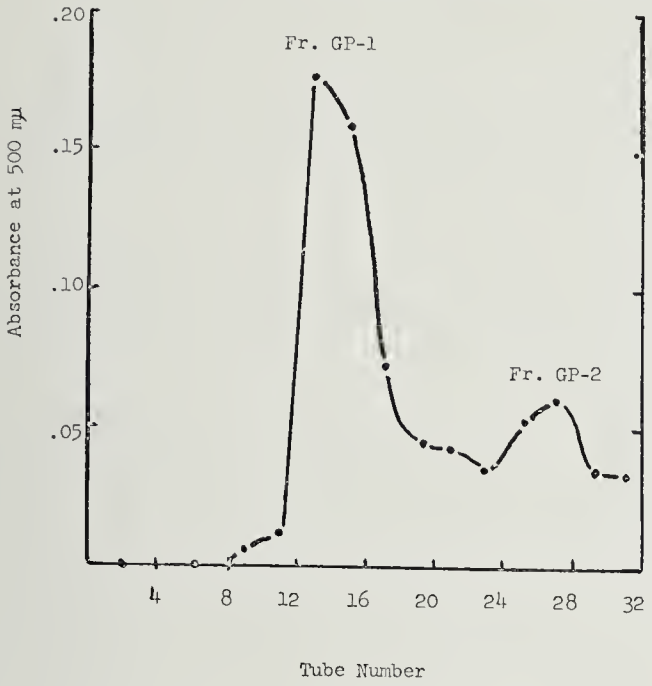

FIGURE 1. Sephadex G-200 Column Chromatography of Fraction GP. Volume collected 3 ml per tube. Column (1.5 x 60 cm) was monitored by protein analysis (Lowry method). Similar result was obtained when column was monitored by carbohydrate analysis (anthrone method).

nent (Fr. GP-1) on Sepharose 4 B failed to provide further fractionation.

The chemical composition of the subfractions are presented in Table 2. It appears that the fatty acids and carbohydrates removed in the phenol phase are eventually recovered in the form of complex molecule(s). The analytical data show that the phenol-soluble material from the endotoxin of *S. marcescens* contained at least two components. The major component has the essential characteristics of bacterial lipopolysaccharide (Alaupovic, *et al.*, 1966), while the minor component is possibly a lipoglycoprotein (60% protein, 14% fatty acids, and 33% total carbohydrates) which is very similar to that of the phenol-soluble material isolated from the endotoxin of the wild-type chromogenic *S. marcescens* 08 (70% protein, 2% fatty acids, and 4% carbohydrate) (Wober and Alaupovic, 1969). However, there are some chemical as well as physical chemical differences between these two preparations (fraction GP-2 and lipoglycoprotein from 08 strain). The lipoglycoprotein from the wild-type (08) contained a pigment, prodigiosin, and was insoluble in most acids and bases, while fraction GP-2 was readily soluble in most aqueous solvents. Recently, a prodigiosin containing cell envelope glycoprotein was isolated from the cell walls of *S. marcescens* 08 (Tsang, *et al.*, 1971; Tsang and Kallvy, 1971). It is not certain whether this glycoprotein is related functionally or structurally to the phenol-soluble lipoglycoproteins from the endotoxin of either the wild-type (08) or the mutant (Bizio).

Since the starting endotoxin had been shown to be heterogeneous (Tsang and Rilett, 1970), it is difficult to speculate at this stage the structural relationship of the isolated fractions (Fr. GP-1 and Fr. GP-2) to the endotoxin complex. However, the possible isolation of the lipoglycoprotein (Fr. GP-2) from the endotoxin complex may suggest

TABLE 2.—Chemical Composition of Phenol-Soluble Material (Fr. GP) and Its Sub-fractions.

	Fr. GP %	Fr. GP-1 %	Fr. GP-2 %
Protein	30.5	26.5	60.0
Fatty Acids	22.8	20.6	14.1
Anthrone-Positive Carbohydrates	40.0	42.0	25.0
Glucosamine	3.6	3.3	1.8
Uronic Acids	10.6	11.8	6.0
Heptose	6.3	5.6	4.5
KDO	+	+	+
Phosphorus	2.25	1.50	1.80

that the protein moiety of the endotoxin may be covalently linked to the lipopolysaccharide complex rather than associated through ionic or hydrophobic linkages. In other words, it is possible that the protein moiety in bacterial endotoxin is specific to the endotoxin molecule and not of non-specific cellular origin. It is hoped, however, that studies on the immunochemical cross-reactivity of the various fractions may reveal their possible functional and structural relationships to each other and to the endotoxin complex, as well as to the cell envelope glycoproteins.

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THE RESPONSE OF SOUTHERN ILLINOIS BARREN VEGETATION TO PRESCRIBED BURNING

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ABSTRACT.—Permanent quadrats were used to study the effect of prescribed burning on an area of barren vegetation that was being over-grown by honeysuckle (*Lonicera japonica*), trees, and shrubs. The site was burned in the spring of 1969 and 1970. Herbaceous and woody plants were sampled on three occasions; in the fall before burning, and in August following each burn. Several species of prairie plants responded favorably to the burning. The prairie willow *Salix humilus* appeared to be spreading as a result of the burning. The only species to have tree size individuals (greater than 3.5 in. dbh) killed by the fire was *Juniperus virginiana*. However, seedlings of *Juniperus virginiana* and *Betula nigra* were eliminated. Following the second burn, but not the first, the frequency of honeysuckle was reduced by one-half. The second burn occurred later in the spring than the first after the honeysuckle had already begun growth.

Forests covered most of southern Illinois before settlement. Prairies were of limited occurrence and generally did not extend much further south than Carbondale (Anderson, 1970; 1970a). "Barrens" occurred south of the Illinoian glacial limit and beyond the major area of prairies. Barrens apparently had more trees and shrubs than prairies and lacked some characteristic prairie species (Vestal, 1936) and might best be described as forest-prairie transitions. At presettlement times these areas were probably degraded forests that had been invaded by prairie plants as a result of fires.

A few of the broader ridges and stream valleys in the unglaciated portions of southern Illinois undoubtedly supported these barrens as indicated by occasional stands of some prairie species. In the extreme southeastern portion of the state some of the areas recorded as barrens in the original land survey now support closed forest communities, a pattern repeated in many areas throughout the midwest as the result of the cessation of nearly annual fires set by aboriginals that had maintained these communities (Curtis, 1959; Vogl, 1964; Muir, 1965).

A vegetation map based on the surface soil color apparently provides the best published information about these barren areas (Fehrenbacher and Alexander, 1958). The soils are transitional between dark prairie and the lighter forest soils.

The purpose of this study was to carefully document changes in plant composition in response to fire using permanent quadrats. If the barren vegetation existed in presettlement times as the result of fire, then burning should tend to perpetuate this community. Fire might increase the dominance of prairie species; however, the woody plants should persist. The area studied was about 1/2 acre, on level ground adjacent to Burke Branch Creek in southern Pope County (S.W.1/4 sect. 4, T 15S, R6E). The area was badly encroached by *Lonicera japonica* (Japanese honeysuckle) in addition to other woody species. A further objective was to determine the effectiveness of fire as a management tool to control honeysuckle.

METHODS

The area to be burned was sampled in November, 1968, and fired in the early spring March 16, 1969. A control plot was established and sampled with the burned area dur-

ing August, 1969. In 1970 the burning was done later, April 5. Both the control and burned areas were resampled during the summer after the second burn, on the first of August. Throughout the study the control area showed little shift in composition or dominance of the plant species.

To record the response of woody plants to fire, the diameter of all tree species greater than 3.5 in. dbh were measured in four 1/40 acre and one 1/100 acre permanently marked circular quadrats. Seedlings and saplings (tree species less than 3.5 in. dbh) as well as shrubs were sampled in five 1/100 acre circular quadrats that were nested within the tree quadrats. The seedlings, saplings, and shrubs were placed into one of five size classes: (1) > 6 in. tall < 4.5 ft., (2) > 4.5 ft. tall < .5 in. dbh, (3) > .5 in. < 1.5 in. dbh, (4) > 1.5 in. < 2.5 in. dbh, (5) > 2.5 in. < 3.5 in. dbh.

Ten one meter square quadrats were nested within the 1/40 acre tree plots to sample herbs and woody plants less than six inches tall. In the 1/100 acre tree plot only four meter square quadrats were located. The honeysuckle was sampled by nesting five decimeter square quadrats within each of the square meter quadrats.

Results and Discussion

The quadrat frequency (per cent quadrats of occurrence) for the burned area in 1970, after two burns, is given in Table I for species having a frequency of 10 per cent or greater. Changes in frequency for selected species from 1968 to 1970 is shown in Figure 1 and discussed in detail later. The area is diverse with a total species list of 125 herbs and 26 trees and shrubs on about one-half acre. The flora is mixed, containing prairie species, woodland and forest edge species as might be expected for barren vegetation. The two bluestems, *Andropogon gerardi* and *A. scoparius*, were the dominant prairie grasses; however, *Sorghastrum nutans* was prominent in scattered areas. *Cassia fasciculata* and *C. nictitans* were the dominant forbs.

The density of trees per acre on the permanent plots was 51.8 in 1968 and it remained unchanged throughout the study. The total tree basal area was 21.1 in 1971 and it increased slightly from 20.0 sq. ft. in 1968. Only four species of trees *Ulmus alata*, *Juglans nigra*, *Platanus occidentalis*, and *Betula nigra* had individuals greater than 3.5 in. dbh in the permanent tree quadrats. None of the trees of these species, ranging in diameter from 3.7 to 10.6 in. (dbh), were killed by the fire. However, tree sized red cedars, outside of the permanent plots, were eliminated by the burning.

Table 1.—Frequency values for the burned area (1970) after two burns.

Andropogon gerardi	77.3	Solidago altissima	18.2
Cassia fasiculata	63.6	Elymus virginicus	15.9
Andropogon scoparius	61.4	Cirsium altisimum	15.9
Cassia nictitans	56.8	Stylosanthes biflora	13.6
Panicum anceps	47.7	Tradescantia ohiensis	11.4
Coreopsis tripteris	38.6	Galium pilosum	11.4
Acalypha gracilens	36.4	Croton glandulosus	11.4
Solidago nemoralis	34.1	Panicum lanuginosum	11.4
Ambrosia artemisiifolia	31.8	Erigeron strigosus	11.4
Potentilla simplex	29.5	Anemone virginiana	11.4
Sorghastrum nutans	22.7	Uniola latifolia	11.4
Euphorbia corollata	22.7	Strophostyles leiosperma	11.4
Rudbeckia hirta	20.5	Eupatorium serotinum	11.4

Burning decreased the number of woody stems less than 3.5 in. dbh in all but the smallest size class (6″ tall but less than 4.5 feet tall) where there was a marked increase because of resprouting, Figure 2. The total number of woody stems per acre in the smallest size class increased from 5,800 in 1969, to 9,100 in 1969, and in 1970 there were 10,160 stems per acre. However, the seedlings (size classes 1 and 2) of red cedar and river birch, were completely eliminated.

Three species were primarily responsible for the increase of woody stems in the first size class, as a result of resprouting: *Salix humilis*, *Cornus ammomum*, and *Juglans nigra*. *Salix humilis*, the prairie willow, increased the most of any of the species and showed a strong tendency to spread as the willow clumps became larger with repeated burning. The total number of willow stems, in the first size class, increased from 1,240 in 1968 to nearly 6,000 in 1970. The density of *Rubus* (probably *R. ostryifolius*) stems greater than 6″ tall remained about the same.

Herbaceous species that showed a marked increase in frequency after burning include several legumes, *Cassia fasciculata*, *C. nictitans*, *Stylosanthes biflora*, *Strophostyles leiosperma*, two goldenrods *Solidago nemoralis* and *S. altissima*, and tall tick seed, *Coreopsis tripteris*, Figure 1. Ragweed (*Ambrosia artemisiifolia*) increased after the first year of burning; however, it declined after the second burn in 1970. The initial increase in ragweed and annual legumes may be in response to the more open environment following fire.

In another study still in progress (Van Valkenburg, 1971) the two species of *Cassia* showed a similar response and in one area increased from 300 individuals per acre to 33,000 with a single fire. Other legumes, including perennial ones, increased following the burn. Similarly, Wahlenberg *et al.* (1939) working in Mississippi found that the number of legumes per acre was 41,500 on burned plots compared to 23,900 on unburned areas after nine years of annual burning. Lemon (1967) working in Africa also reported an increase in legume density in response to fire. The work being done in Southern Illinois by us and others (Johnson, 1969) indicates that there is an increase in the density of legumes following fire.

However, on the Tucker Prairie in Missouri, an increase in grass dominance with repeated burning was reported by Kucera and Koelling (1964) and Kucera (1970). They did not find an increase in legumes with burning.

Several of the species that show a marked reduction in frequency in response to fire included *Panicum polyanthes*, *P. clandestinum*, *Eragrostis spectabilis* and *Rubus* sp. The *Rubus* is probably *R. flagellaris* that is less than six inches tall and was treated as a herb. Steyermark (1968) lists the two species of *Panicum* as occurring on moist ground and on wet prairies.

Figure 1 shows that *Gaura biennis* increased slightly in response to fire. These results do not show the actual response to fire by this species. In several areas outside of the sampling plots *Gaura* increased much more than the data indicates. *Veronicastrum virginicum* also increased markedly outside of the area sampled.

Gentiana flavida (yellow prairie gentian) was restricted to one small clump that was being badly encroached by honeysuckle at the initial sampling in 1968. A permanent quadrat was located so that the gentian clone was included within it. The gentian responded well to

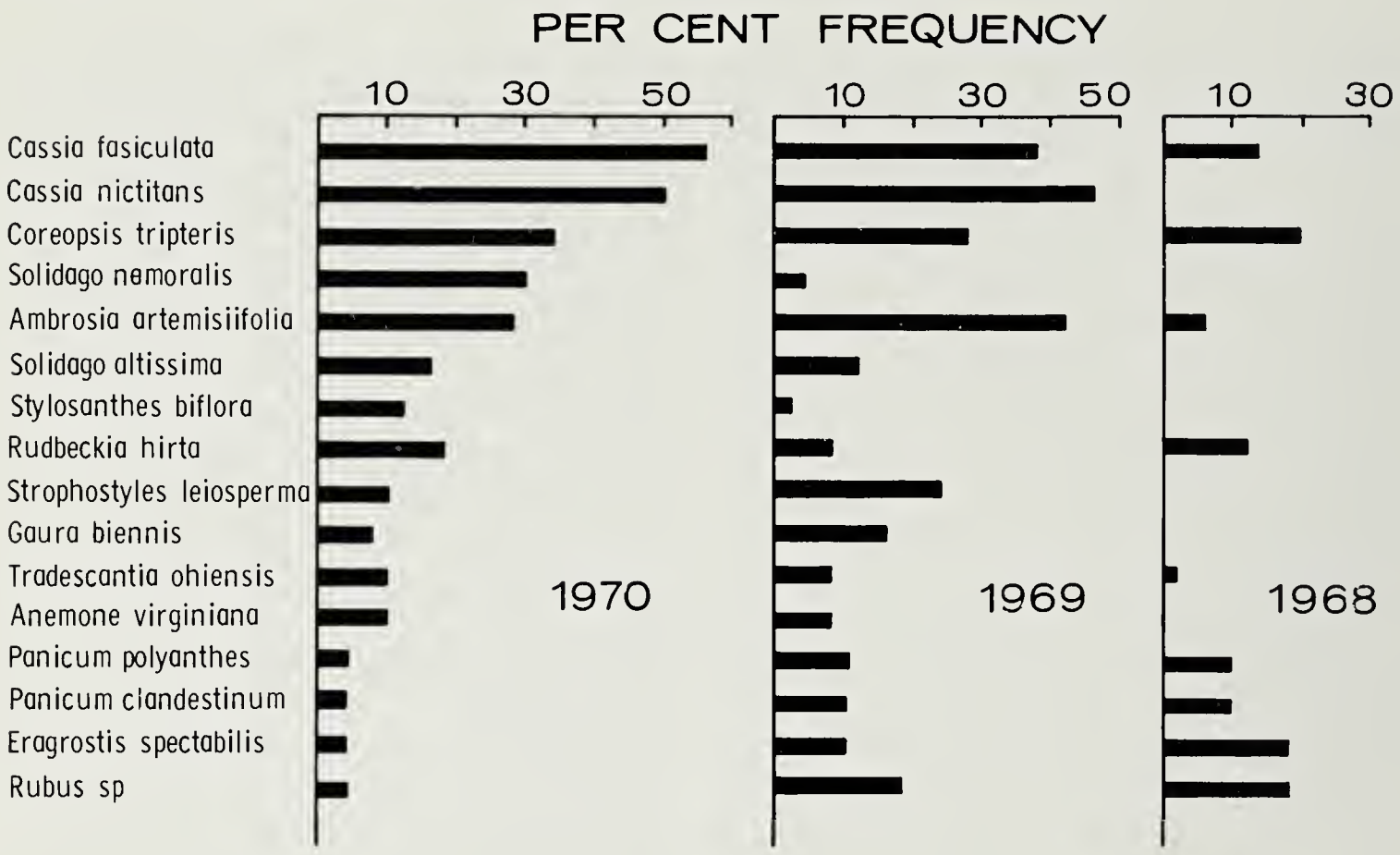

FIGURE 1. Changes in quadrat frequency for selected species in the study area.

the fire and the number of stems in the quadrat increased each year. In 1968 there were 5 gentian stems, in 1969, 10, and in 1970 there was more than a three-fold increase over 1968, 17 stems were in the quadrat.

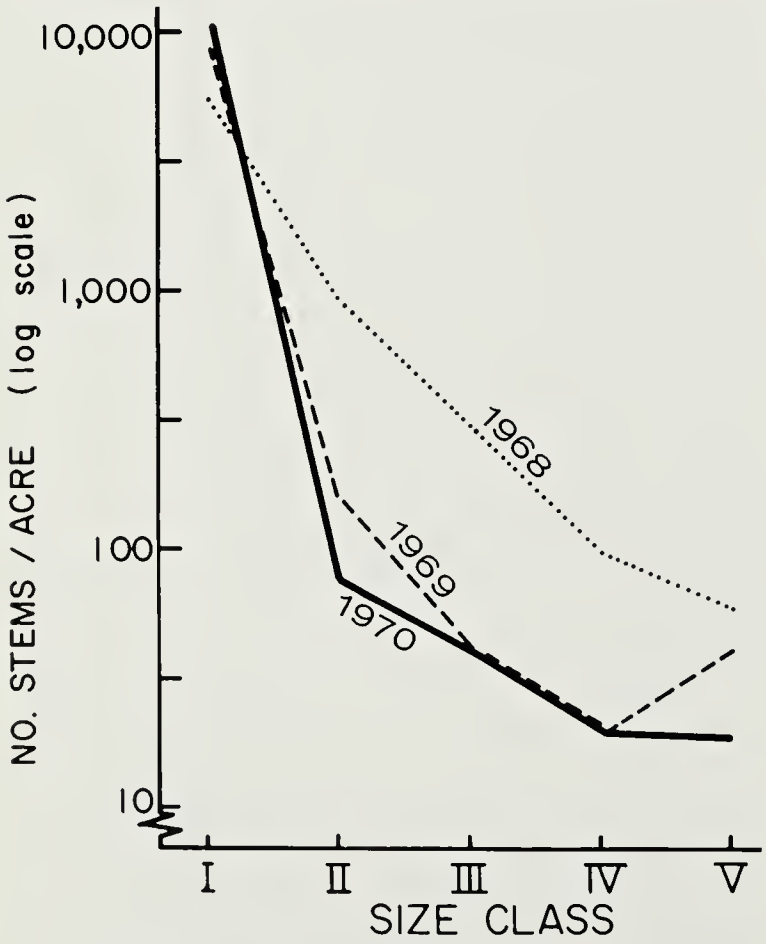

FIGURE 2. The change in the total number of woody stems by size class.

An area that lacked any prairie vegetation grew up to a dense patch of fireweed (*Erechtites hieracifolia*). After the second burn this same area was dominated by *Cirsium altissimum*. However, the fireweed showed little tendency to invade areas that had prairie species well established.

A large portion of the viney honeysuckle that had climbed up into the trees was removed by the first burn. The fire burned up the vines on the trees to heights of 10-15 feet. While most of the honeysuckle in the trees was removed and did not reestablish itself on the trees, after the first fire, there was no decrease in quadrat frequency as much of the honeysuckle resprouted.

The first burn was in early spring, March 16, 1969. The following year the study area was burned on April 5, 1970, after the buds on the honeysuckle had just begun to burst. The frequency of honeysuckle was reduced by about 1/2 from 24 per cent to about 12 per cent, Figure 3. On the control area, established in

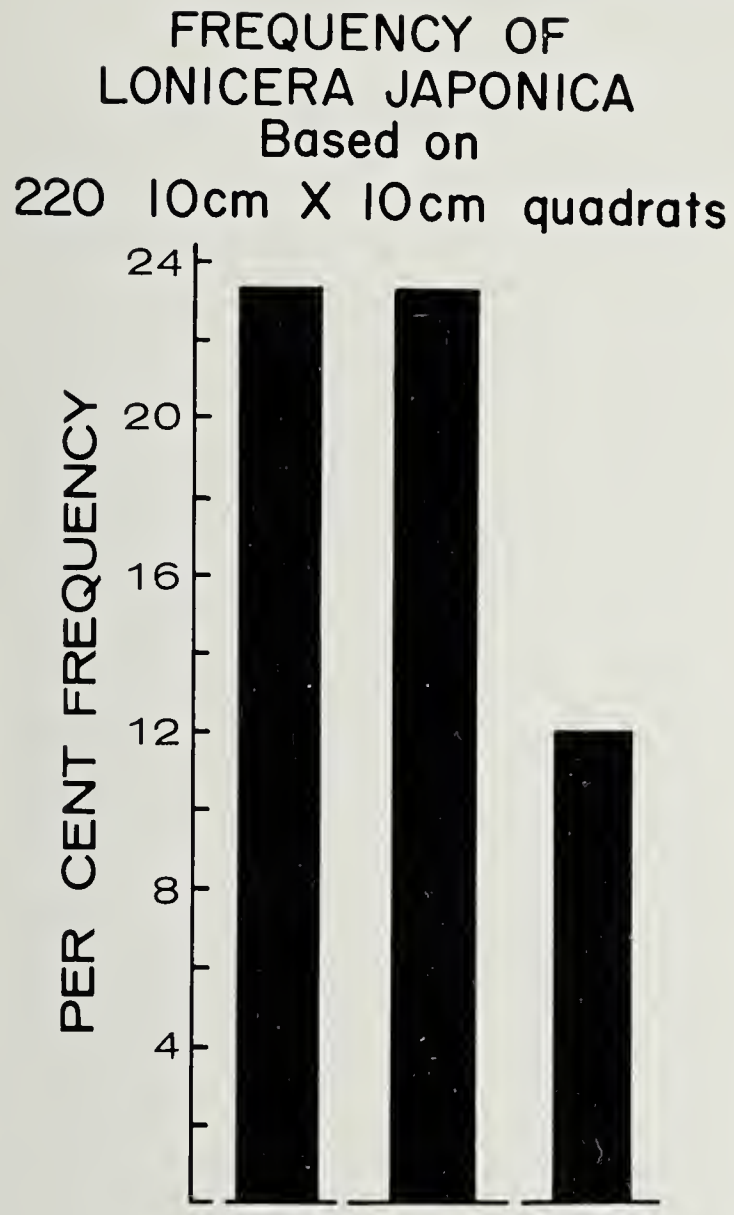

FIGURE 3. The response of *Lonicera japonica* to repeated fires.

1969, 50 decimeter quadrats were used to sample the honeysuckle. Its frequency remained almost the same, 68 per cent in 1969 compared to 66 per cent in 1970.

Prescribed burns appears to be an effective tool for controlling honeysuckle in locations where there is a vegetation such as prairie that will respond favorably to the burning and offer the honeysuckle increased competition. A burn as late as possible in spring, preferably after the honeysuckle has begun growth but while the prairie plants are still dormant would seem to be the most effective.

ACKNOWLEDGEMENTS

The authors acknowledge the U. S. Forest Service for permitting the study to be done on the Shawnee National Forest (Headquarters Harrisburg, Illinois) and for the loan of fire fighting equipment. Thanks are also due to Mrs. M. Rebecca Anderson for her assistance in data collection and Professor Grant Cottam (Univ. of Wis., Botany) for his review of the manuscript.

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ANALYSIS OF THE ELECTRON DENSITY AND POTENTIAL OF SOLID BENZENE

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ABSTRACT.—The electron density distribution of the benzene molecule is analyzed in terms of the selected electron shell (SES) method. With this method the electron density is split into two terms corresponding to the inner and outer electrons, respectively. The validity of subtracting a Gaussian distribution for the inner electrons is checked against the equivalence of subtracting the $1s^2$ contribution. The potential function for the benzene crystal is calculated from experimental x-ray diffraction data. A low potential barrier is observed between the atoms in the benzene ring indicating the presence of delocalized electrons. Also the high potential barrier between molecules is in accordance with the idea that molecules are closed electron systems.

Benzene occupies a central position in organic chemistry and in molecular theory. The basic electronic structure of the molecule is well understood in terms of the molecular orbital theory, which in essence, considers the bonding electrons to be distributed into the α, or localized, and α, or delocalized, types. The molecule of benzene consists accordingly of a framework built up by the trigonal hybrid sp^2 orbitals of the separated atoms overlapping with themselves or with the 1s orbitals of hydrogen. The linear combination of the pz atomic orbitals of the carbon atoms build up the α molecular orbitals. The electron density of benzene has been worked out theoretically by March (1952) by applying the Thomas-Fermi and the molecular-orbital method, but there is no other analysis of the experimentally observed electron density except the work done by Cox et al. (1958). One of the problems in the study of the electron density via x-ray diffraction methods is that it is difficult to get rid of the series termination effect. Another problem is that the most important contribution to the total electron density derives from the electrons near the nucleus of the atom, forbiding any detailed analysis of the experimental maps.

In a series of papers, the authors have shown (Amoros and Canut-Amoros, 1968, 1969, 1970) that it is possible to separate the contribution of the inner and outer electrons to the atomic scattering factor. As a consequence, the analysis of the density distribution from an experimental point of view can be done. The method, that has been called the selected electron shell (SES) method, is based on the possibility of an analytical representation of the atomic scattering factor as a Gaussian polynomial of positive terms, each one corresponding to what we call an electron shell. The method proved to be powerful in the sharpening of the Patterson of a molecular crystal, and it was tested successfully in the analysis of the electron distribution of the outer electrons in hexamine. It seemed, therefore, appropriate to extend the SES analysis to the molecule of solid benzene.

CRYSTAL STRUCTURE OF SOLID BENZENE

Benzene has a fairly simple orthorhombic crystal structure, for which good x-ray diffraction data have been provided by Cox, Cruickshank and Smith (1958) from experiments at −3°C. Solid benzene belongs to the space group Pbca of unit cell dimensions a = 7.46, b = 9.66, c = 7.03 Å. It contains four molecules located on the centers of symmetry at 000, 0,1/2,1/2;1/2,0,

1/2, and 1/2,1/2,0, as first given by Cox (1932). The refinement of the structure by the forementioned authors gave well resolved atomic positions for the three independent carbon atoms. The positions of the hydrogen atoms were assumed to be at a radial distance of 1.084 Å from the carbon atom to which the hydrogen is associated. The atomic coordinates of the hydrogen atoms were calculated in this way by Harada and Shimanouchi (1966).

An overall reliability factor of 10.5% was obtained by Cox and collaborators after five differential cycles of refinement, introducing anisotropic temperature factors. Those temperature factors were further utilized by the same authors to determine the translational and librational components of the amplitudes of vibration of the rigid benzene molecule. The possibility of this analysis showed us that the observed amplitudes and the whole crystal structure were reliable, and that the SES method could be applied to this crystal. In our study we shall assume that the atomic coordinates as determined by Cox, Cruickshank and Smith are correct.

Application of the SES Method

In our previous work on hexamine (Amoros and Canut-Amoros, 1969) it was shown that the innermost electrons of carbon could be approximated by a Gaussian function whose Gs was taken as 2, the number of electrons in the inner shell. Also it was shown that the parameter g's, which contains both the form and temperature factors of the Gaussian function, can be refined by using the structure factors that correspond to reflections with $|r^*| > .9$ Å^{-1}. The SES method utilizes a difference Fourier summation of the type

$$\rho_{outer}(r) = \frac{1}{v} \sum_h (F_{obs} - (F_{inner}) \exp(2\mu i\, r.r_h^*) \quad (|r_h^*| \leq R_L^* \lim) \qquad (1)$$

where v is the unit cell volume, F_{obs} are the observed structure amplitudes, and F_{inner} are the calculated structure amplitudes for the inner electrons alone. Eq. (1) can be calculated by evaluating the contribution of the inner electrons once the parameter g'inner has been determined for each independent carbon atom. In the case of benzene 110 observations were used in the region of the reciprocal space beyond $|r^*| = .9$ A°$^{-1}$. In the refinement of ginner the accepted atomic coordinates were used. The contribution of the hydrogen atoms in this region of reciprocal space is negligible and it was disregarded. Equation (1) is then calculated using all terms within a region of the reciprocal space limited by a sphere of radius $|r^*| = 1.$A°$^{-1}$, the limit of influence of the outer electrons of carbon. Series (1) contains therefore the contribution of the outer electrons of carbon and the hydrogen atoms and is free of series termination effect.

Once the Gaussian functions that describe the electron density of the inner electrons are known, the electron density distribution of the inner electrons in the benzene molecule can be calculated either by convolution or by a Fourier summation of the normal type whose coefficients are the calculated ones. Due to the high temperature factor of the carbon atoms in benzene, the Fourier series method is applicable. The series is practically convergent, and no important series termination effect is observed as

discussed later on. Finally, the density map of the outer electrons of the carbon atoms alone can be calculated by subtracting from Eq. (1) the contribution of the hydrogen atoms.

At this stage one may question the legitimacy of subtracting a Gaussian function as the corresponding function of the electron distribution of the innermost electrons in carbon. In benzene we have the carbon 1s, 2s, and 2p, and the hydrogen 1s functions. The approximation used by the SES method must be equivalent to subtract the 1s state of carbon. In order to be sure that this is the case, the contribution of the 1s function was subtracted using the values of the shell scattering of 1s 1/2 given by Cromer and Waber (1964) taking into account that two electrons belong to this state. The procedure followed in this new calculation was similar to the previous one. First the anisotropic temperature factors for the 1s shell were deter-

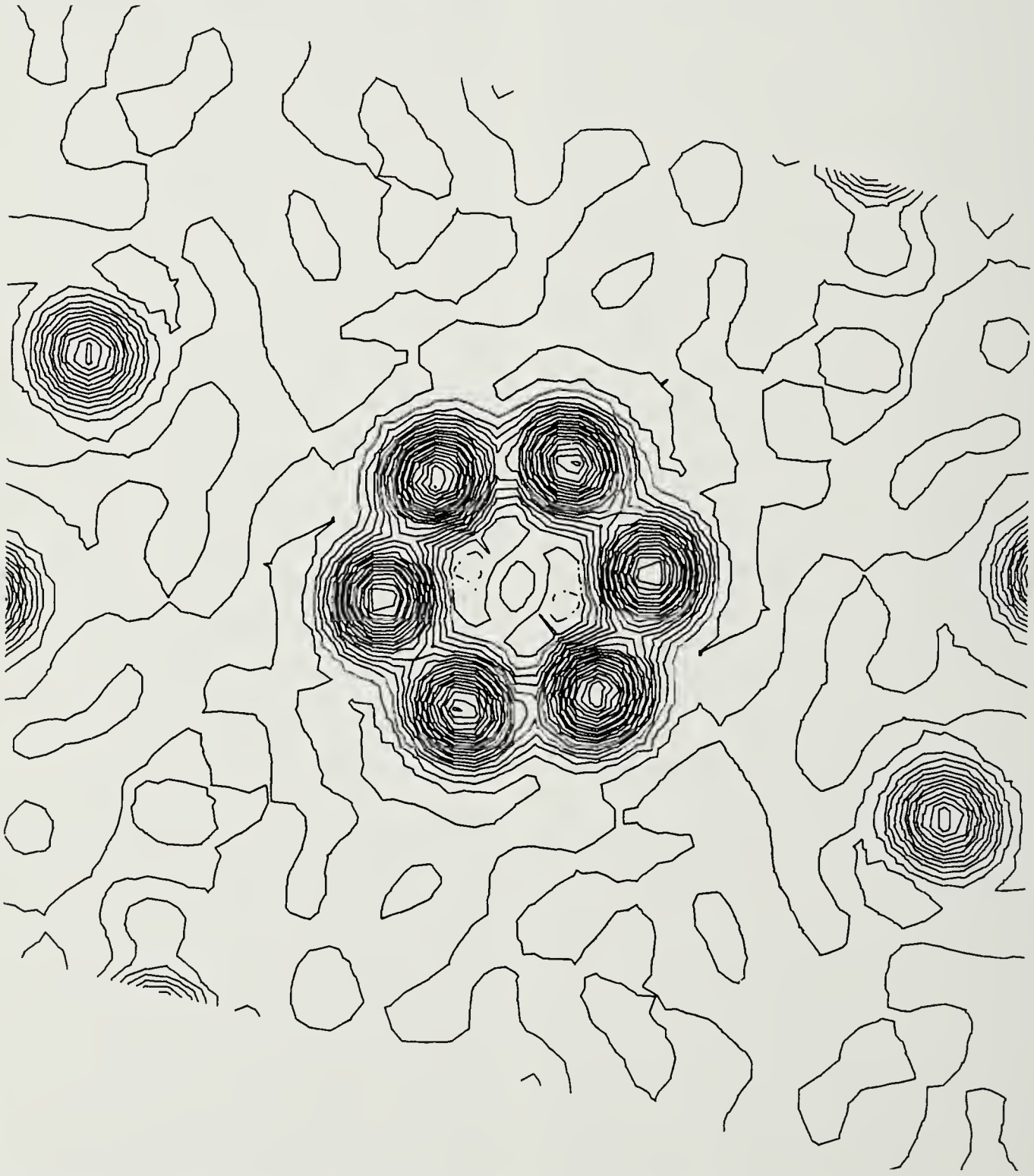

FIGURE 1a. Electron density distribution of benzene. Section through the molecular plane. Contour lines at increments of 0.2 e A^{-3}. Linear scale 1 cm = 1A. Carbon inner electrons only. The first contour line 0.0 e A^{-3} is not significative.

mined using the observed values for $|r^*| > .9$ A°$^{-1}$. Then a difference Fourier series of the type of Eq. (1) was performed in which the F_{inner} were computed in terms of the 1s electrons alone.

Analysis of the Results

The most interesting maps are those corresponding to the section through the molecular plane. According to our theory, the electron density can be split into two terms: the inner and outer electron density. Figure 1 gives the SES maps.

The first map (Fig. 1a) corresponds to the inner electrons. The zero line is very well defined, and no values higher than than 0.1 e. A°$^{-3}$ are observed in the map outside the molecule. The fluctuation

Figure 1b. Benzene outer electrons (a Gaussian core for carbon atoms has been subtracted). First contour line 0.2 e A-^{3}.

of the background is so small that the map is practically free of series termination effect. The electron density shows maxima of about 3.65 e. A°$^{-3}$ centered at the sites of the six carbon atoms of the molecule. Thermal motion causes an overlap of the electron density at mid-point between two adjacent carbon atoms, and due to this a reading of 0.9 e. A°$^{-3}$ is observed at that mid-point.

Figure 1c. Benzene outer electrons (1s isotropic state for carbon atoms has been subtracted). First contour line 0.2 e A-^{3}.

The outer electrons density maps are particularly interesting. Two kinds of maps have been computed. One map was calculated subtracting only the inner electrons as given by the Gaussian approximation (Fig. 1b). The map contains then the electron distribution of the outer electrons of carbon and the hydrogen electron. The map clearly shows the distortion of the basic hexagon of carbons produced by the presence of hydrogens. It is interesting to note that the outer electrons of carbon behave like a doughnut of almost constant density of about 1.4 e. A°$^{-3}$ showing very small maxima of 1.5 e. A°$^{-3}$. In order to check the validity of the Gaussian approximation for the inner electrons, a similar map was computed by subtracting the contribution of the 1s orbital. The resulting map (Fig. 1c) is identical to the previous one (Fig. 1b), the only difference being that the small maxima of 1.5 e. A^{-3} at the atomic positions are more de-

fined. The size of the van der Waals envelope and distribution of contour lines is identical in both cases, as well as the count of 0.2 e. A^{-3} at the center of the benzene ring. From this follows that the Gaussian approximation for the inner electrons is a realistic one. The maps are very similar to the ones calculated by March (1952), corresponding to the molecular orbital electron-density contours in a plane parallel to the plane of the ring at a height of 0.35 Å above the plane of the ring, which could be expected from the averaging effect of thermal vibration in the real molecule.

FIGURE 1d. Carbon outer electrons only. First contour line 0.2 e A^{-3}.

The last map corresponds to the outer electrons of the carbon atoms alone (Fig. 1d). In the calculations the contribution of the inner electrons of the carbon and the hydrogen atoms have been subtracted. The consequences of existing delocalized electrons is the existence of a region of almost constant electron density. In fact, the map shows very clearly that this is the case in benzene. It is important to note that the subtraction of the hydrogen atoms did not alter the electron density in the benzene ring nor the inner part, and only the humps due to the hydrogen atoms have disappeared.

Better information can be obtained by observing the graph of Fig. 2. In the graph the electron density in the plane of the molecule has been plotted along a radial line uniting two opposite carbon atoms of the ring. The electron density curves of the inner, outer and hydrogen electrons are clearly visible. Also, the positions of the C and H as deduced from x-rays by Cox, Cruikshank and Smith (1958) and neutron diffraction experiments by Bacon, Curry and Wilson (1964) are included. Thermal motion forces the observed peak positions of the carbon atoms as determined from x-ray experiments to move toward the center of the benzene ring. It is rewarding to note that the zero electrons contour line in our maps coincide with the effective size of the van der Waals envelope for carbon atoms or for hydrogen + carbon atoms determined from the intermolecular potential (Newman, private communication).

THE POTENTIAL FUNCTION

The study of the nature of the chemical bond can also be conveniently done by analyzing the potential distribution in the unit cell. It was shown by Ewald (1938) and Laue (1940) that the electrostatic potential can be calculated from the Fourier series

$$(r) = \frac{e}{\eta v} \sum_h \frac{F_h}{r^{*2}_h} \exp\left(2_\eta i\, r.r^*_h\right) \quad (2)$$

where e is the electron charge. This series has the advantage that converges very rapidly, especially in molecular compounds where the temperature factor is normally high. Further advantage of Eq. (2) is that it can be calculated directly from

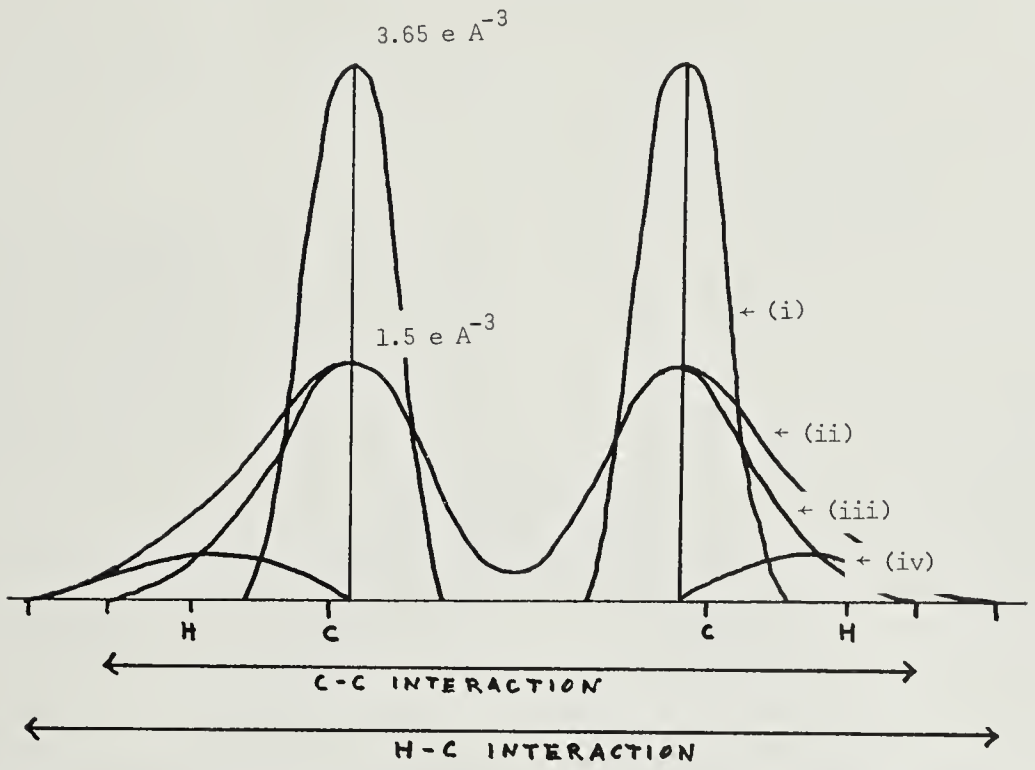

FIGURE 2. Electron density along two diametrally opposite carbon atoms. Linear dimension 1 cm = 1A.

(i) Inner electrons of carbon atoms only.
(ii) Outer electrons with hydrogen atoms.
(iii) Outer electrons without hydrogen atoms.
(iv) Electron density of hydrogen atoms.

experimental data. Series (2) has been used for the analysis of the potential in semiconductors (see, for instance, papers published in Sirota (1968). However, no calculations have been done for molecular crystals, although this kind of function has great interest because it gives the experimental form of the van der Waals envelope of a molecule and enables the determination of the potential barrier between molecules. With this in mind, the observed potential function for benzene was computed.

The most significant map is the section through the molecular plane, which is given in Fig. 3. The atomic positions are determined by well defined potential wells separated by low potential barriers. The barrier has a potential of 1.6 eV. The low value of the potential barrier is in agreement with the view of existence of delocalized electrons in the benzene ring. The center of the molecule shows a barrier of 4.8 eV. with respect the atomic well, which indicates the possibility of some electron density in the center of the molecule. This is in agreement with the SES map of the outer electrons that shows an electron density of 0.2 e. A^{-3} at that point.

One of the most interesting features of the map is the clear limitation of the benzene molecule, that is bound by a high potential barrier. The lowest value of this barrier corresponds to about 14 eV with respect to the atomic positions. This value corresponds to the line along the nearest intermolecular distance in the plane of the section. This indicates that strong repulsion between the electron clouds of neighboring molecules exist and that the molecule must be considered as a closed system of electrons in agreement with current ideas of molecular crystals.

The value of the potential and potential barriers obtained from Eq. (2) depends upon the temperature factor. Therefore, the actual values given here must be taken as

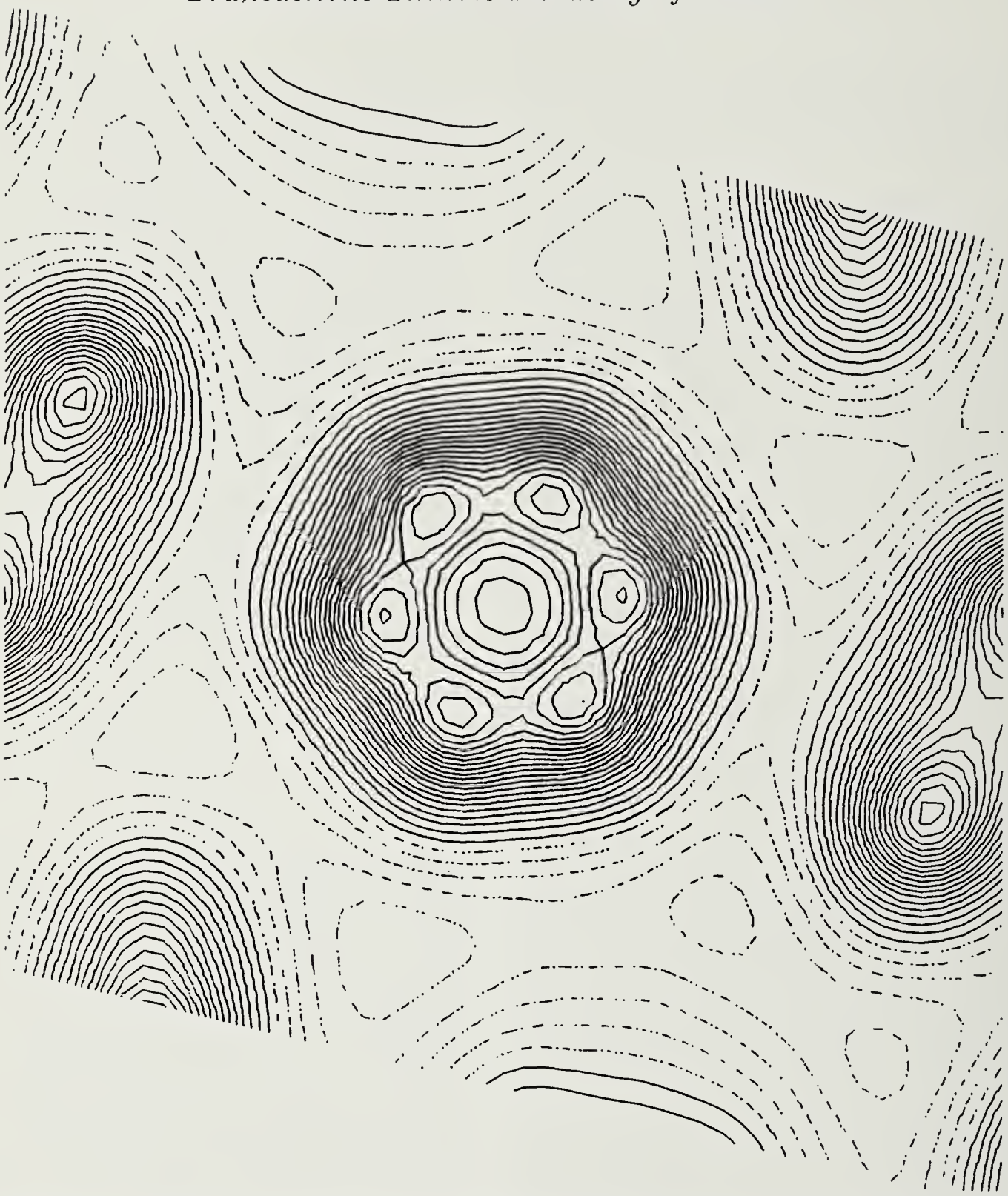

FIGURE 3. Electronic potential distribution through the benzene molecular plane. The first continuous line corresponds to O e V. Contours at increments of 0.3 e V.

having qualitative rather than quantitative significance. However, a significative picture of the existence of low and high potential barriers can be obtained from electron-potential maps.

The study of intermolecular potential barriers is of great interest for a better understanding of the chemistry of molecular solids. Further work on this sense is therefore necessary.

COMPUTATIONAL WORK

The computational work was made in an IBM 7044 and the plots in the digital incremental CalComp 565 plotter with off-line magnetic tape unit 470.

The least squares refinements and structure factor calculations were done with the ORFLS program of Busing, Martin and Levy (1962). The Fourier series were computed with the FORTRAN program of Zalkin (1962). Details of the actual computations involved in the SES method have been given by Canut-Amoros, Casper, Walters and Amoros (1970) and the computing

programs described in the Computing Report of Canut-Amoros et al. (1968).

Acknowledgements

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PLANT INVESTIGATIONS II. STUDIES ON THE HEXANE EXTRACT OF *CIRSIUM ARVENSE*

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ABSTRACT.—The hexane extract of *Cirsium arvense* was investigated by application of column, thin layer, and gas chromatography and spectroscopic methods. A C_{18}-C_{31} alkane mixture, B-amyrin, taraxasterol, taraxasterol acetate, tetracosanol, hexacosanol, and octacosanol were positively identified.

Cirsium arvense, or Canadian thistle, the curse of the Midwestern farmer, is a plant which has received little attention by the natural product chemist (Shelyuta et al., 1970). Throughout the chemical literature the multitude of articles about it has centered upon its eradication although there have been scattered reports on its potential as a supply of blood coagulants (Mazzanti, 1954), seed growth inhibitors (Helgeson and Konzak, 1950), and meat-curing solution additives (Jensen and Hess, 1951). The little phytochemical work done with the *Cirsium* genus has been concerned only with the isolation of flavanoids (Wagner et al., 1960; Nakaoki and Morita, 1959; Ibid., 1960; Morita and Shimizu, 1963) and the detection of alkaloids, tannins, and flavanoids (Ismailov, 1958; Bandyukova and Shinkarenko, 1965; Kolodziejshi, et al., 1966).

In this paper we describe our preliminary investigations on the alkane, n-alcohol, and terpene components of *Cirsium arvense*.

EXPERIMENTAL

General—Melting points (mp) were taken on a Fisher-Johns apparatus and are corrected. Infrared spectra were recorded with a Beckmann IR-8, nuclear magnetic resonance spectra (n.m.r.), with a Varian A-60 on $CDCl_3$ solutions, and mass spectra with a Perkin-Elmer-Hitachi RMU-6E instrument. Silica gel HF_{254} (Merck) at a thickness of 0.5 mm was used for all thin layer chromatography (TLC). A Varian Aerograph Series 200 instrument with a flame ionization detector was used for gas liquid chromatography (GLC). The instrument was utilized at an injector temperature of 300°, a detector temperature of 325°, and a nitrogen rate of 80% flow at 65 psi. A 5 ft. by 1/8 in. SS column packed with 5% SE-30 on acid-washed dmcs 60/80 chromosorb W was used for all separations.

Preliminary Separation—The aerial portions of *C. arvense* were collected during June 1968 in DeKalb. The air-dried material (2 kg) was powdered and then exhaustively extracted with hexane in a Soxhlet apparatus. Removal of the hexane gave a residue (89 g) which was treated in 400 ml. of tetrahydrofuran with 120 ml. of 10% KOH. After heating on the steam bath for 15 minutes the resulting solution was diluted with water, and the organic materials were recovered with ether. The residue (63 g.) was then dissolved in hexane and the hexane removed *in vacuo*. This process was repeated twice more to ensure full removal of all other solvents.

The treated extract was dissolved in hexane and chromatographed on 900 g of Alcoa F-20 alumina which had been neutralized to Activity II-III by stirring it under hexane for 3 days with 27 ml. of 10% acetic acid. The column was eluted with 1.5 liter fractions. The solvent used and the fractions are as fol-

lows: hexane 1-8, 2% benzene-hexane 9-11, 5% benzene-hexane 12-14, 10% benzene-hexane 15-17, 25% benzene-hexane 18-48, 35% benzene-hexane 49-59, 50% benzene-hexane 60-68, benzene 69-75, 1% chloroform-benzene 76-80. The chromatogram had been continued with other solvents but no identifiable material was isolated.

Taraxasterol Acetate—A white solid which was shown by TLC analysis to be essentially one compound was found in Fractions 5-9. The material was chromatographed preparatively on three TLC plates with benzene. Recrystallization of the recovered material (400 mg.) from methylene chloride gave colorless crystals, mp 220-225°; $[\alpha]_D^{22}$, 96.7° (C,0.2); max(in KBr) 1735, 1642, 898 cm-^{1}; n.m.r. 278 (d,2H,J=1.0), 122(s,3H), 63, 61, 58, 57, 51 c.p.s.; m/e 468(M+), 453, 408, 399, 393, 357, 339, 297, 249, 218, 205, 204, 203, 191, 190, 189 (base).

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.70; H, 11.12.

Reported mp and $[\alpha]_D$ values for taraxasterol acetate have been 234-248° and 95.0° (Kasprzyk and Pyrek, 1968); 238-240° and 96.0° (Chaudbury and Ghosh, 1970); 256-257° and 100° (Simonsen and Ross, 1957).

n-Alcohols—Fractions 20 (410 mg) and 21 (430 mg) were purified by treatment with Norit A. Crystallization of each from benzene-methanol yielded material melting at 75-77.5° and 80-82°, respectively. GLC analysis of both at a column temperature of 253° gave peaks corresponding to 1-tetracosanol at 146 sec., 1-hexacosanol at 238 sec., and 1-octacosanol at 394 sec. Both fractions consisted mainly of 1-octacosanol and were almost identical in alcohol proportions. Identification of latter two alcohols was made by comparing their GLC behavior to a commercial sample of 1-hexacosanol and the 1-octacosanol sample obtained in the first paper of this series (Piatak and Reimann, 1970). The 1-tetracosanol structure was inferred from the retention time pattern.

Taraxasterol—By TLC analysis fractions 23-48(7.0g.) were found to contain one compound. Recrystallization of the material from benzene, acetone, and chloroform-methanol after Norit A treatment gave 1.1 g. of colorless crystals, mp 203-205° (normal) and 213-215° (evacuated capillary); $[\alpha]^{22}D$ 90.9° (C,3.0); *v* max (in KBr) 3400, 1642, 885 cm-^{1}; n.m.r. 277 (d,2H, J = 2.00), 64, 61, 58, 56, 51, 46 c.p.s.; m/c 426(M^+), 411, 408, 357, 315, 218, 207, 189 (base).

Anal. Calcd. for $C_{30}HO_{50}$: C, 84.44; 11.81. Found: C, 84.37; H, 11.76.

Literature values for the melting point and specific rotation of taraxasterol were 213-219° and 82.0° (Kasprzyk and Pyrek, 1968); 222-225° and 93.5° (Atherinos et al., 1962).

The taraxasterol was converted to an acetate which was identical to the taraxasterol acetate obtained above and to a benzoate, mp 230-232 [reported mp 242-244° (Simpson, 1944)].

β-Amyrin—TLC analysis was used to determine that fractions 49-59 (1.5 g.), 60-68(1.2 g.), and 69-80 (0.9 g.) contained taraxasterol and another major component. The two were separated by preparative TLC using 25% ethyl acetate-hexane. GLC of the new compound at a column temperature of 235° revealed that the material consisted of two closely related compounds with retention times of 954 sec. and 1096 sec. The latter compound was identified as β -amyrin by comparison of its retention time with authentic material and by noting an

increase in the amount of response for the second peak when authentic material was added to the mixture. The more mobile material was not investigated further when comparison of its GLC characteristics to several triterpenes failed to identify it.

n-*Alkanes*—Fractions 1-4 (24g.) were rechromatographed on 720 g of Woelm neutral alumina of Activity I. The first three fractions eluted (3 x 500 ml. hexane) were combined to give 500 mg. of semi-crystalline material, ^{max} no C=O absorption. GLC analysis with a programmed column temperature of 90-280° at a rate of 4-6°/min indicated the presence of 18 separate substances.

The mixture was dissolved in benzene and treated with a drop of bromine. Reanalysis of the material after removal of the solvent showed 15 substances. By comparing the remaining peaks to a set of standards identified by mass spectral analysis of gas chromatographically separated material (Reimann, 1970) and extrapolation of the regularity in the retention times, the alkanes were identified. The mixture was found to consist of the following n-alkanes: C_{18}(4.4%), C_{19}(<1), C_{20} (<1), C_{21}(<1), C_{22}(<1), C_{23}(5.8), C_{24}(1.0), C_{25}(4.1), C_{26}(1.9), C_{27}(25.3), C_{28}(2.8), C_{29}(37.8), C_{30}(1.7), C_{31} (12.4).

Discussion

Our work with *Cirsium arvense* appears to be the first report concerned with the identification of individual compounds in this species. Although Shelyuta et al. (1970) have explored this plant phytochemically, they had only concerned themselves with the classes of compounds present or not present.

Thus far, we have identified several known compounds. The first compound isolated was taraxasterol acetate, a derivative of a pentacyclic triterpene commonly found throughout the plant world. In fact, taraxasterol seems to be the major pentacyclic triterpene in *C. arvense*, since it was detected in a number of the chromatography fractions as the free alcohol or its acetate.

Taraxasterol itself appeared much further along in the column chromatography fractions. It was not readily identified at first even though it gave a single peak in GLC analysis, because the mp and specific rotation data were widely divergent from literature values. However, spectroscopic data did point to taraxasterol or a similar triterpene since an exocyclic methylene group was indicated by n.m.r. and infrared spectra, a hydroxyl group by an infrared spectrum, and a pentacyclic skeleton by a mass spectrum. Some derivatives were prepared, but the physical constants could not be fully reconciled with literature values.

The structure was finally resolved by a comparison of taraxasterol acetate with samples provided by other workers in India and Poland. Although a mixture m.p. with these samples had the same value as our material, the mass spectra and GLC characteristics of each were in full agreement, thereby fully establishing the structure.

The taraxasterol acetate isolated from the column appears to be an artifact produced by the action of acetic acid used to neutralize the column alumina on taraxasterol. Normally, acetates of triterpenes are not found readily in any quantity in plants. Since we did hydrolize the plant extract with base before chromatography, it is even less likely the acetate was originally present.

The isolation and identification of the mixture of straight chain primary alcohols obtained from the column after taraxasterol acetate

was easily accomplished by various chromatographic techniques. Although the fractions were mixtures of practically the same composition, the difference in their mp's and the sharpness of the mp's were not totally unexpected. In the first paper of this series (Piatak and Reimann, 1970) it had been found that mp's of alcohols were not satisfactory criteria for identification and that GLC comparison to material fully established by a mass spectrum was best.

A second pentacyclic triterpene, β-amyrin, was also uncovered in this plant. This terpene, however, could not be isolated owing to the presence of another compound. Although several different methods, e.g. silver nitrate impregnated TLC plates and double-dip TLC techniques, were attempted, a clear cut separation between the two could not be gotten. The -amyrin nevertheless was verified by the standard GLC technique of adding authentic material to the chromatographic sample and observing an increase in the response for that material.

The last series of compounds to be investigated were the n-alkanes. Even though these compounds were contained in the first four column fractions, they were mixed with several other materials. A second column chromatography employing a larger alumina to material ratio did give a colorless, crystalline mixture of alkanes containing a few unsaturated compounds. The unsaturated hydrocarbons could be removed readily by bromine treatment. Since the bp of the bromo addition product would be quite high, the net result of the bromine treatment is a removal of all unsaturated compounds. Of course, the peaks obtained for each alkane must be examined carefully to see if any have been increased by the addition products; but, thus far, this has not been our experience.

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LONGEVITY RECORD FOR PIPISTRELLUS SUBFLAVUS

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ABSTRACT.—Longevity record for *Pipistrellus subflavus* extended from 11.2 years to 14.8 years, with remarks on tooth wear.

The longevity of American bats has recently been reviewed by Paridiso & Greenhall (1967) from data compiled by the U. S. Fish & Wildlife Service banding returns. Fourteen species, representing six genera are cited. The oldest present known longevity record is for a specimen of *Myotis lucifugus* recovered 24 years after banding (Griffin & Hitchcock, 1965), while Hall, et al. (1957) recorded 18.5 years for *Myotis keenii*. Paridiso & Greenhall (1967) gave equally long dates for *Eptesicus fuscus*, 19 years and 16.5 years for *Plecotus townsendii*.

On 14 February 1971 and again on 25 February 1971, while recording recoveries of *Pipistrellus subflavus* in South Blackball Mine, 1.75 miles west of Utica, LaSalle Co., Illinois, a male *P. subflavus* was found carrying the U. S. Fish & Wildlife Service Band Number 57-04984, which was banded on 16 February 1957 at South Blackball Mine, by Dr. Wayne H. Davis. This would give this bat a minimum age of 14.8 years, since the young of these bats are born in June or early July. Paridiso & Greenhall (1967) gave 11.2 years as the greatest age for *P. subflavus*, while Davis (1966) using multiple regression analysis showed that *P. subflavus* could live up to 13 years, but gave no indication of having recovered a specimen of this age.

It is interesting to note that all of the species having exceedingly long life spans (*M. lucifugus*, *M. keenii*, *M. sodalis*, *Plecotus townsendii*, and *Eptesicus fuscus* in eastern United States) bear only one young. *Pipistrellus subflavus* which is smaller than any of the above noted species has two young, and possibly this could be a major factor in its being shorter-lived.

In examining the canine teeth of this specimen on 25 February 1971, I was surprised to find only slight wear in this individual. This would agree with Twente (1955) for five year old bats. Hall, et al. (1957) have shown that tooth wear in *M. lucifugus* and *M. keenii*, as proposed by Twente (1955) and Stegeman (1956) for other species, is a highly unreliable criterion for age determination. The tooth wear criterion is apparently unreliable for *P. subflavus* also.

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NOTES ON ILLINOIS AND WISCONSIN RESUPINATE BASIDIOMYCETES

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ABSTRACT.—Four species of resupinate Basidiomycetes are reported from either Illinois or Wisconsin for the first time.

Collections of fungi that extend their known North American range are noted here. These collections were made either by me (AEL) or by R. M. Miller (RMM) and are now deposited in the mycological herbarium at Illinois State University (ISU).

Cerinomyces pallidus Martin

On decayed deciduous wood, Funks Grove, McLean County, Ill., VI. 20. 1969, AEL 1470 (ISU 1210); near Crab Orchard Lake, Williamson County, Ill., XI.23.1963, AEL 557 (ISU 688).

The reported North American range of this species now includes, in addition to Illinois, Iowa and Ontario (Martin, 1952) as well as Wisconsin and Colorado (Liberta, 1965, 1966).

Galzinia incrustans (Hoehn. & Litsch.) Parm.

On decayed deciduous wood, Funks Grove, McLean County, Ill., XII. 1.1970, RMM 70-21 (ISU 1260).

The only other midwestern state where collections of this species have been reported is Missouri, under the binomial *Corticium roseopallens* Burt (1926).

Hyphodontia alutacea (Fr.) Eriks.

On decayed wood (*Tsuga?*), near Sayner, Vilas County, Wisconsin, VII.16. 1964. AEL 493 (ISU 1209).

This is the first report of the species in Wisconsin. Larsen (1964) has reported it from several eastern and western states as well as from Canada. Miller and Boyle (1943) reported its occurrence in Iowa.

Xenasma grisellum (Bourd.) Liberta

On decayed deciduous wood, near Crab Orchard Lake, Williamson County, Illinois, XI.23.1963, AEL 1454 (ISU 1211).

This species is apparently wide spread in Europe, but the only previous North American report was from the province of Quebec, Canada (Liberta, 1966a). The collection of this species in Illinois considerably extends its known North American range.

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MORRIS M. LEIGHTON
1887-1971

Dr. Morris M. Leighton, widely-known geological scientist and administrator, died Thursday, January 7, 1971, at Urbana, Illinois, at the age of 83, after a long illness. He was Chief of the Illinois State Geological Survey from 1923 until his retirement in 1954, during which time the Survey came to a preeminent position among state surveys.

Dr. Leighton was born in 1887 at Wellman, Iowa, a son of Stephen T. and Jane Leighton. He was educated at the University of Iowa, where he received B.A. and M.S. degrees in 1912 and 1913, and at the University of Chicago, where he received his doctorate in 1916.

Between 1915 and 1923, Dr. Leighton was on the geology faculty at the University of Washington, Iowa State Teachers College, Ohio State University, and the University of Illinois. During the period 1919-1923 he was also on the staff of the Illinois State Geological Survey. After his retirement he remained active professionally until shortly before his death.

Dr. Leighton published numerous scientific articles over the past 53 years and is recognized especially for his publications on Pleistocene glacial deposits. Particularly noteworthy was his contribution on the concept of a state geological survey as a research institution. The Illinois State Geological Survey was the first state survey to emphasize a coordinated research effort to bring together the various disciplines, e.g., geology, chemistry, physics, and engineering, and apply them to natural resources for the shaping of sound policies of conservation.

Dr. Leighton was a member of many scientific and technical societies including the Society of Economic Geologists of which he was past president and honorary member; the Geological Society of America, fellow and councilor; the American Association of State Geologists, honorary member and past president; the American Geological Institute, director and past president; Illinois State Museum Board, member and chairman; Illinois Postwar Planning Commission, member and vice chairman; Advisory committee, U. S. Geological Survey; Coordinating Committee on National Water Policy; Chicago Geographic Society, fellow; American Association for the Advancement of Science, past vice president; Illinois State Academy of Science, past president; and Illinois Mining Institute, past president.

Dr. Leighton was also made an honorary member of the American Association of Petroleum Geologists, the Chicago Academy of Sciences, and the Illinois Mining Institute. He also served as business editor for "Economic Geology" for many years, and as editor of the State Geologists Journal.

Among his awards and honors, he was named Distinguished Alumnus at the University of Iowa in 1947, was elected a fellow of the American Academy of Arts and Sciences in 1952, was awarded an Honorary Doctor of Science Degree from Southern Illinois University in 1954, and was a member of the United States Delegation to the Twentieth International Geological Congress held in Mexico City in 1956.

He was a member of the Wesley United Methodist Church and of the Chaos, Dial, and University Clubs.

Dr. Leighton was preceded in death by five brothers and one sister. He leaves his widow, Ada B. Leighton; a sister, Mrs. Golda Jenkinson of Tulsa, Oklahoma; three sons, F. Beach of Hacienda Heights, California; Morris W. of Seaforth, New South Wales, Australia; and Richard T. of Rockford; and 10 grandchildren.—*Boris Musulin, Department of Chemistry, Southern Illinois University, Carbondale, Illinois.*

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GILBERT H. CADY
1882-1970

Gilbert Haven Cady, world-renowned coal scientist, died Friday, December 25, 1970, at Champaign, Illinois, at the age of 88, following a short illness.

Dr. Cady served as Senior Geologist and Head of Coal Section of the Illinois State Geological Survey from 1926 until his retirement in 1951 but remained active professionally until two weeks before his death. He was born in 1882 in Chicago. He studied at the Lewis Institute, Chicago, and Northwestern and Yale Universities and the University of Illinois, and received his doctorate from the University of Chicago in 1917.

He worked with the Illinois State Geological Survey from 1907 to 1919, leaving to spend a year as a geological consultant in the interior of China. From 1920 to 1926, he was head of the geology department at the University of Arkansas.

His many scientific papers, published over a period of nearly 60 years, concentrated on the field of coal geology. A high percentage of coal scientists in North America from time to time had professional association with Dr. Cady.

Dr. Cady was a member of many other scientific and technical societies, including the Society of Economic Geologists, of which he was past president and councilor; Geological Society of America, fellow and counsilor, and American Association for the Advancement of Science, fellow. He was a member of Phi Beta Kappa and Sigma Xi.

Dr. Cady was honored with a life membership in the Illinois Mining Institute. In 1963, the International Committee for Coal Petrology recognized his many years of contributions to this field with the presentation of the Reinhardt Thiessen Medal for Coal Petrology. He was the third recipient of the award, established in 1953, and the only American so honored to date.

North American coal petrographers gathered in Urbana in 1964 for the presentation of a formal testimonial to Dr. Cady, and in 1967 he was the recipient of the Penrose Medal of the Society of Economic Geologists for "unusual original work in the earth sciences."

Colleagues throughout North America are planning to establish a memorial fund to be used for recognition of contributions in coal geology.

Dr. Cady was a member of the Wesley United Methodist Church and the Urbana Exchange Club. He was preceded in death by his wife, Marian, and two sons, Gilbert and Allan. He leaves two daughters, Ruth Adams of Urbana and Mary Johnson of Las Vegas, Nevada, and two grandsons, Derek and Cady Johnson, of Las Vegas.—*Boris Musulin, Department of Chemistry, Southern Illinois University, Carbondale, Illinois.*

Manuscript received March 1, 1971.

Erratum:

In the article "The first record in Illinois of a population of *Stethaulax marmoratus* (Say) (Hemiptera: Scutelleridae) with information on life history. 64:198-200, the photograph for Figure 1 is reversed, the male is to the left.

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CONTENTS

NOTES

EFFECT OF ETHYLENE AND 2,3,5-TRIIODOBENZOIC ACID ON SOYBEAN SEEDLINGS GROWN IN HYDROPONIC CULTURE

E. PATRICK LIRA AND ARTHUR H. FREYTAG
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Libertyville, Illinois 60048

ABSTRACT—Soybean seedling, Glycine max (L) Wayne, grown in hydroponic culture were treated with ethylene and 2,3,5-triiodobenzoic acid by root application. Significant aerial shoot elongation with minimal lateral bud initiation and growth resulted from the ethylene treatment. The concomitant addition of 2,3,5-triiodobenzoic acid to the ethylene treatment had an overriding effect on these growth responses.

Treatment of root systems of plants with growth regulators has been the subject of several reports (Scott and Norris, 1970; Sherbeck, 1967). Ethylene treatment of excised root sections, root tips and intact seedlings has been reported to cause effects which resemble auxin response (Chadwick and Burg, 1967, 1970; Radin and Loomis, 1969). Recent observations indicate this may not be generally true (Scott and Norris, 1970; Andreae, *et al.*, 1968). The exogeneous ethylene treatment of root systems of intact plants without concomitant treatment of the aerial portions of the plant has been reported recently (Smith and Russell, 1969). We can now describe a previously unreported ethylene-induced response of soybean seedlings, Glycine max (L) var. Wayne, grown in hydroponic culture to which ethylene was added and removed without contamination of the surrounding atmosphere. Under these conditions significant aerial shoot elongation with minimal lateral bud initiation and growth were observed. We have also overridden this shoot elongation by the addition of 2,3,5-triiodobenzoic acid (TIBA) to the nutrient solution.

MATERIALS AND METHODS

Soybean seeds planted in vermiculite were germinated in the greenhouse and grown to the unifoliate leaf stage. Two healthy seedlings were then transplanted to a 3.7 liter widemouth glass jar covered by a five-holed, grooved, opaque plastic lid. The jar contained 3.6 liters of half strength Hoagland solution (Hoagland and Arnon, 1938), continually aerated with carbon-filtered air. The container was covered completely with an opaque plastic film. The seedlings were supported by sponge rubber gaskets. Two sintered glass tubes were inserted in the lid in rubber stoppers, one for aeration and the other for ethylene, and an exhaust tube was extended from the remaining opening.

Each test consisted of four treatments replicated five times. The treatments were: control, ethylene, TIBA, and ethylene plus TIBA. Although four tests varying the TIBA concentration (1, 2.5, 5 and 10 ppm) were run, only the data of the tests at 1 and 5 ppm are reported, because all except the lowest concentration gave similar results. The treatments were initiated when the seedlings were transplanted and were terminated at the seven-eight trifoliate leaf stage. The nutrient solution and the TIBA were replaced weekly. Ethylene was added daily for a two-hour period at the same time each day. Distilled water was added daily to maintain liquid level.

Research grade ethylene from a

high pressure cylinder was distributed equally to the jars by a manifold and flow meter at a rate of four liters/minute. The exhaust gas was collected by a manifold and vented to the exterior of the greenhouse. During the addition of ethylene this process was assisted by a low volume vacuum pump.

The ethylene concentration in the aqueous solution was determined by removing 2 ml solution with a syringe fitted with a six inch No. 20 needle. Samples were taken at 0.5, 2.0, 4.0, 7.0 and 24.0 h after initiation of ethylene addition. The samples were transferred to septum-capped 250 ml erlenmeyer flasks containing 1 g lithium chloride and were allowed to equilibrate for 24 hours. The vapors were analyzed by gas chromatography (Burg and Burg, 1966).

Results and Discussion

The aqueous ethylene concentration was calculated by application of the simple gas laws. The concentration varied with time (Figure 1). The solution was saturated (McAuliffe, 1966) during the second h of addition. Then there was a rapid decrease to 10 ppm after 4 h followed by a more gradual reduction to 1 ppm after 7 h. Ethylene was still detectable (0.01 to 0.001 ppm) after 24 hr. The observed elongation of the main shoot of soybean seedlings when ethylene treatment is rigorously confined to the root zone has not been previously described (Holm, *et al.*, 1970). It can

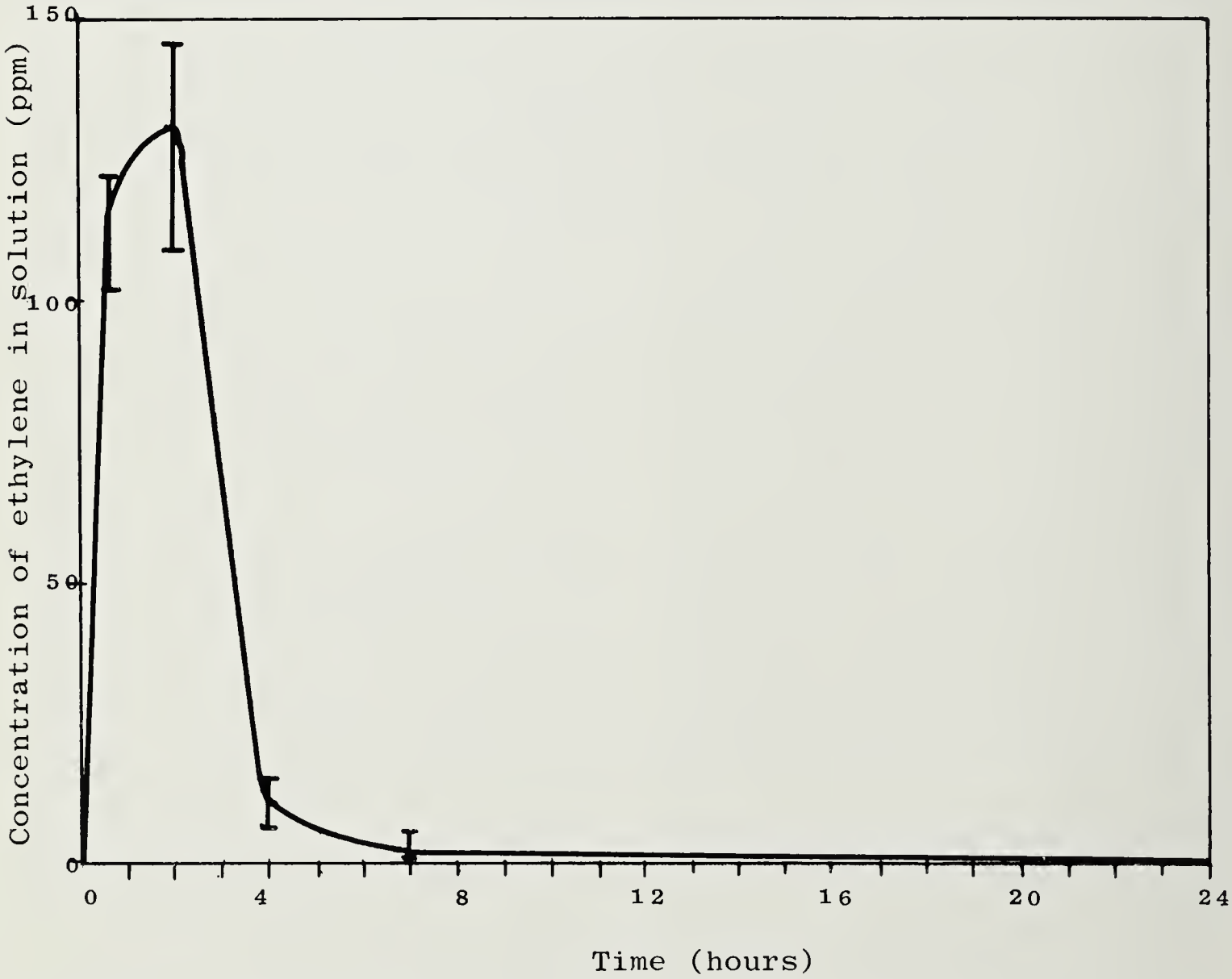

Figure 1. Variation of aqueous ethylene concentration with time. The variation between samples is illustrated by the vertical bars. The results are the composite of thirty samples at each sampling period. This cycle is repeated each day.

TABLE 1. Growth of Soybean Seedlings Treated with Ethylene and Tiba Grown in Hydroponic Culture

Treatment*	Height*	Lateral Bud No.	Lateral Length	Green Weight	
				Roots	Aerials
Control*	100	100	100	100	100
Test I					
Ethylene	+25a	−290b	−300c	−53b	−53bc
TIBA (5 ppm)	−21b	−1a	+80a	−26b	−22b
Ethylene + TIBA (5 ppm)	−5b	−15a	+3b	−32c	−170c
Test II					
Ethylene	+32a	−900c	−160c	−24b	−25b
TIBA (1 ppm)	−9c	+3a	+6a	−17ab	−41b
Ethylene + TIBA (1 ppm)	+8b	−21b	−9ab	−62c	−91c

*Each datum is the mean of 10 plants. Two plants per treatment (jar) replicated five times. (See text for complete description of procedure.) The datum is expressed as a percent of control. Numbers for each test within a column followed by a different letter are significantly different at the 5% level using Duncan's multiple range test.

be seen (Table 1) that the treated plants are between 25 and 32% taller than the controls. The two other unreported tests terminated at the three-four and thirteen trifoliate leaf stages showed height increases of 52 and 12%, respectively. It appears that this response is most pronounced during the earlier stages of growth while at later stages the controls may actually be growing faster. A further observation is the dramatic reduction in the number of lateral buds being formed and their subsequent growth. These results may be due to an increase in the production and distribution of gibberellins in the roots (Jones and Phillips, 1967) or perhaps to a redistribution of auxin (Beyer and Morgan, 1970; Abeles, 1966).

The response of soybean seedlings to TIBA in hydroponic culture has been described (Ohki, 1968) and our results (Table I) generally agree. When TIBA, an auxin transport inhibitor (Huffman, *et al.*, 1967) is added at 5 ppm to the ethylene solution, elongation is completely overridden and lateral bud formation is enhanced. However, TIBA at 1 ppm only partially reverses these ethylene-induced effects. These TIBA-ethylene interaction observations tend to indicate that root-applied ethylene is influencing the auxin transport system rather than a specific gibberellin system.

The overall growth in all the treatments was less than the control. In general, the combined treatment caused a greater reduction in growth. The ethylene-treated roots gave a typical auxin growth response (Scott and Norris, 1970). Finally, in one of our tests (unreported) the seedlings became chlorotic, but the ethylene treated plants appeared significantly greener.

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EFFECTS OF SOIL MOISTURE STRESS ON FOLIAR NUTRIENTS OF LOBLOLLY PINE

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ABSTRACT—Loblolly pine (*Pinus taeda* L.) seedlings were subjected to three soil moisture stresses during their second growing season. Foliar nitrogen was higher and foliar potassium, calcium, and magnesium were lower in those seedlings grown in the drier treatment. Foliar phosphorus was not affected by soil moisture stress.

Leaf analysis as a means of evaluating the nutritive status of forest trees has met with varying success. Even where a correlation has been found between nutrient concentrations in the leaf and in the soil, there is always the question of how accurately such data reflect the nutrient supplying power of a given site. Many variables, in addition to soil fertility, affect the concentration of chemical elements in the leaf, such as physiological maturity, position in tree, sampling date, time of day sampled, and others (Leyton and Armson, 1955).

These reported studies and conceptions indicate that soil-moisture stress must play an important role in the nutrient concentrations in tree foliage. In an attempt to clarify these findings and concepts, loblolly pine seedlings were grown during their second year in the greenhouse under varying soil-moisture stresses and the elements in the needles correlated with soil moisture.

METHODS

The study consisted of two phases: The first phase was conducted with one-year-old loblolly pine seedlings planted in pots filled with a silt loam soil. This soil (Ap horizon) was developed from loess and is medium to high in K (.02 percent); low in P (trace), N (.01 percent), and organic matter (0.90 percent), and moderately to strongly acid (pH 5.2). The equivalent of 1,000 kilograms per hectare of a 12-12-12 (N, P, K) fertilizer was applied to each pot at planting time. The second phase of the study conducted the following year was with year-old seedlings grown from seed in pots (three seed per pot) filled with the same soil as used in the first phase but not fertilized. During both years, the soil with seedlings was allowed to dry until about 30 percent, 50 percent, or 70 percent of the available soil moisture was exhausted as determined by weighing, and then watered to above field capacity. Available moisture in this study is that moisture held in the soil between 1/3 and 15 bars as determined by the pressure pot and pressure membrane apparatus. There was 23 percent moisture in the soil at 1/3 bar and 8 percent moisture in the soil at 15 bars for this soil. Hereafter, these three soil-moisture levels will be known as low, medium, and high soil-moisture stresses, respectively.

Thirty seedlings were planted in individual pots in each of the three treatments in the first phase. Mortality reduced the number in the medium and high soil-moisture stress treatments to 18 and 8 seedlings, respectively. Twenty-five pots were used in each treatment in the second phase, but the number of seedlings per pot varied from one to three. At the termination of the second experiment, there were 44, 47, and 40 seedlings in the low, medium, and high soil-moisture stress treatments, respectively.

At the end of both growing sea-

sons, plants were removed from the pots and dried at 75° C. Foliar nitrogen was determined by a semimicro Kjeldahl procedure (Ranker, 1927), phosphorus by phosphomolybdic acid (A.O.A.C., 1945), potassium by flame emission, and calcium and magnesium by the EDTA method (Malmstadt and Hadjiioannou, 1959).

Analysis of variance was made of the five elements in the needles with soil-moisture stresses.

Results

Shoot growth of seedlings during the study period varied by moisture treatments. In both phases of the study, seedlings subject to the drier conditions grew about 90 percent (as a percent of total height at beginning of season), whereas those in the moist treatment (low soil moisture stress) grew about 120 percent.

Table 1 shows the average nutrient levels in the foliage under high fertility in the first phase. When the soil was supplied with adequate moisture (low moisture stress), the nitrogen content of the needles was significantly lower than in seedlings growing at higher soil-moisture stresses. At this relatively high soil fertility, soil moisture did not influence the concentration of phosphorus in the needles, but plants growing at low moisture stress had more potassium in the needles than plants growing under drier conditions.

Table 1. Foliar Nutrient Concentrations of Seedlings Grown under Different Moisture Stresses in the First Phase

Variable	Moisture stress		
	Low	Medium	High
	Percent of O.D. Weight		
N[1]	2.566	2.823	2.759
P	0.135	0.128	0.110
K[1]	0.280	0.241	0.243

[1]Low is significantly different from other stresses at 5 percent level.

Nutrient concentration in the needles are shown in Table 2 for the second phase. The results for N, P, and K were very similar to those obtained in the first phase, except that the concentrations of N and P were much lower and K higher. Calcium and magnesium concentrations were higher at the low moisture stress than in the

Table 2. Average Foliar Concentrations and Ratios of Concentrations in Second Phase of Study

Variable	Moisture stress			F ratio[1]
	Low	Medium	High	
	Percent of O.D. weight			
N	0.862	1.093	1.118	16.32
P	0.072	0.078	0.071	1.85
K	0.319	0.301	0.290	3.45
Ca	0.286	0.266	0.264	4.07
Mg	0.231	0.208	0.204	12.40

[1]Significant differences between stresses at 1 percent level, 2.99.

higher stresses in this phase of the study.

Discussion and Conclusions

The increase in foliar nitrogen in the high moisture stress treatment probably means that when growth was limited by soil moisture, nitrogen tends to accumulate within the plant because the rate of entry is approximately maintained in conjunction with the decreased rate of utilization in growth processes. The general tendency for potassium, calcium, and magnesium to be relatively low in plants on drier soil (high moisture stress) indicates that the rates of entry into the plant of these elements are less than the rates of utilization in the slow growing plants.

The increase in foliar potassium in this study with decreasing soil moisture stress agrees with results obtained for herbaceous plants and for 26-year-old red pine growing on a coarse sandy soil in New York State (Jurgensen and Leaf, 1965). But the small difference in foliar potassium for the two years is difficult to explain, as it was thought that the potassium contents of seedlings grown in the well-fertilized soil would be greater than they were.

It is well known that fertilizers interact with soil moisture stresses (Schomaker, 1969) and that the nutrient concentration of plants grown at different fertility levels may not always conform to a set pattern. These results tend to confirm the thought that many factors influence an element in a plant, and it is difficult to predict how any one factor can affect the concentration of an element within a plant.

It is recognized that the method used in reporting the concentration of nutrients in this paper (as a percentage of weight of oven-dry foliage) has its limitation, and there is a possibility that some other method such as total uptake might be superior. This possibility has been suggested for red pine in western Massachusetts (Hoyle and Mader, 1964) and in the Adirondack region of New York (White and Leaf, 1965).

The most useful role that foliar analysis can play at the present time is in the nutrition of forest trees on depleted soils where nutrient deficiencies have been indicated. This technique has been used quite successfully in New York on potassium-deficient soils (Madgwick, 1964) and in Florida on phosphorus-deficient soils (Pritchett and Llewellyn, 1966).

Acknowledgements

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CONTRIBUTIONS TO AN ILLINOIS FLORA No. 4. COMPOSITAE II. (TRIBE HELIANTHEAE, PART I—DYSSODIA, HELENIUM, GAILLARDIA, HYMENOXYS, HYMENOPAPPUS, AND POLYMNIA).

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ABSTRACT.—A key to the 23 Illinois genera of the tribe Heliantheae and keys, descriptions, and distributional maps for the Illinois species of *Dyssodia*, *Helenium*, *Gaillardia*, *Hymenoxys*, *Hymenopappus*, and *Polymnia* are given.

This is the second of a series on the Compositae in Illinois. The first, a treatment of the tribe Vernonieae, has been published previously (Wunderlin, 1968).

This study includes only a key to the Illinois genera of the tribe Heliantheae and a treatment of the first six genera, *Dyssodia*, *Helenium*, *Gaillardia*, *Hymenoxys*, *Hymenopappus*, and *Polymnia*, in Illinois. Treatments of the other genera in the tribe will follow in subsequent publications. The magnitude of this tribe as well as the desirability of making the works available without too much delay has made it desirable to publish it in several parts.

The tribe Heliantheae is characterized by having opposite (rarely alternate or whorled) leaves, predominately radiate heads, the style-branches usually hispidulous and with the stigmatic lines poorly defined, and the anther bases obtuse to sagittate (rarely caudate).

The tribe Helenieae has been traditionally distinguished from the Heliantheae by a single technical character, the absence of chaff on the receptacle. This character is not always clear-cut; *e.g.*, *Gaillardia* has a bristly rather than a naked receptacle. The tribe Helenieae is now considered by most Compositae specialists as an artificial taxonomic group. The subtribes of the Helenieae apparently have been derived independently from the Heliantheae in several different lines. Therefore, the Helenieae should not be considered as a separate tribe but should be included in the Heliantheae as has been done in this treatment. The subtribe Ambrosiinae has become adapted to wind pollination and differs from the rest of the Compositae in its much reduced corollas and free or nearly free anthers. This group might justifiably be considered as a separate tribe or even a separate family as it has been treated by several other authors. However, the Melampodinae furnishes a good series of transitional forms between the typical Heliantheae and the less modified genera, *e.g.*, *Iva*, of the Ambrosiinae. Thus the Ambrosiinae are treated as part of the Heliantheae as is more customary.

The Heliantheae in Illinois is composed of 23 genera of which three, *Gaillardia*, *Cosmos*, and *Galinsoga*, have been introduced. *Gaillardia* is from the west, *Cosmos* is from the southern United States, and *Galinsoga* is from the tropics. Several species of other genera have been introduced or are adventive in Illinois.

The distributional maps are based upon specimens housed in the following herbaria: Eastern Illinois University, the Field Museum of Natural History, the Illinois Natural History Survey, the Illinois State Museum, the Missouri Botanical Garden, Southern Illinois University, the University of Illinois, and Western Illinois University.

The author is grateful to the curators of the herbaria used in this study for allowing him to study their specimens. The author also gratefully acknowledges Dr. James R. Wells, Cranbrook Institute of Science, Bloomfield Hills, Michigan, for his assistance and comments on the treatment of *Polymnia*, Dr. Warren P. Stoutamire, the University of Akron, Akron, Ohio, for his comments on *Gaillardia*, and Dr. Robert H. Mohlenbrock, Southern Illinois University, for his critical reading of the entire manuscript.

Systematic Treatment

Key to the Illinois Genera of the Tribe Heliantheae

(This key is admittedly partially technical so that each genus is keyed out only once. A strictly artificial key would result in many genera being keyed out in several places and would be greatly increased in size and complexity.)

1. Heads with ligulate or discoid flowers; corolla regularly developed.
   2. Receptacle without chaff (merely bristly in *Gaillardia*).
      3. Inner phyllaries united at base, glandular-dotted..... 1. *Dyssodia*
      3. Inner phyllaries free at base, not glandular-dotted.
         4. Phyllaries herbaceous; heads radiate.
            5. Plants with leafy stems.
               6. Leaves decurrent on stem or, if not, then linear-filiform..... 2. *Helenium*
               6. Leaves not decurrent on stem nor linear-filiform. 3. *Gaillardia*
            5. Plants scapose..... 4. *Hymenoxys*
         4. Phyllaries petaloid, scarious; heads discoid..... 5. *Hymenopappus*
   2. Receptacle definitely with chaff.
      7. Disc-flowers sterile (styles undivided, ovary reduced).
         8. Cypselas* thick, scarcely flattened..... 6. *Polymnia*
         8. Cypselas compressed dorso-ventrally.
            9. Flowers yellow, in large corymbose-panicled heads.. 7. *Silphium*
            9. Flowers white, in small corymbose heads..... 8. *Parthenium*
      7. Disc-flowers fertile (styles divided, ovary normal sized).
         10. Cypselas turbinate, 5-angled..... 9. *Galinsoga*
         10. Cypselas flat, 4-angled, or, if 5-angled, then subterete and linear.
            11. Ligules persistent on cypselas, chartaceous..... 10. *Heliopsis*
            11. Ligules promptly deciduous or absent.
               12. Cypselas compressed dorso-ventrally (terete in *Bidens beckii*, an aquatic with filiform-dissected leaves); phyllaries dimorphic.
                  13. Pappus of 2 short teeth or awns, barbed upward or smooth, or a mere border, or absent; cypselas wing-margined (except *C. tinctoria*)..... 11. *Coreopsis*
                  13. Pappus of 2-6 awns or teeth, these barbed or hispid, usually retrorsely (rarely smooth or absent); cypselas not wing-margined.
                     14. Cypselas beaked..... 12. *Cosmos*
                     14. Cypselas not beaked..... 13. *Bidens*
               12. Cypselas scarcely flattened or sometimes compressed laterally; phyllaries not dimorphic.
                  15. Heads discoid, appearing gray because of black-tipped anthers..... 14. *Melanthera*

15. Heads radiate (inconspicuously in *Eclipta*), not gray.
    16. Heads small, with short white ligules subequal to phyllaries; receptacle chaff bristleform; small weak annuals with short-stalked axillary heads.....15. *Eclipta*
    16. Heads large, with yellow or pink to purple ligules much longer than phyllaries; receptacle chaff broader; stout perennials, biennials, or sometimes annuals with peduncled or terminal heads
        17. Receptacle conical or columnar (if conical, then leaves not decurrent).
            18. Cypselas compressed laterally.....16. *Ratibida*
            18. Cypselas 4-angled.
                19. Flowers yellow (or mottled with brown).....17. *Rudbeckia*
                19. Flowers pink to purple (in ours).....18. *Echinacea*
        17. Receptacle flat to convex (rarely conical in *Verbesina*).
            20. Leaves decurrent on stem; cypselas compressed laterally; pappus of 2-3 persistent awns.....19. *Verbesina*
            20. Leaves not decurrent on stem; cypselas 3- to 4-angled; pappus of 2-4 caducous scales.....20. *Helianthus*

1. Heads without ligulate flowers; pistillate flowers without corolla or with corolla reduced to a tube or ring around base of style; staminate flowers with regularly developed corolla.
    21. Heads all alike; pistillate flowers few, marginal; staminate flowers many, central; involucre of few rounded phyllaries..21. *Iva*
    21. Heads of two kinds, pistillate with tuberculate or bur-like involucre.
        22. Staminate involucre with united phyllaries.....22. *Ambrosia*
        22. Staminate involucre with distinct phyllaries.....23. *Xanthium*

*Characteristic fruit of the Compositae. Similar to an achene but bicarpellate rather than unicarpellate and formed from an inferior ovary with adherent floral tissues on outside wall.

1. DYSSODIA Cav., Anal. Cienc. Nat. 6:334. 1802.

Willdenowa Cav., Ic. 1:61. 1791.
Boebera Willd., Sp. Pl. 3:2125. 1804.
Schlechtendalia Willd., Sp. Pl. 3:2125. 1804, non Less., 1830.
Adenophyllum Pers., Syn. Pl. 2:458. 1807.
Thymophylla Lag., Gen. & Sp. Nov. 25. 1816.
Hymenatherum Cass., Bull. Soc. Philom. 1817:12. 1817.
Clomenocoma Cass., Dict. Sci. Nat. 9:416. 1817.
Lebetina Cass., Dict. Sci. Nat. 25:395. 1822.
Rosilla Less., Syn. Gen. Comp. 245. 1832.
Syncephalantha Bartl., Ind. Sem. Hort. Goett. 6.1836, ex Linnaea 12:80. 1838.
Gnaphaliopsis DC., Prodr. 7:258. 1838.
Lowellia Gray, Mem. Amer. Acad. II. 4:89. 1849.
Aciphyllaea (DC.) Gray, Mem. Amer. Acad. II. 4:91. 1849.
Comaclinium Scheidw. & Planch. ex Planch., Fl. Seres 8:19. 1852.

Urbinella Greenman, Proc. Amer. Acad. Arts 39:117. 1903.
Gymnolaena (DC.) Rydb., N. Amer. Fl. 34:160. 1915.
Boeberastrum (Gray) Rydb., N. Amer. Fl. 34:161. 1915.
Trichaetolepis Rydb., N. Amer. Fl. 34: 170. 1915.
Dysodiopsis (Gray) Rydb., N. Amer. Fl. 34:170. 1915.

Annual or perennial herbs; leaves opposite or alternate, entire to pinnately dissected, glandular-dotted. Heads several, terminal, radiate or rarely discoid; involucre in one or two series, conspicuously glandular-dotted, inner united at base or above; receptacle flat or nearly so, naked or nearly so; ray-flowers pistillate, fertile, ligule yellow or orange; disc-flowers numerous, perfect; anthers narrow at base; style-branches flattened, truncate, or with elongate pubescent appendages; pappus of 10-20 scales, each divided to middle or below into several bristles or entire. Cypselas narrow, substriate.

The genus *Dyssodia* consists of about 40 species native to the western hemisphere. It is represented in Illinois by the following single species.

1. DYSSODIA PAPPUSA (Vent.) Hitchc., Trans. Acad. St. Louis 5:503. 1891.
Tagetes papposa Vent., Descr. Cels. *pl. 36*. 1802.
Boebera chrysanthemoides Willd., Sp. Pl. 3:2125. 1804.
Tagets pumila Willd., Sp. Pl. 3:2126. 1804, pro syn.
Boebera glandulosa (Cav.) Pers., Syn. Pl. 2:459. 1807, nom nud.
Dyssodia chrysanthemoides (Willd.) Lag., Gen. et Sp. Nov. 29. 1816.
Dyssodia fastigiata DC., Prodr. 5:640. 1836, non *D. fastigiata* HBK., 1820.
Dyssodia chrysanthemifolia Steud., Nom. Bot. II. 2:660. 1841.
Boebera papposa (Vent.) Rydb., in Bretton, Man. Fl. N. States & Canada 1012. 1901.
Boebera ciliosa Rydb., N. Amer. Fl. 34:168. 1915.
Boebera roseata Rydb., N. Amer. Fl. 34: 169. 1915.
Dyssodia ciliosa (Rydb.) Standl., Field Mus. Pub. Bot. 4:299. 1929.
Dyssodia roseata (Rydb.) Gentry., Los Pastizales de Durango 331. 1957.

Much branched, ill-scented annual; stems 0.5-4.0 dm tall, puberulent to glabrous; leaves opposite, 2-5 cm long, pinnatifid or bipinnatifid into linear or filiform segments. Heads sessile or subsessile, numerous; involucre campanulate, 6-8 mm high, biseriate, outer phyllaries linear, subherbaceous, two-thirds or as long as the wider more chartaceous inner, inner phyllaries united at base, with conspicuous elliptic glandular dots; ray-flowers few, ligules oval, inconspicuous, erect, up to 1.5 mm long; pappus scales divided to near base into 5-10 bristles, about 3 mm long. Cypselas subangled, compressed, about 3 mm long, pubescent. $2n = 26$ (Smith, 1964).

Dyssodia papposa occurs from Ohio to Montana, south to Arizona and Louisiana and is adventive north and east of this range. It occurs infrequently along roadsides and in fields throughout Illinois (Fig. 1). It flowers from September to October.

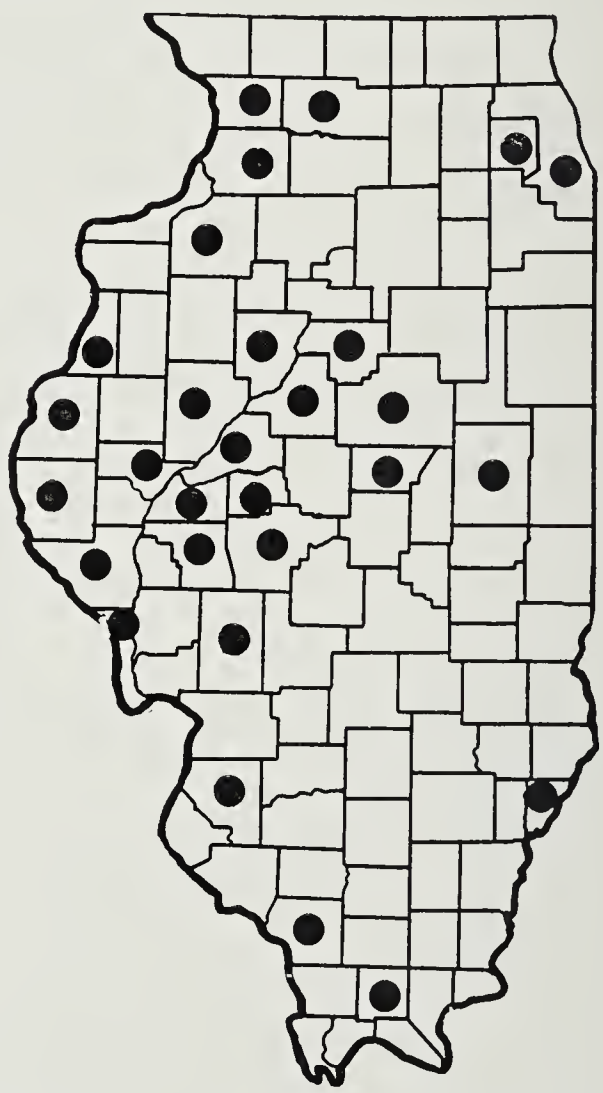

FIGURE 1. Distribution of *Dyssodia papposa* in Illinois.

2. HELENIUM L., Sp. Pl. 886. 1753.
Helenia L., Gen. Pl. ed. 5. 377. 1754.
Brassavola Adans., Fam. 2:127. 1763.
Actinea Juss., Ann. Mus. Paris 2:425. 1803.
Mesodetra Raf., Fl. Ludov. 141. 1817.
Leptopoda Nutt., Gen. N. Amer. Pl. 2:174. 1818.
Leptophora Raf., Am. Mo. Mag. Crit. Rev. 4:195. 1819.
Tetrodus Cass., Dict. Sci. Nat. 55:264. 1828.
Dugaldia Cass., Dict. Sci. Nat. 55:270. 1828.

Hecubaea DC., Prodr. 5:665. 1836.
Amblyolepis DC., Prodr. 5:667. 1836.
Leptocarpha Raf. ex Endl. Gen 1383. 1841, pro syn.
Oxylepis Benth., Pl. Hartw. 87. 1841.
Expeletiopsis Schultz-Bip. ex Benth. & Hook., Gen. 2:414. 1873.
Heleniastrum (Vaillant) Kuntze, Rev. Gen. 341. 1891.

Annual or perennial herbs; leaves alternate, impressed-punctuate, usually decurrent. Heads corymbiform or solitary, radiate, rarely discoid: involucre in 2-3 series, subequal or the inner shorter, narrow, herbaceous or subherbaceous, soon deflexed, outer sometimes joined at base; receptacle convex to ovoid or conic, naked; ray-flowers pistillate or sterile, yellow or occasionally purplish at base, ligule cuneate, 3- to 4-lobate, few; disc-flowers perfect, yellow or purple, numerous; anthers minutely auriculate or sagittate; style-branches flattened, tips dilated, subtruncate, penicillate; pappus scarious, hyaline, often awn-tipped scales. Cypselas 4- to 5-angled, with as many intermediate ribs, pubescent or glabrous.

The genus *Helenium* consists of about 40 species native to the western hemisphere with three species occurring in Illinois.

The pappus characters given for the species are variable. These often unusual variations are not considered to be of taxonomic importance.

Key to the Illinois Species of *Helenium*

1. Leaves linear to linear-filiform, not decurrent.....1. *H. amarum*
1. Leaves linear-lanceolate to ovate, decurrent.
   2. Disc depressed-globose; disc-flowers yellow... .....2. *H. autumnale*
   2. Disc globose; disc-flowers purplish...3. *H. flexuosum*

1. HELENIUM AMARUM (Raf.) Rock, Rhodora 59:131. 1957.
Galardia amarum Raf., Fl. Ludov. 69. 1817.
Helenium tenuifolium Nutt., Jour. Acad. Phila. 7:66. 1834.
Heleniastrum tenuifolium (Nutt.) Kuntze, Rev. Gen. 342. 1891.

Erect annuals; stems up to 5 cm tall, glabrous or nearly so; leaves linear to linear-filiform, entire, 1-8 cm long, 1-2 mm wide, not decurrent, glabrous. Heads corymbose; phyllaries linear, punctate, herbaceous, soon deflexed; disc depressed-globose, 0.5-1.0 cm in diameter; ray-flowers 5-10, pistillate, yellow, ligule 0.5-1.0 cm long, 3-logate; disc-flowers yellow; pappus ovate to obovate, hyaline, with awn as long as body. Cypselas about 1 mm long, hispid on angles. $2n = 30$ (Turner & Ellison, 1960; Jackson, 1962).

Helenium amarum occurs from Virginia south to Florida, west to Texas, and north to Kansas and Missouri. It is occasionally introduced or adventive elsewhere. It is frequent in fields and waste ground in southern Illinois, extending north to Pike and Champaign counties (Fig. 2). It flowers from August to October.

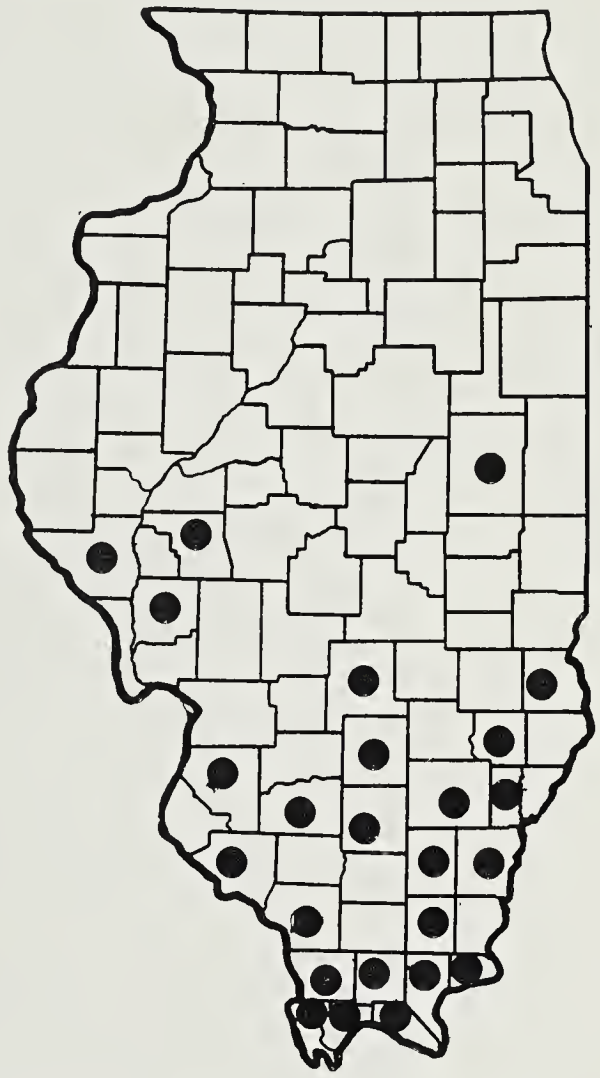

FIGURE 2. Distribution of *Helenium amarum* in Illinois.

2. HELENIUM AUTUMNALE L., Sp. Pl. 886. 1753, non Gray, 1857.
Helenium latifolium Mill., Gard. Dict. ed. 8. *Helenium* no. 2. 1768.
Helenia autumnalis (L.) Hill, Hort. Kew. 6. 1769.
Helenium pubescens Ait., Hort. Kew. 3:227. 1789, non H. & A., 1838.
Helenium canaliculatum Lam., Jour. Hist. Nat. 2:213. 1792.
Helenia decurrens Moench, Meth. 598. 1794.
Helenium longifolium Smith, in Rees, Cycl. 17: *Helenium* no. 2. 1811.
Helenium pumilum Willd., Enum. Suppl. 60. 1813.
Helenium altissimum Link, Ind. Sem. Berol. 1840:21. 1840. nom. nud.

Helenium commutatum Link, Ind. Sem. Berol. 1840:21. 1840, nom. nud.
Helenium parviflorum Nutt., Trans. Amer. Phil. Soc. II. 7:384. 1841.
Helenium autumnale L. var. *canaliculatum* (Lam.) T.&G., Fl. N. Amer. 2:284. 1843.
Heleniastrum autumnale (L.) Kuntze, Rev. Gen. 342. 1891.
Heleniastrum parviflorum (Nutt.) Kuntze, Rev. Gen. 342. 1891.
Helenium autumnale L. var. *pubescens* (Ait.) Britton, Mem. Torr. Club 5:339. 1894.
Helenium altissimum Link ex Rydb., N. Amer. Fl. 34:126. 1915.
Helenium huronense Britton ex Rydb., N. Amer. Fl. 34:127. 1915, nom. nud.
Helenium autumnale L. var. *parviflorum* (Nutt.) Fern., Rhodora 45:492. 1943.

Erect perennials; stems up to 1.5 m tall, glabrous or finely strigose or puberulent; leaves linear-lanceolate to elliptical, acute, narrowed to sessile or subsessile base, decurrent along stem, 4-16 cm long, 0.5-5.5 cm wide, serrate to subentire, glabrous or occasionally puberulent. Heads corymbose; phyllaries lanceolate-subulate, strigose or puberulent, soon deflexed; disc depressed-globose, 1-2 cm in diameter; ray-flowers 10-20, pistillate or occassionally neutral, yellow, ligule 0.5-1.5 cm long, 3- to 4-lobate; disc-flowers yellow; pappus ovate to lanceolate, with awn up to 1 mm long. Cypselas about 1.5 mm long, hispid on ribs. $2n = 34$ (Janaki-Ammal, 1945).

Helenium autumnale occurs from Quebec, south to Florida, west to Arizona, and north to British Columbia. It is common in wet meadows and along ditches, streams, and ponds throughout Illinois (Fig. 3). It flowers from August to October.

A number of varieties are recognized by various authors. These are believed by the author to be variations within a polymorphic species and merit no taxonomic segregation.

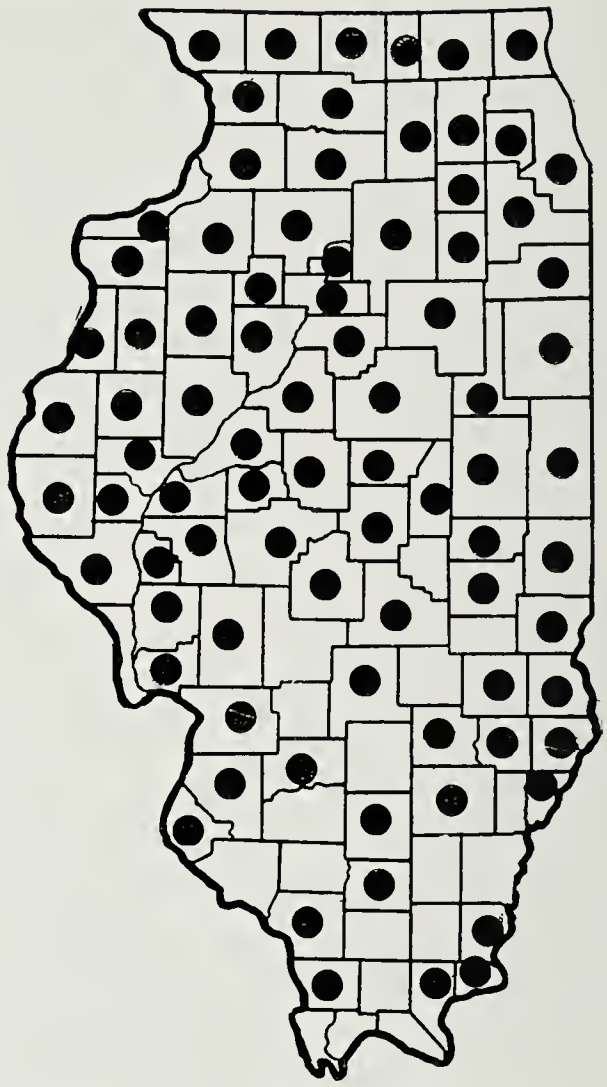

FIGURE 3. Distribution of *Helenium autumnale* in Illinois.

3. HELENIUM FLEXUOSUM Raf., New Fl. N. Amer. 81. 1838.
Helenium quadridentatum Hook., Comp. Bot. Mag. 1:98. 1835, non Labill, 1792.
Helenium dichotomum Raf., New Fl. N. Amer. 81. 1838.
Helenium nudiflorum Nutt., Trans. Amer. Phil. Soc. II. 7:384. 1841.
Helenium micranthum Nutt., Trans. Amer. Phil. Soc. II. 7:385. 1841.
Leptopoda brachypoda T.& G., Fl. N. Amer. 2:388. 1842.
Heleninum purpureum Hale ex T.& G., Fl. N. Amer. 2:388. 1842. pro syn.
Leptopoda brachypoda T.& G., var. B T.& G., Fl. N. Amer. 2:388. 1842.
Helenium atropurpureum Kth. & Bouche, Ind. Sem. Hort. Berol. Anno 1845, Collectorum 12. 1845.
Helenium atropurpureum Kth. & Bouche var. *grandicephalum* Lamaire, Ill. Hort. 10:375. 1863.
Helenium brachypoda (T.& G.) A. Wood, Am. Bot. Fl. 182. 1870.
Helenium seminariense Featherman, La. Univ. Rep. 1870:74. 1871.
Helenium nudiflorum Nutt. var. *purpurea* (Hale ex T.& G.) Gray, Proc. Amer. Acad. Arts 9:203. 1871.
Heleniastrum nudflorum (Nutt.) Kuntze, Rev. Gen. 342. 1891.
Helenium polyphyllum Small, Fl. S. E. U.S. 1291. 1903.
Helenium floridanum Fern., Rhodora 45: 494. 1943.
Helenium godfreyi Fern., Rhodora 45:494. 1943.

Erect perennials; stem up to 1 m tall, subpuberulent; leaves oblong to linear-lanceolate, entire or subentire, sessile, decurrent along stem, 3-12 cm long, 0.5-2.0 cm wide. Heads corymbose; phyllaries lanceolate to linear-lanceolate, puberulent, soon deflexed; disc globose, 6-14 mm in diameter; ray-flowers 10-20, neutral, yellow or sometimes purplish; pappus

ovate to lanceolate, with awn up to 0.5 mm long. Cypselas about 1 mm long, hispid. $2n = 28$ (Turner, 1959; Jackson, 1962).

Helenium flexuosum occurs from New England, south to Florida, west to Texas, and north to Michigan. It is local along roadsides, in meadows, and in pastures in Illinois but is more common in the southern one-half of the state (Fig. 4). It flowers from June to September.

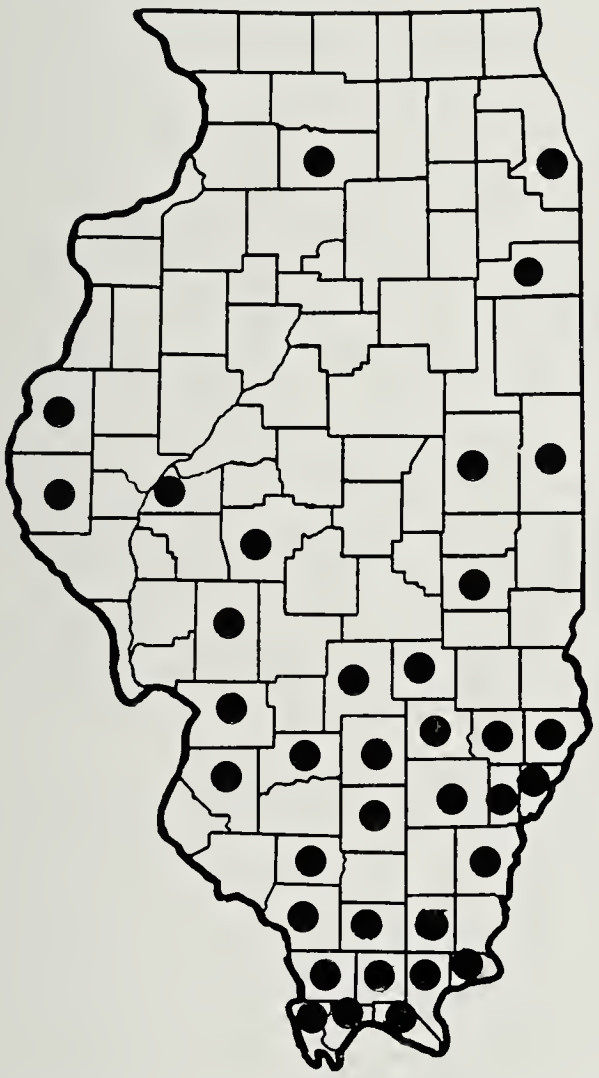

FIGURE 4. Distribution of *Helenium flexuosum* in Illinois.

3. GAILLARDIA Foug., Mem. Acad. Sci. Paris 1786:5. 1788.
Gaillarda Foug., Obs. Phys. 29:55. 1786. sine sp.
Galardia Lam., Encyc. 2:590. 1788.
Calonnea Buchoz ex Lam., Encyc. 2:590. pro syn.
Virgilia L'Her., Virgilia 1788.
Polatherus Raf., Amer. Mo. Mag. 2:268. 1818.
Guentheria Spreng., Syst. 3:356. 1826.
Cerostylis Less., Syn. Comp. 239. 1832.
Agassizia Gray & Engelm. ex Gray, Proc. Amer. Acad. Arts 1:49. 1847, non Chav., 1830.

Annual, biennial, or perennial herbs, rarely suffruticose at base. Leaves alternate or basal, entire to pinnatifid. Heads radiate or discoid; phyllaries in 2-3 series, ovate to lanceolate, at least upper reflexed in fruit; receptacle convex to subglobose, alveolate, usually fimbrillate, fimbrillae soft and short conic to stiff and spine-like; ray-flowers usually neutral, often wanting; ligules, if present, broad, cuneate or flabelliform, deeply 3-lobed, yellow and/or purple; disc-flowers bisexual and fertile, 5-lobate; anthers auriculate at base; style-branches with glabrous and short to hispidulous and filiform appendages; pappus of about 6- to 10-awned scales. Cypselas broadly obpyramidal, wholly or partly covered by long stiff, ascending hairs.

The genus *Gaillardia* consists of about 12 species native to western North America. The genus is represented in Illinois by *G. pulchella* Foug., an escape from cultivation. *Gaillardia aestivalis* (Walt.) Rock (=*G. lutea* Greene; *G. lanceolata* Michx.) has been attributed to Illinois by Biddulph (1944) on the basis of a single specimen collected by Otto Kuntze supposedly near Cairo, Alexander County. The Kuntze specimen deposited in the herbarium of the New York Botanical Garden has the following on its label: "*G. lanceolata* 2868, 9/9/74" (in one handwriting) "2868, Cairo U. St." (in a second handwriting). Kuntze (Rev. Gen. 339. 1891) states the following: "*Gaillardia lanceolata* Michx. U. St.: Cairo, Miss." Thus there is no mention of Illinois either on the label of Kuntze's specimen or in his publication although Biddulph specifically cites it from Cairo, Alexander County, Illinois. If the species was once in Illinois it has not been collected since 1874 although on the other hand, it may never have been collected in Illinois at all but at some other location. In view of the uncertain occurrence of *G. aestivalis* in Illinois, the author has chosen not to include it in this treatment.

1. GAILLARDIA PULCHELLA Foug., Mem. Acad. Sci. Paris 1786:5. 1788.
Gaillardia bicolor Lam., Encyc. 2:590. 1788, pro syn.
Virgilia heliodes L'Her., Virgilia. 1788.
Gaillardia lobata Buckl., Prod. Acad. Phila. 1861:459. 1862.

Branched annual herbs; stems 2-6 dm tall; striate, short-hirsute with ascending hairs; lower leaves oblanceolate, 4-8 cm long, 0.5-2.0 cm wide, bluntly toothed or lobed, short-petiolate, upper leaves oblong-lanceolate, 2-6 cm long, 0.5-1.5 cm wide, acute, base sessile, often somewhat clasping, densely hispid beneath, sparsely long pubescent above. Heads terminal, 3-6 cm wide; peduncles 5-15 cm long; phyllaries lanceolate, long-acuminate, herbaceous with chartaceous bases, hirsute

and ciliate above; receptacle fimbrillae subulate, stiff, longer than achenes; ray-flowers neutral, ligules 1-2 cm long, 3-lobate, yellow with purple base or wholly purple; disc-flowers yellow below, purple above; pappus of lanceolate scales 5-6 mm long, gradually tapering into an awn equaling body. Cypselas 2.0-2.5 mm long, densely hirsute. $2n = 34$ (Biddulph, 1944, Stoutamire, 1955, 1958, 1960); $2n = 36$ (Biddulph, 1944).

Gaillardia pulchella occurs naturally in dry sandy prairies and openings from Colorado and New Mexico, east to Minnesota, Nebraska, Missouri, and Louisiana and as an escape along roadsides and in waste ground east to the Atlantic states. It occurs as an escape locally throughout Illinois (Fig. 5). It flowers from June to July.

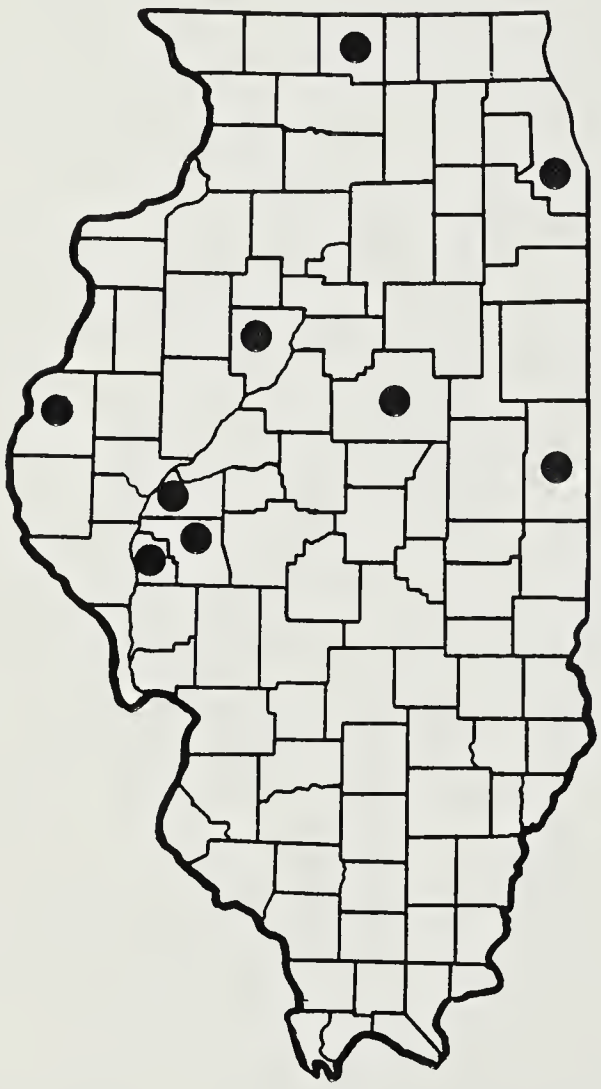

FIGURE 5. Distribution of *Gaillardia pulchella* in Illinois.

4. HYMENOXYS Cass., Dict. Sci. Nat. 55:278. 1828.

Actinella Nutt., Gen. 2:113. 1818, in part, non Pers., 1807.
Picradenia Hook., Fl. Bor.-Am. 1:317. 1833.
Phileozera Buckl., Proc. Acad. Phila. 1861:459. 1862.
Tetraneuris Greene, Pittona 3:265. 1898.
Rydbergia Greene, Pittonia 3:270. 1898.
Macdougalia A. Heller, Bull. Torrey Club 25:629. 1898.
Plateilema (Gray) Cockerell, Bull. Torrey Club 31:462. 1904.

Aromatic annual or perennial herbs; leaves alternate or all basal (ours), entire or occasionally pinnately lobed. Heads solitary or few, radiate; involucres 2- to 3-seriate, subequal or slightly imbricate, appressed, herbaceous but often scarious margined; receptacle hemispherical or conic, naked; disc-flowers perfect, fertile; ray-flowers 10-20 pistillate, yellow; anthers entire or sagittate at base; style branches flattened, truncate, penicillate; pappus of 5-12 hyaline, often aristate scales. Cypselas turbinate, mostly 5-angled, villous or sericeous.

The genus *Hymenoxys* consists of about 15 species native to the western hemisphere and is represented in Illinois by the following single taxon.

1. HYMENOXYS ACAULIS (Pursh) Parker var. GLABRA (Gray) Parker, Madroño 10:159. 1950.

Actinella scaposa (DC.) Nutt. var. *glabra* Gray, Man. Bot. ed. 5. 263. 1867.
Actinella acaulis (Pursh) Nutt. var. *glabra* (Gray) Gray, Syn. Fl. 2:345. 1884.
Tetraneuris herbacea Greene, Pittonia 3: 268. 1898.
Actinea herbacea (Greene) B. L. Robins., Rhodora 10:68. 1908.
Actinea acaulis (Pursh) Spreng. var. *glabra* (Gray) Cronquist, Rhodora 47:403. 1945.

Perennial scapose herbs; stems 0.5-2.5 cm tall; leaves narrowly to broadly oblanceolate, 1-8 cm long, 1.5-10.0 mm wide, villous when young, soon glabrate, strongly punctate. Head solitary, 3.5-4.0 cm wide; involucre pubescent to subglabrate, 7-8 mm high; phyllaries broadly rounded; ligules 5-20 mm long, yellow; pappus ovate, acute or obtuse, about 2 mm long. Cypselas turbinate, about 3 mm long. Chromosome number unknown.

Hymenoxys acaulis var. *glabra* is very rare and occurs in dry, gravelly banks, stony fields, limestone hills, sandy fields and prairies, only in Mason and Will Counties, Illinois (Fig. 6), Ottawa County, Ohio, and southern Ontario, Canada. It flowers from May to July.

5. HYMENOPAPPUS L'Her., Hymenop. 1. 1788.

Rothia Lam., Jour. Nat. Hist. Paris 1:16. 1792, non Schreber, 1791, nec Borkhausen, 1792; nec Pers., 1807.

Biennial or perennial scapose to leafy-stemmed herbs; leaves alternate, pinnatifid or bipinnatifid to rarely simple, reduced upwards. Heads several to numer-

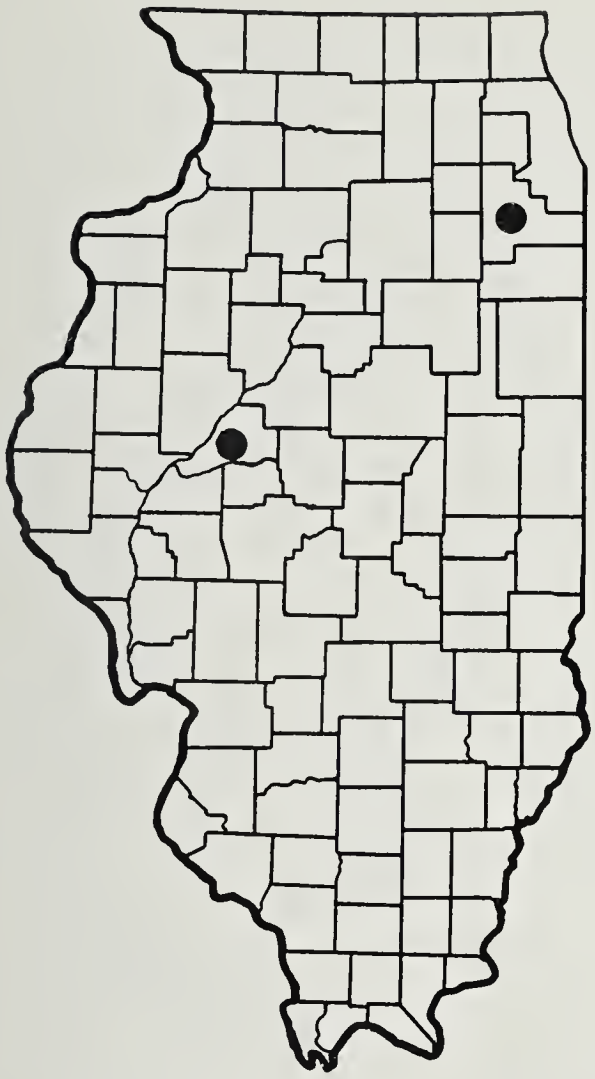

FIGURE 6. Distribution of *Hymenoxys acaulis* var. *glabra* in Illinois.

ous in a corymbiform panicle, radiate or discoid; involucre 2- to 3-seriate, inner usually with broad, scarious, petaloid, yellowish or whitish tips, outer herbaceous; receptacle convex or nearly flat, naked or rarely with chaff; ray-flowers, when present, pistillate, fertile, ligules white; disc-flowers perfect, lobes reflexed, yellow or whitish (rarely purple); anthers cordate-sagittate at base; style branches flattened, with obtuse, papillose appendages; pappus of 12-22 linear to ovate, obtuse, membranous or hyaline scales (rarely wanting). Cypselas turbinate, 4-angles, often striate.

The genus *Hymenopappus* consists of about 15 species native to North America and is represented in Illinois by the following single species.

1. HYMENOPAPPUS SCABIOSAEUS L'Her., Hymenop. 1. 1788.
Rothia carolinensis Lam., Jour. Hist. Nat. Paris 1:17. 1792.
Hymenopappus laxiflorus L'Her., DC. Prodr. 5:658. 1836, pro syn.
Hymenopappus carolinensis (Lam.) Porter, Mem. Torrey Club 5:338. 1894.

Biennial herbs; stems 3-15 dm tall, floccose-tomentose, becoming glabrate below, villous above; leaves pinnatifid or bipinnatifid, subpersistently floccose-tomentose below, glabrate above, lower 8-25 cm long, 3-12 cm wide, reduced upwards. Heads several to numerous in open corymbiform inflorescences, 7-12 mm wide; involucre, 7-15 mm high; phyllaries broad, scarious, petaloid white or yellowish; disc-flowers 5-lobed, lobes reflexed, half as long as tube or longer, tube stipitate-glandular, white or yellowish; pappus of 14-18 hyaline obovate scales, up to 1 mm long. Cypselas turbinate, 3.5-5.0 mm long, 4-angled, striate, hirsute principally on angles. $2n = 34$ (Raven & Kyhos, 1961).

Hymenopappus scabiosaeus occurs from Florida to Texas, north to South Carolina, Indiana, Illinois, and Kansas. It is rare in Illinois, known only from Cass, Iroquois, Kankakee, and Mason counties (Fig. 7) where it occurs in open sandy woods and prairies. It flowers from May to June.

6. POLYMNIA L., Sp. Pl. 2:926. 1753.
Alymnia Neck., Elem. Bot. 1:31. 1790.
Polyniastrum Lam., Tabl. Enc. t. 712. 1797.
Smallanthus Mackenz., in Small, Man. S.E. Fl. 1406. 1933.

Erect perennial herbs (ours); stems to 3 m tall; leaves opposite, pinnately or palmately veined, sessile or petiolate. Heads panicled corymbs, radiate, involu-

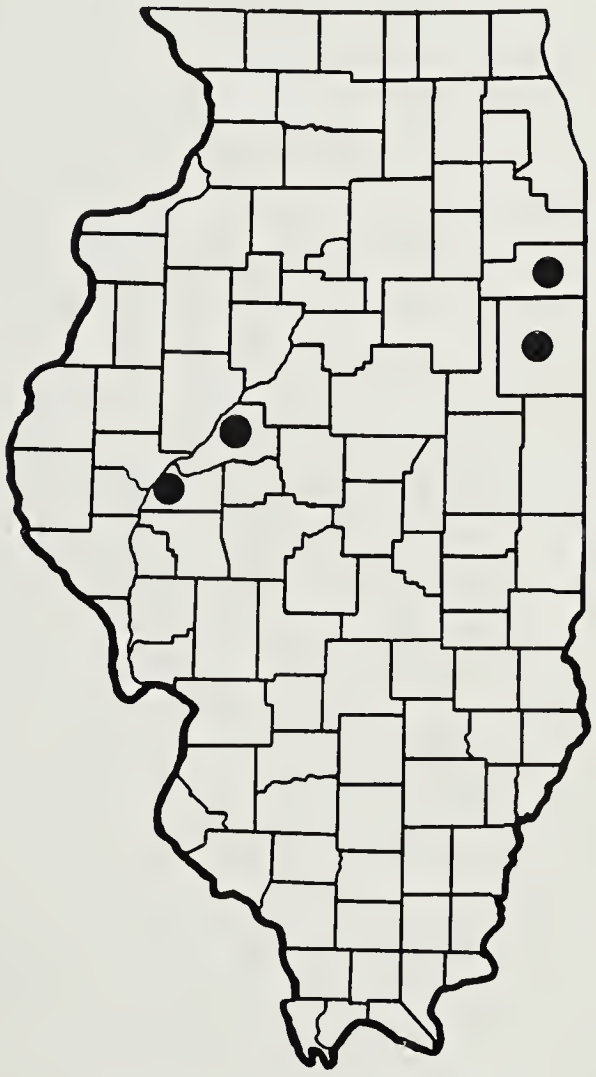

FIGURE 7. Distribution of *Hymenopappus scabiosaeus* in Illinois.

cre subfoliaceous, receptacle flat to convex; ray-flowers pistillate, ligule 2- 3-lobate to entire, sometimes wanting, white or yellow; disc-flowers staminate, yellow; pappus wanting. Cypselas obovoid or spherical, slightly flattened laterally or 3- to 5-angled.

The genus *Polymnia* consists of approximately 20 species native to the western hemisphere with two species occurring in Illinois.

Key to the Illinois Species of *Polymnia*

1. Leaves pinnately lobed; cypselas 3-angled, not striate.....1. *P. canadensis*
1. Leaves palmantely lobed; cypselas slightly flattened laterally, striate.....2. *P. uvedalia*

1. POLYMNIA CANADENSIS L., Sp. Pl. 2:926. 1753.

Polymnia variabilis Poir, Enc. Meth. 5:505. 1804.

Polymnia canadensis L. var. *discoidea* Gray, Gray's Lessons in Bot. & Veg. Physio. 248. 1881.

Polymnia canadensis L. var. *radiata* Gray, Syn. Fl. N. Am. 1:238. 1884.

Polymnia radiata (Gray) Small, Fl. S.E. U.S. 1340. 1903.

Osteospermum canadense (L.) House, Bull. N.Y. State Mus. 243:63. 1923.

Polymnia canadensis L. f. *radiata* (Gray) Fassett, Rhodora 34:96. 1932.

Erect perennial herbs; stems 0.5-1.5 m tall, glandular-pubescent; lower leaves deeply pinnately lobed, up to 4 cm long, 3 cm wide, petiolate, upper triangular-ovate, entire to 3- to 5-lobed, smaller, petiolate. Heads in panicled corymbs, phyllaries 4-6, glandular-pubescent, subovate; ray-flowers 4-6, white or pale yellow, wanting or up to 1.5 cm long, 3-lobate, paleae ovate to ovate-lanceolate; disc-flowers white or pale yellow, paleae elliptic to oblanceolate, nearly equaling disc-flowers. Cypselas asymmetrically obovoid, 3-angled, not striate, 3-4 mm long, about 2-3 mm wide, dark brown to black. $2n = 30$ (Wells, 1965).

Polymnia canadensis occurs from New England, Ontario, and Minnesota, south to Oklahoma, Louisiana, and Georgia. It is common in moist woods throughout Illinois (Fig. 8). It flowers from June to November.

Infraspecific categories based on ligule length of the ray-flowers have been named. The author, like Wells (1965), has chosen to treat these as variants of the species.

2. POLYMNIA UVEDALIA (L.) L., Sp. Pl. ed. 2. 2:1303. 1764.

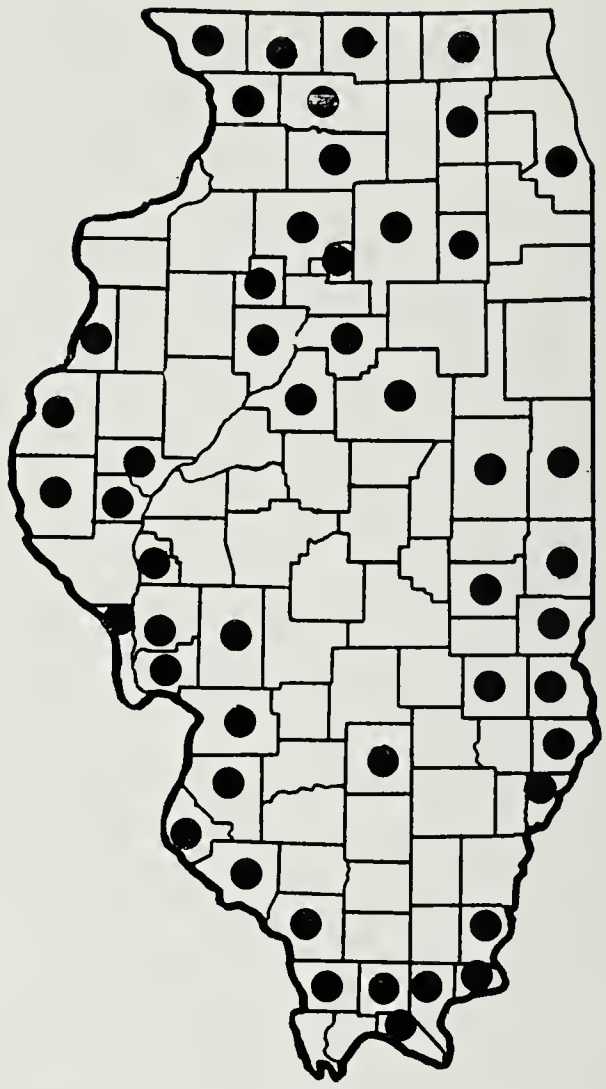

FIGURE 8. Distribution of *Polymnia canadensis* in Illinois.

Osteospermum uvedalia L., Sp. Pl. 2:923. 1753.

Polymnia macrophylla Raf., Fl. Ludov. 70. 1817.

Polymniastrum uvedalia (L.) Small, in Small and Carter, Fl. Landcaster Co. 302. 1913.

Polymnia uvedalia (L.) L. var. *genuina* Blake, Rhodora 19:47. 1917.

Polymnia uvedalia (L.) L. var. *densipilis* Blake, Rhodora 19:48. 1917.

Polymnia uvedalia (L.) L. var. *floridana* Blake, Rhodora 19:48. 1917.

Smallanthus uvedalia (L.) Mack., in Small, Man. S.E. Fl. 1406. 1933.

Erect perennial herbs; stems up to 3 m tall, glabrous to densely glandular-pubescent; lower leaves deeply palmately 3- to 5-lobed, to 7 cm long, 4 cm wide, sessile or with winged petioles, upper leaves ovate, entire or toothed, sessile. Heads in panicled-corymbs, phyllaries 4-6, 20 mm long, ovate to ovate-lanceolate; ray-flowers 7-13, yellow, ligule about 3 cm long, paleae ovate, acuminate; disc-flowers yellow, paleae lanceolate. Cypselas asymmetrically obovoid, laterally compressed, striate, 5-6 mm long, 3-4 mm wide. $2n = 32$ (Wells, 1965).

Polymnia uvedalis occurs from New

England west to Missouri, south to Texas and Florida. It has recently been introduced into Bermuda (probably between 1883 and 1904 or 1905, cf. Wells, 1965). It is found in rich woods and is uncommon in southern Illinois (Fig. 9). It flowers from July to September.

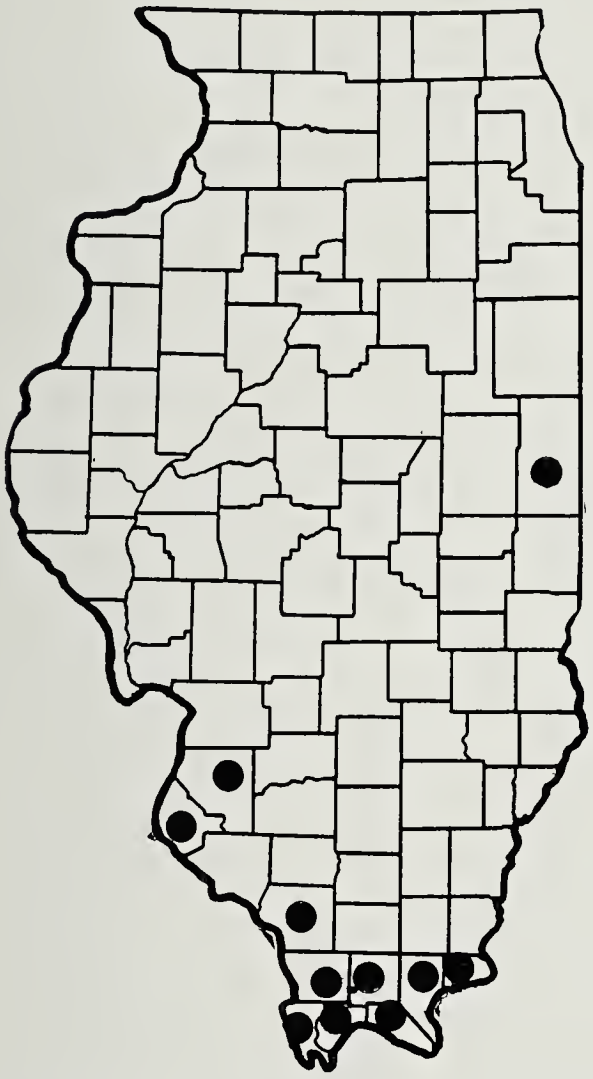

FIGURE 9. Distribution of *Polymnia uvedalia* in Illinois.

The three varieties proposed by Blake (1917) and recognized by some authors are differentiated primarily on peduncle vestiture as well as geographical distribution. Because of the broad overlap in their ranges and the lack of clear-cut distinctions among them, the author does not treat these variations as varietal entities of the species. This treatment of the species is also now advocated by Wells (pers. comm.).

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WELLS, J. R. 1965. A taxonomic study of *Polymnia* (Compositae). Brittonia 17: 144-150.

WUNDERLIN, R. P. 1968. Contributions to an Illinois Flora no. 2. Compositae I (Tribe Vernonieae). Trans. Ill. Acad. Sci. 61:132-138.

Manuscript received April 30, 1971.

CONTRIBUTIONS TO AN ILLINOIS FLORA No. 5. COMPOSITAE III. (TRIBE HELIANTHEAE, PART II—THE GENUS COREOPSIS)

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ABSTRACT.—Seven species of Coreopsis are reported for Illinois. Keys, descriptions, and distributional maps are provided for each species.

This is the second part of a treatment of the tribe Heliantheae in Illinois. The first part, consisting of a key to the 23 Illinois genera and a treatment of the genera *Dyssodia, Helenium, Gaillardia, Hymenoxys, Hymenopappus,* and *Polymnia,* has been published previously (Wunderlin, 1971). Treatments of the other genera will follow in subsequent publications.

Coreopsis consists of about 100 species native to North and South America, Hawaii, and Africa. Seven species occur in Illinois, three of which are adventive or escaped from cultivation.

Taxonomically the genus approaches *Bidens* on one hand and *Cosmos* on the other, but it is generally well demarcated as a genus in having wing-margined cypselas (rarely absent) and a pappus of two short teeth or awns, a short crown, or absent.

Many members of the genus are widely cultivated as ornamentals in temperate regions, several of which have escaped and have become naturalized.

The synonymy in this work essentially follows that of Sherff (1955).

The distributional maps are based upon specimens housed in the following herbaria: Eastern Illinois University, the Field Museum of Natural History, the Illinois Natural History Survey, the Illinois State Museum, the Missouri Botanical Garden, Southern Illinois University, the University of Illinois, and Western Illinois University. The author is grateful to the curators of these herbaria for allowing him to study their specimens of *Coreopsis*. The author also gratefully acknowledges Dr. Robert H. Mohlenbrock, Southern Illinois University, for his critical reading of the manuscript.

SYSTEMATIC TREATMENT

11. COREOPSIS L., Sp. Pl. 907. 1753.
Acispermum Neck., Elem. 1:34. 1790.
Coreopsoides Moench, Meth. 594. 1794.
Anacis Schrank, Denkschr. Akad. Munchen 5 (Math. Naturw.):5. 1817.
Leachis Cass., Dict. Sci. Nat. 25:388. 1822.
Chrysomelea Tausch, Hort. Canal. (15). 1823.
Diplosastera Tausch, Hort. Canal. (15). 1823.
Calliopsis Reichenb., Ic. Pl. Cult. *pl. 70.* 1823.
Chrysostemma Less., Syn. Gen. Comp. 227. 1823.
Electra DC., Prodr. 5:63. 1832.
Leptosyne DC., Prodr. 5:331. 1836.
Agaristra DC., Prodr. 5:569. 1836.
Epilepis Benth., Pl. Hartw. 17. 1839.
Tuckermannia Nutt., Trans. Amer. Phil. Soc. II. 7:363. 1841.
Pugiopappus A. Gray, in Torr., Pacif. Railr. Rep. 4:104. 1857.

Annual or perennial herbs; leaves opposite or rarely alternate, simple, entire to deeply pinnately or palmately lobed or divided. Heads solitary or loosely corymbose-paniculate, radiate; involucre double, outer foliaceous, usually spreading, inner wider, submembranous, appressed; receptacle flat; palea membranous, striate, deciduous with fruit; ray-flowers usually neutral, ligule yellow, variegated, or rarely purple; disc-flowers bisexual, fertile, regular, 5-dentate, throat with annulus; anthers entire or subauriculate; style-branches truncate, conic, or caudate; pappus 2-toothed, 2-awned, or absent. Cypselas (cf. Wunderlin, 1971) flat, obcompressed, often winged.

Key to the Illinois Species of *Coreopsis*

1. Leaves undivided or rarely with 1 or 2 short lateral lobes.
   2. Leaves mostly basal, linear to oblanceolate.....1. *C. lanceolata*
   2. Leaves produced to middle of stem or higher, ovate to elliptic-lanceolate.....2. *C. pubescens*
1. Leaves 3- to 5-lobed or divided.
   3. Leaves sessile, deeply 3-lobed to or below middle.....3. *C. palmata*
   3. Leaves usually petiolate, divided into 3-5 segments.
      4. Ligules of ray-flowers reddish-brown at base or throughout; disc-flowers reddish-brown.
         5. Cypselas linear-oblong, wingless; leaf segments linear to linear-lanceolate..4. *C. tinctoria*
         5. Cypselas obovate, cartilaginous-margined; leaf segments lanceolate to elliptic-oblong to orbicular..5. *C. basalis*
      4. Ligules of ray-flowers yellow; disc-flowers yellow or reddish-brown.
         6. Leaf segments elliptic-lanceolate; cypselas 5-7 mm long; disc-flowers yellow or reddish-brown.....6. *C. tripteris*
         6. Leaf segments linear to linear-lanceolate; cypselas 1-4 mm long; disc-flowers yellow .....7. *C. grandiflora*

1. COREOPSIS LANCEOLATA L., Sp. Pl. 908. 1753.

Coreopsis crassifolia Ait., Hort. Kew. 3:253. 1787, non Sesse & Moc., 1894.
Coreopsoides lanceolata (L.) Moench, Meth. 594. 1794.
Coreopsis lanceolata L. var. *glabella* Michx., Fl. Bor.-Amer. 2:137. 1803.
Coreopsis lanceolata L. var. *villosa* Michx., Fl. Bor.-Amer. 2:137. 1803.
Leachia lanceolata (L.) Cass., Dict. Sci. Nat. 25:388. 1822.
Chrysomelea laneolata (L.) Tausch, Hort. Canal. (15). 1823.
Coreopsis oblongifolia Nutt., Jour. Acad. Phila. 7:76. 1834.
Coreopsis lanceolata L. var. *succisaefolia* DC., Prodr. 5:570. 1836.
Coreopsis lanceolata L. var. *crassifolia* (Ait.) Heynh., Nom. 1:219. 1840.
Coreopsis lanceolata L. var. *angustifolia* T. & G., Fl. N. Amer. 2:344. 1842.
Coreopsis heterogyna Fern., Rhodora 40: 475. 1938.

Erect or ascending perennial herbs; stems 2-8 dm tall, scapiform, subangular, glabrous or pubescent; leaves opposite, simple or rarely with 1-2 small lateral lobes, linear to oblanceolate, 5-10 cm long, 0.5-2.0 cm wide, lower long-petiolate, upper sessile, pubescent or with ciliolate bases only. Heads usually solitary, radiate, 3-6 cm wide; peduncles 2-4 cm long; outer phyllaries lanceolate to oblong-ovate, 4-8 mm long, inner ovate, 8-12 mm long; ray-flowers about 8, yellow, 1.3-3.0 cm long, ligule obovate or cuneate, 3-lobate; palea linear-oblong with filiform tip, 4-6 mm long; disc-flowers yellow; style branches caudate; pappus with 2 small fimbriolate teeth. Cypselas obcompressed, orbicular, 2.3-3.0 mm long, black calloused excrescences at ends, margin winged. $2n = 24, 48$ (Bilquez, 1955); $2n = 26$ (Turner, 1960).

Coreopsis lanceolata occurs naturally from Michigan and the Lake Superior region south to Florida and New Mexico and is escaped from cultivation and becoming naturalized northeast to New England. It occurs locally in dry sandy or rocky soils throughout Illinois (Fig. 1), where it flowers from June to July.

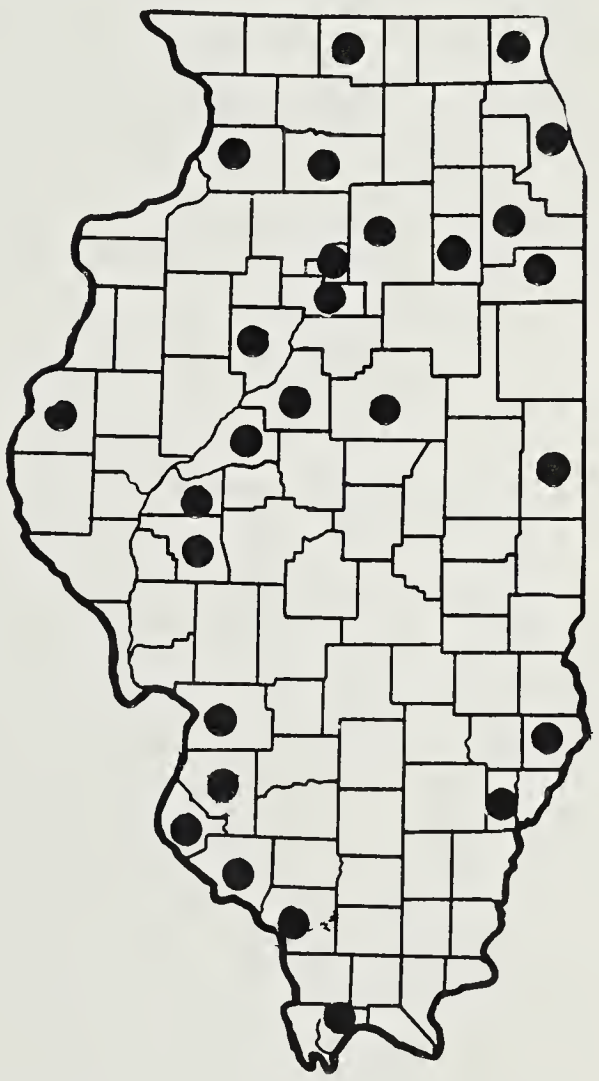

FIGURE 1. Distribution of *Coreopsis lanceolata* in Illinois.

Coreopsis lanceolata var. *villosa* has been segregated by some authors. In Illinois it does not appear to have any distinct geographical range and both varieties frequently grow together with intermediate forms present. It therefore is not recognized in this study.

2. COREOPSIS PUBESCENS Ell., Bot. S. C. & Ga. 2:441. 1823.
Coreopsis auriculata L. var. T. & G., Fl. N. Amer. 2:343. 1842.
Coreopsis auriculata L. var. T. & G., Fl. N. Amer. 2:344. 1842, excl. syn.
Coreopsis pubescens Ell. var. *typica* Sherff, Brittonia 6:341. 1948.

Erect perennial herbs; stems 5-13 dm tall, pubescent; leaves opposite, lower ovate to obovate, 2-5 cm long, 1-4 cm wide, petiolate, upper ovate to elliptic-lanceolate, entire or 3- to 5-parted, 5-10 cm long, 1-7 cm wide, sessile. Heads usually solitary, radiate, 3-5 cm wide; peduncles 1-2 cm long; outer phyllaries linear-lanceolate, 7-10 mm long, inner ovate, subequal; ray-flowers about 8, yellow, 1.0-2.3 cm long, ligule cuneate to oblong-cuneate, 3-lobate; palea elongate-filliform, 6-8 mm long; disc-flowers yellow; style-branches abruptly narrowed, linear-appendaged; pappus with 2 fimbriate teeth. Cypselas obcompressed, suborbicular, 2.8-3.0 mm long, black, glabrous or papillate, calloused excrescences at ends, margin winged. $2n = 28$ (Snoad, 1952; Turner, 1960).

Coreopsis pubescens occurs naturally from Virginia, south to Florida, west to Oklahoma, and north to Missouri and is adventive or escaped from cultivation elsewhere. It occurs locally in open woods, fields, and along roadsides in southern Illinois (Fig. 2). It flowers from June to September.

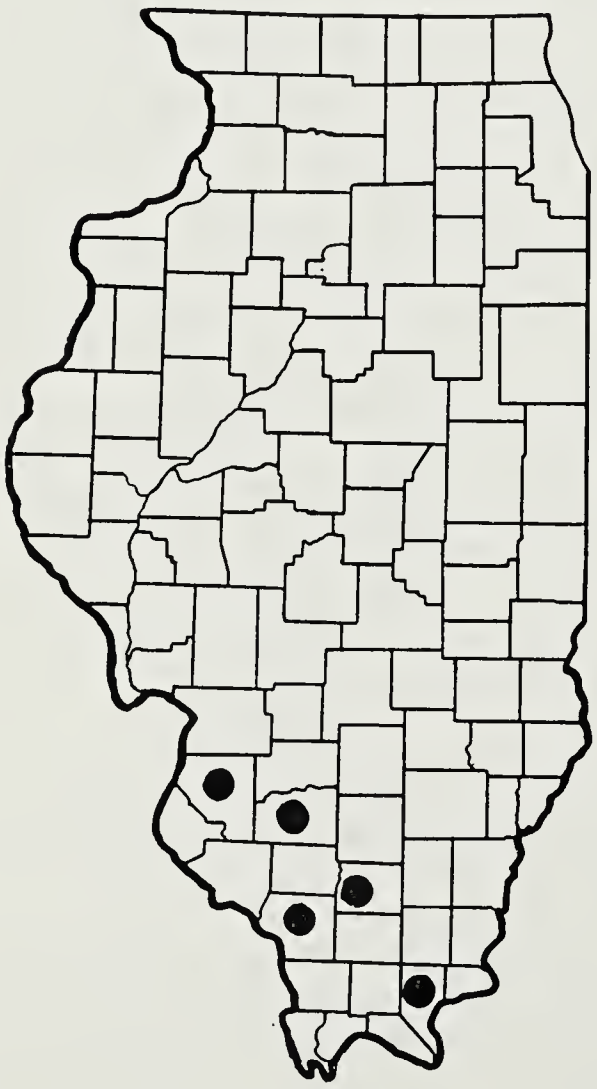

FIGURE 2. Distribution of *Coreopsis pubescens* in Illinois.

3. COREOPSIS PALMATA Nutt., Gen. 2:180. 1818.
Calliopsis palmata (Nutt.) Spreng., Syst. 3:611. 1826.
Coreopsis pauciflora Lehm. ex Schlecht., Linnaea 10 (Litt.-Ber.):76. 1836.
Coreopsis praecox Fresen. ex Schlecht., Linnaea 10 (Litt.-Ber.):76. 1836.

Erect perennial herbs; stems 5-9 dm tall, glabrous or hirsute at nodes; leaves opposite, principal palmately 3-parted into entire or irregularly lobed oblong or oblong-linear segments, 0.4-2.5 cm long, 3-6 mm wide, sessile. Heads 1-3 (-6), radiate, 2.5-6.0 cm wide; outer phyllaries linear-oblong, obtuse, margins scabrous-ciliolate; ray-flowers about 8, yellow, 1.5-2.7 cm long, ligule oblong-obovate, slightly dentate; palea filiform, 6-7 mm long; disc-flowers yellow; style-branches narrowly conic; pappus 2-toothed. Cypselas obcompressed, elliptic-oblong, 5.0-6.5 mm long, black, glabrous, narrowly winged. Chromosome number unknown.

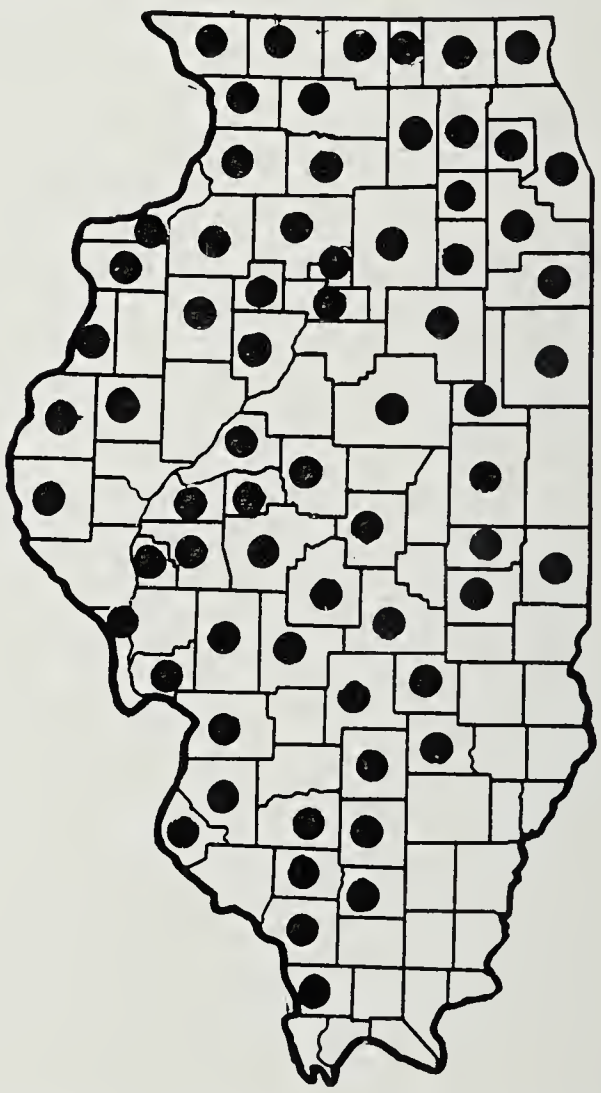

FIGURE 3. Distribution of *Coreopsis palmata* in Illinois.

Coreopsis palmata occurs from Wisconsin and Manitoba south to Indiana, Missouri, and Oklahoma. It occurs in prairies, open woods, and along roadsides nearly throughout Illinois (Fig. 3). It flowers from June to July.

4. COREOPSIS TINCTORIA Nutt., Jour. Acad. Phila. 2:114. 1821.
Calliopsis bicolor Reichenb., Ic. Pl. Cult. *pl. 70*. 1823.
Diplosastera tinctoria (Nutt.) Tausch, Hort. Canal. (16). 1823.
Calliopsis tinctoria (Nutt.) DC., Prodr. 5:568. 1836.
Calliopsis tinctoria (Nutt.) DC. var. *atropurpurea* Hook., Curtis's Bot. Mag. 63:*pl. 3511*. 1836.
Coreopsis bicolor Bosse ex Buch., Linnaea 25:630. 1853, nom. nud.
Coreopsis elegans Hort. ex Wieg., in Bailey, Stand. Cyc. Hort. ed. 2. 2:845. 1914, pro syn.
Coreopsis nigra Hort. ex Wieg., in Bailey, Stand. Cyc. Hort. ed. 2. 2:845. 1914, pro syn.
Coreopsis marmorata Hort. ex Wieg., in Bailey, Stand. Cyc. Hort. ed. 2. 2:845. 1914, pro syn.
Coreopsis tinctoria Nutt. var. *nana* Hort. ex Wieg., in Bailey, Stand. Cyc. Hort. ed. 2. 2:845. 1914.
Coreopsis radiata Hort. ex Wieg., in Bailey, Stand. Cyc. Hort. ed. 2. 2:846. 1914, non Mill., 1768.
Coreopsis tinctoria Nutt. f. *atropurpurea* (Hook.) Fern., Rhodora 44:477. 1942.
Coreopsis tinctoria Nutt. var. *atropurpurea* Hook. f. *tinctoria* Sherff, Brittonia 6: 341. 1948.
Coreopsis tinctoria Nutt. var. *atropurpurea* Hook. f. *atropurpurea* Fern., ex Sherff, Brittonia 6:341. 1948.

Erect annual herbs; stems 6-12 dm tall, glabrous, subquadrangulate; leaves opposite, subsessile, basal and lower pinnately divided, segments linear, 5-10 cm long, 1-2 mm wide, upper entire. Heads numerous, subcorymbose, radiate, 1.5-3.0 cm wide; peduncles slender, 4-10 cm long; outer phyllaries linear-oblong to triangular, about 2 mm long, edges scarious, inner ovate, 5-7 cm long; ray-flowers 7-8, yellow with reddish-brown bases, 0.7-1.5 cm long, ligule obovate, usually 3-lobate; palea subfiliform, 4.0-4.5 mm long; disc-flowers reddish-brown; style-branches obtuse; epappose. Cypselas obcompressed, linear-oblong, 1.5-4.0 mm long, black, papillose below, wingless. $2n = 24$ (Turner, 1960).

Coreopsis tinctoria occurs naturally in low ground from Minnesota, south to Louisiana, west to California, and north to Washington and is adventive or escaped from cultivation east to the Atlantic coast. It occurs locally along roadsides, railroads, and in waste ground in Illinois as an adventive or escape (Fig. 4). It flowers from July to September.

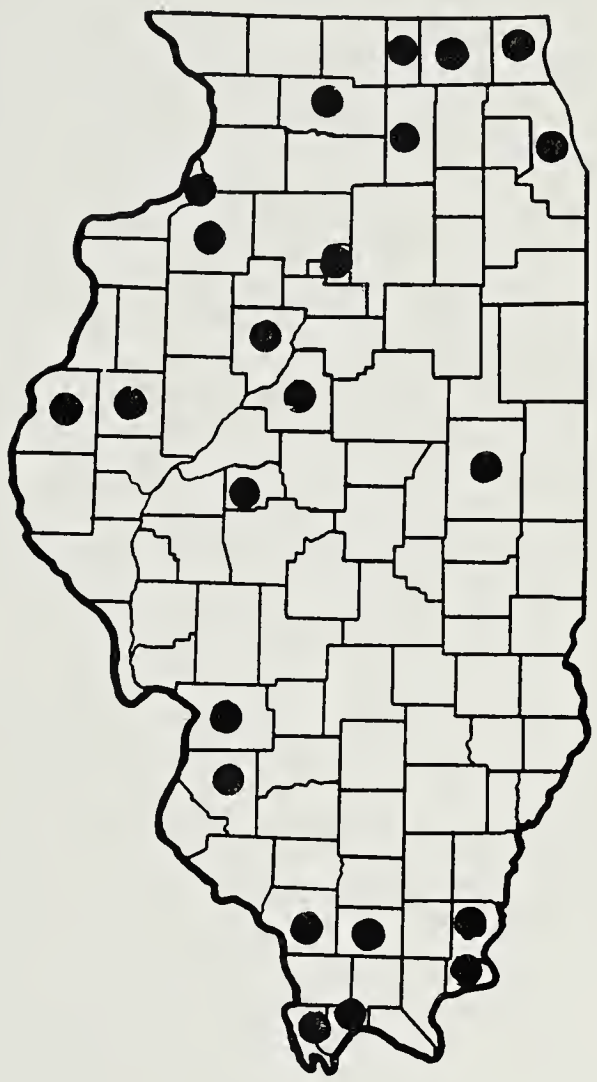

FIGURE 4. Distribution of *Coreopsis tinctoria* in Illinois.

Coreopsis tinctoria f. *atropurpurea* is segregated by some authors. Since it intergrades with f. *tinctoria* and is a cultigen its validity as a distinct taxon is in the opinion of the author not warranted.

5. COREOPSIS BASALIS (Otto & Dietr.) Blake, Proc. Amer. Acad. 51: 525. 1916.
Calliopsis basalis Otto & Dietr., Allg. Gart. 3:329. 1835. (17 Oct.).
Calliopsis drummondii D. Don, in Sweet, Brit. Fl. Gard. II. *pl. 315*. 1835. (1 Dec.).
Coreopsis diversifolia Hook., Curtis' Bot. Mag. 63:*pl. 3474*. 1836, excl. syn.
Coreopsis picta Hort. ex Sieb. & Voss, in Vilmorin, Blumengartnerei ed. 3. 1: 487. 1894.
Coreopsis basalis (Otto & Dietr.) Blake var. *typica* Sherff, Brittonia 6:341. 1948.

Erect annual herbs; stems 2-4 dm tall, subglabrous to pubescent; leaves opposite, basal and lower 1- to 3-pinnate, segments lanceolate to elliptic-oblong to or-

bicular, 3-8 cm long, 2-4 cm wide; petioles 1-5 cm long. Heads numerous, subcorymbose, radiate, 3-6 cm wide; peduncles slender, naked, 5-15 cm long; outer phyllaries linear-lanceolate, 5-9 mm long, inner ovate, 6-10 mm long; ray-flowers about 8, yellow with reddish-brown bases, 1.3-2.3 cm long, ligule cuneate-obovate, 2- to 3-lobate; palea linear, 6-10 mm long; disc-flowers reddish-brown; style-branches obtusely conic; epappose. Cypselas obcompressed, obovate, 1.4-1.8 (-2.0) mm long, black, papillate, incurved margins thickened and cartilaginous. $2n = 26$ (Gelin, 1934).

Coreopsis basalis occurs naturally in dry rocky soils only in Texas although it occurs as an escape from cultivation elsewhere in the eastern United States. It is known in Illinois only from the following collection: LAKE CO.: Cedar Lake, Lake Villa, *Fuller 13272* (ISM) (Fig. 5).

6. COREOPSIS TRIPTERIS L., Sp. Pl. 908. 1753.

Anacis tripteris (L.) Shrank, Denkschr. Akad. Munchen 5 (Math. Naturw.):7. 1817.

Chrysostemma tripteris (L.) Less., Syn. Gen. Comp. 227. 1832.

Coreopsis tripteris L. var. T. & G., Fl. N. Amer. 2:341. 1842.

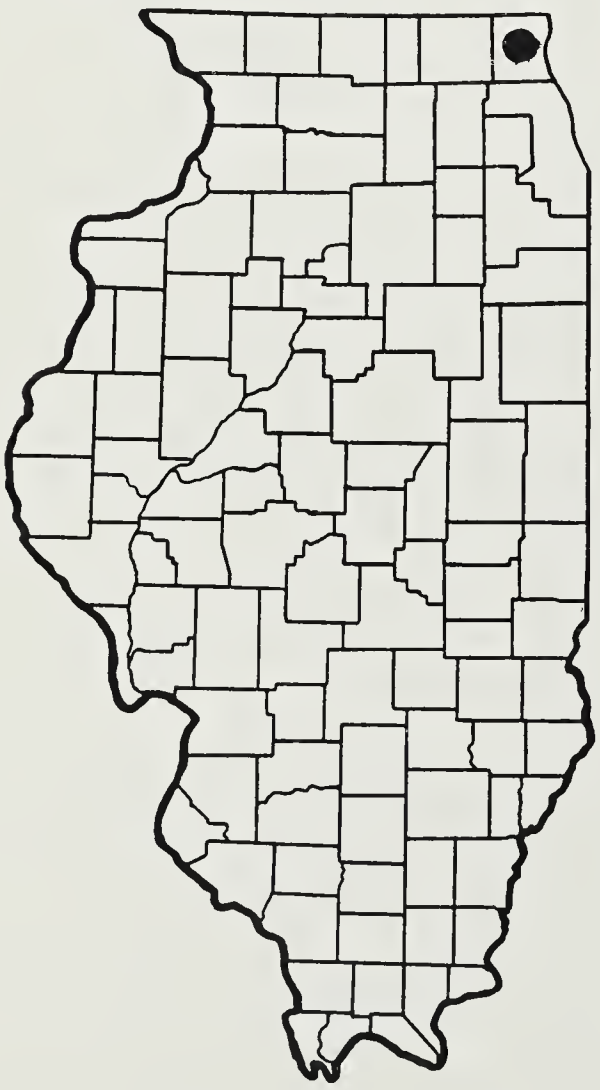

FIGURE 5. Distribution of *Coreopsis basalis* in Illinois.

Coreopsis tripteris L. var. *deamii* Standl., Rhodora 32:33. 1930.

Coreopsis tripteris L. var. *intercedens* Standl., Rhodora 32:34. 1930.

Erect perennial herbs; stems 1-3 m tall, glabrous or rarely pubescent; leaves opposite, principal ones pinnately 3- to 5-parted, segments elliptic-lanceolate, 3-10 cm long, 1-3 cm wide, petioles 0.5-3.0 cm long, rameal leaves usually simple, sessile. Heads subcorymbose, radiate, 3-5 cm wide; peduncles 3-8 cm long; outer bracts oblong-linear, obtuse, 2-3 mm long, inner oblong-ovate, 4-6 mm long; ray-flowers 7-8, yellow, 1.2-2.4 cm long, ligule ellip-

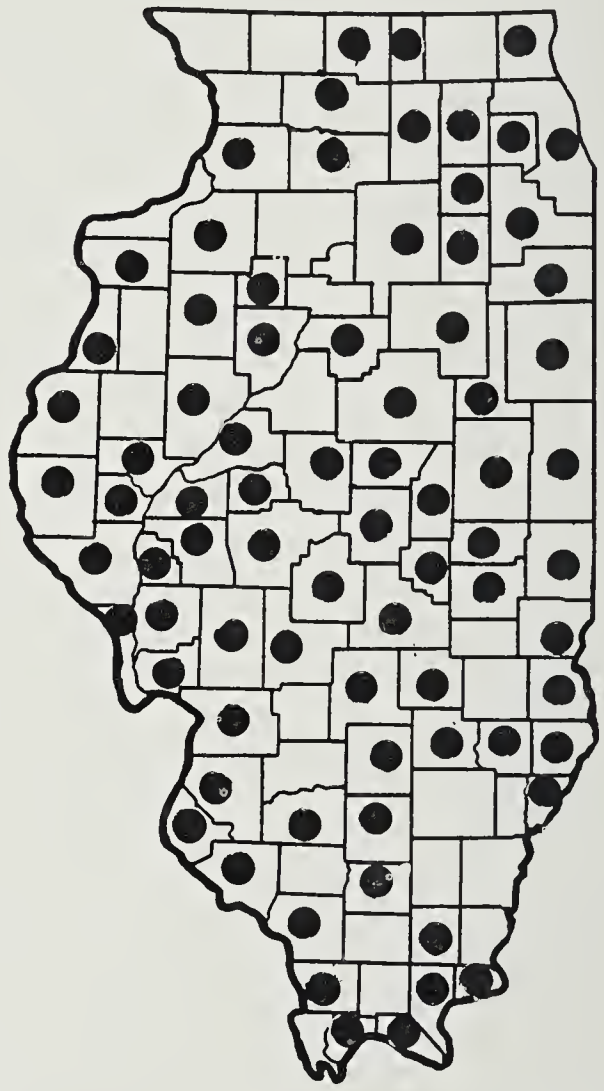

FIGURE 6. Distribution of *Coreopsis tripteris* in Illinois.

tic-oblong, subentire or dentate; palea filiform, purple-striate, 5-7 mm long; disc-flowers yellow to reddish-brown; style-branches caudate; epappose. Cypselas obcompressed, cuneate, 5-7 mm long, brownish-black, glabrous, margin winged. $2n = 26$ (Gelin, 1934).

Coreopsis tripteris occurs from Ontario to Wisconsin, south to Georgia, Louisiana, and Kansas and is escaped from cultivation northeast to New England. It occurs frequently in open woods and along roadsides throughout Illinois (Fig. 6).

7. COREOPSIS GRANDIFLORA Hogg ex Sweet, Brit. Fl. Gard. *pl. 175*. 1826.

Coreopsis boykiniana Nutt., Trans. Amer. Phil. Soc. II. 7:358. 1841.
Coreopsis heterophylla Nutt., Trans. Amer. Phil. Soc. II. 7:358. 1841, non Cav., 1796, nec Bertol., 1848.
Coreopsis grandiflora Hogg ex Sweet var. *subintergrifolia* T. & G., Fl. N. Amer. 2:345. 1842.

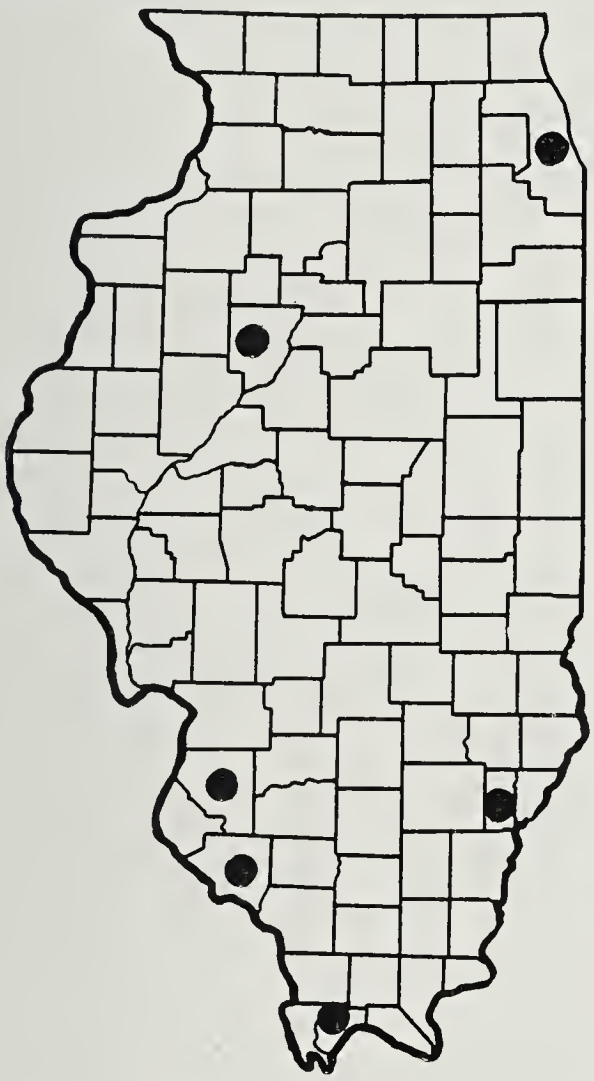

FIGURE 7. Distribution of *Coreopsis grandiflora* in Illinois.

Erect or ascending perennial or rarely annual herbs; stems 3-6 dm tall, subglabrous; leaves opposite, basal and lower simple or irregularly divided, upper 3- to 5-parted, segments linear to linear-lanceolate, 5-10 cm long, 1-5 mm wide; petiole 0.5-4.0 cm long. Heads usually solitary, radiate, 3-6 cm wide; peduncles slender, 1.0-1.5 dm long; outer phyllaries lanceolate, subulate, 5-9 (-18) mm long, margins ciliate, inner ovate, subequal; ray-flowers about 8, yellow, 1.3-2.5 cm long, ligule cuneate-obovate, 3-lobate; palea linear, 6-7 mm long; disc-flowers yellow; style-branches conspicuously cuspidate; pappus small or wanting. Cypselas obcompressed, orbiculate, 1-4 mm long, black, papillate, usually with calloused excrescences at ends, margin winged. $2n = 26$ (Gelin, 1934).

Coreopsis grandiflora occurs naturally in sandy or rocky prairies and thickets from Missouri to Kansas, south to New Mexico and Florida and is adventive or escaped from cultivation in the midwest and New England areas. It occurs locally along roadsides and in waste ground as an adventive or escape in Illinois (Fig. 7).

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ARYL ACETOACETATES—INFRARED AND ULTRAVIOLET SPECTRA

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ABSTRACT.—A series of aryl acetoacetates has been prepared and their infrared and ultraviolet spectra have been determined in order to study the effect of the aryl group upon the keto-enol spectroscopic peak positions.

It is well known that the infrared spectra of beta-ketoesters in the carbonyl region reveal frequencies due to both the keto and enol forms. Although work has been reported correlating spectra of various alkyl acetoacetates and alpha-substituted acetoacetates (Bellamy, 1958), study of the infrared spectra of aryl acetoacetates has not been reported.

The enol form of beta-ketoesters absorbs ultraviolet radiation, and alkyl acetoacetates are reported to absorb in a narrow region around 240 nm (Korte and Wusten, 1961). It is surprising that almost no work has been reported concerning ultraviolet absorption of aryl acetoacetates.

METHODS AND MATERIALS

The aryl acetoacetates were prepared by the method of Zavialov (1965); the physical constants of reported esters agree with values reported by Lacey (1954).

Table 1 gives the data on four unreported esters. Elemental analyses were obtained by Galbraith Laboratories, Knoxville, Tennessee.

Infrared spectra were run in spectral grade chloroform in 0.1mm cells on a Beckman IR8 grating spectrophotometer with an auxiliary recorder used for scale expansion. In order to provide absorption maxima for calibration, the 1944 cm^{-1} peak of polystyrene was recorded and, without stopping the scan, the polystyrene was replaced with the cells and the four peaks of interest were recorded. The cells were removed, again without stopping the scan, and replaced with polystyrene and the 1181 and 1154 cm^{-1} peaks were recorded. The above three polystyrene peaks were used for calibration; the error of measurement is estimated to be ± 3cm^{-1}.

UV spectra were run in absolute ethanol on a Beckman DB spectrophotometer with an estimated error of ± 2nm.

RESULTS AND DISCUSSION

Infrared spectra frequencies measured in chloroform are shown in Table 2 and are arranged in decreasing order of the high frequency bands of the ester carbonyl bond. Assignment of the frequencies to the ester carbonyl (1764-1734 cm^{-1}), keto carbonyl (1720-1713 cm^{-1}), enol ester carbonyl (1673-1630 cm^{-1}) and

TABLE 1. Unreported Aryl Acetoacetates

Aryl Group	mp (°C)	Elemental Analysis			
		Calcd		Found	
		% C	% H	% C	% H
p-nitrophenyl	67-69	53.82	4.06	53.66	4.19
2,4,6-trimethylphenyl	60.5-62	70.89	7.32	70.76	7.34
alpha-phenyl-*p*-cresyl	54-56	75.82	6.36	75.82	6.12
2,6-dimethoxyphenyl	64.5-66	60.50	5.92	60.40	6.02

TABLE 2. Wavenumber Assignment (cm^{-1}) of Keto and Enol Bands of Substituted Aryl Acetoacetates in Chloroform

Aryl Group	Ester C=0	Ketone C=0	Ester C=0	Enol C=C
a-Naphthyl	1764	1720	1649	1627
2,6-Dimethoxyphenyl	1764	1719	1673	1609
a-Phenyl-*p*-cresyl	1759	1723	1668	1629
2,4,6-Trimethylphenyl	1757	1723	1667	1630
p-Tolyl	1750	1718	1666	1614
B-Naphthyl	1741	1717	1659	1630
p-Nitrophenyl	1738	1715	1650	1616
Phenyl	1737	1716	1630	1603
p-Methoxyphenyl	1734	1713	1637	1603

enol carbon-carbon double bond (1630-1603 cm^{-1}) is in accordance with the work of Rasmussen and Brattain (1949) and Leonard *et al.* (1952).

Frequencies due to the ester carbonyl group vary over a range of 30 cm^{-1}. The highest frequencies are exhibited by compounds possessing ortho groups on the aryl group suggestive of a steric effect, however the one exception, alpha-phenyl-*p*-cresyl ester, argues against a steric effect. The keto carbonyl range of values is only 10 cm^{-1}, not unexpected because of the distance between the keto and ester groupings. The trend of the keto carbonyl frequencies, excluding the first two values, parallels that of the ester carbonyl although the significance is lessened because of the closeness to the estimated error of ± 3 cm^{-1} for each frequency. The enol ester carbonyl bands vary by the greatest amount, 43 cm^{-1}, and parallel closely the ester carbonyl frequencies with the glaring exception of alpha-naphthyl and the lesser exception of *p*-methoxyphenyl. Enol carbon-carbon double bond frequencies vary by 27 cm^{-1} and no parallel exists with any of the other three frequency types.

Although alkyl acetoacetates have ultraviolet maxima which, in alcoholic solution, vary little from 240 nm (Korte and Wusten, 1961), the aryl acetoacetates have maxima, as shown in Table 3, which exhibit a wide range of values. The shift toward 220 nm is interesting for it is near 210 nm that alpha-beta unsaturated esters absorb; addition of a hydroxy group on the beta carbon gives the large bathochromic shift to about 240 nm for alkyl acetoacetates (Filler and Naqvi, 1963). All the aryl acetoacetates reported here absorb at a lesser wavelength than alkyl except for the 2,4,6-trimethylphenyl which has a maximum about equal to that of ethyl acetoacetate. Three of the

TABLE 3. Ultraviolet Absorption Maxima of Substituted Aryl Acetoacetates in Absolute Ethanol

Aryl Group	Maxima (nm)	^{a}m
2,4,6-Trimethylphenyl	243	1200
a-Naphthyl	236	2200
p-Nitrophenyl	232	760
B-Naphthyl	229	1300
p-Methoxyphenyl	228	6000
p-Tolyl	225	1600
Phenyl	223	840
2,6-Dimethoxyphenyl	220	2400

esters are p-substituted phenyl compounds with the most electron withdrawing member having the highest value and decreasing for p-methoxy groups, although both are higher than the unsubstituted phenyl. The bulky 2,4,6-trimethylphenyl ester absorbs at the highest wavelength with the less bulky alpha-naphthyl ester following. A steric effect suggested by these two groups is countered by the effect of the 2,6-dimethoxyphenyl ester which has the lowest absorption maxima. However, the electronic effect of two *o*-methoxy groups may be important.

Acknowledgements

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METHODS OF EVALUATING THICKNESS AND TEXTURE OF GLACIAL TILL IN STUDIES OF GROUND-WATER RECHARGE

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ABSTRACT.—The thickness and vertical permeability of the till that overlies the glacial drift and shallow bedrock aquifers in northeastern Illinois were measured to determine their relation to the recharge rate of ground water. The till impedes vertical movement of the water. The average thickness of the till at main pumping centers was determined from maps and from cumulative thickness curves. The saturated thickness of the confining bed was estimated by subtracting depth to the assumed water table from the average thickness of the confining bed. Vertical permeability at the pumping centers was not measured directly but was calculated on the assumption that leakage and pumpage are approximately equal at the pumping centers.

Relative permeability was inferred from textural data obtained from hydrometer analyses of subsurface samples from 52 test holes that penetrated the full thickness of the glacial drift. Textural differences of the confining bed varied regionally. Sand is more plentiful than clay roughly west of the Fox River, indicating relatively high permeabilities, whereas the reverse is true east of the river. The findings, supported by data derived from hydrologic analyses at the pumping centers, indicated thickness and vertical permeability of the till do affect ground-water recharge.

Glacial drift and shallow bedrock aquifers are a major source of ground water in the area of northeastern Illinois that includes Cook, DuPage, Kane, Lake McHenry, and Will Counties. Where glacial drift aquifers (sand and gravel) and shallow bedrock aquifers (principally Silurian dolomite) are overlain by confining beds of glacial till, leaky artesian conditions exist. The till beds impede the vertical movement of ground water to the aquifer.

The withdrawal of ground water from these aquifers has produced a number of cones of depression. The vertical permeability of the confining beds as well as the hydrogeologic character of the aquifer are two of the factors that control the depth and shape of the cone of depression. In this report, the cone of depression is simply called the pumping center.

Several pumping centers were described and studied by Zeizel et al. (1962) and Prickett et al. (1964). The area of a pumping center is measured by drawing flow lines at right angles to the piezometric surface contours. It is rarely symmetrically shaped because production wells are usually unevenly distributed and are pumped at the same rates.

Figure 1 shows the areal distribution of the confining beds and possible sand and gravel deposits of the glacial drift (Piskin, 1963) in seven of these pumping centers. Working in northeastern Illinois, Walton (1960) calculated the quantity of leakage through a confining bed (the Maquoketa Group of Ordovician age) into an aquifer by employing the equation:

$$Q_c = \frac{P'}{m'} \triangle h A_c$$

where Q_c = leakage through the confining bed (gpd)
P' = vertical permeability of the confining bed (gpd/ft^2)
m' = thickness of the confining bed through which leakage occurs (ft)
A_c = area of the confining bed through which leakage occurs (ft^2)

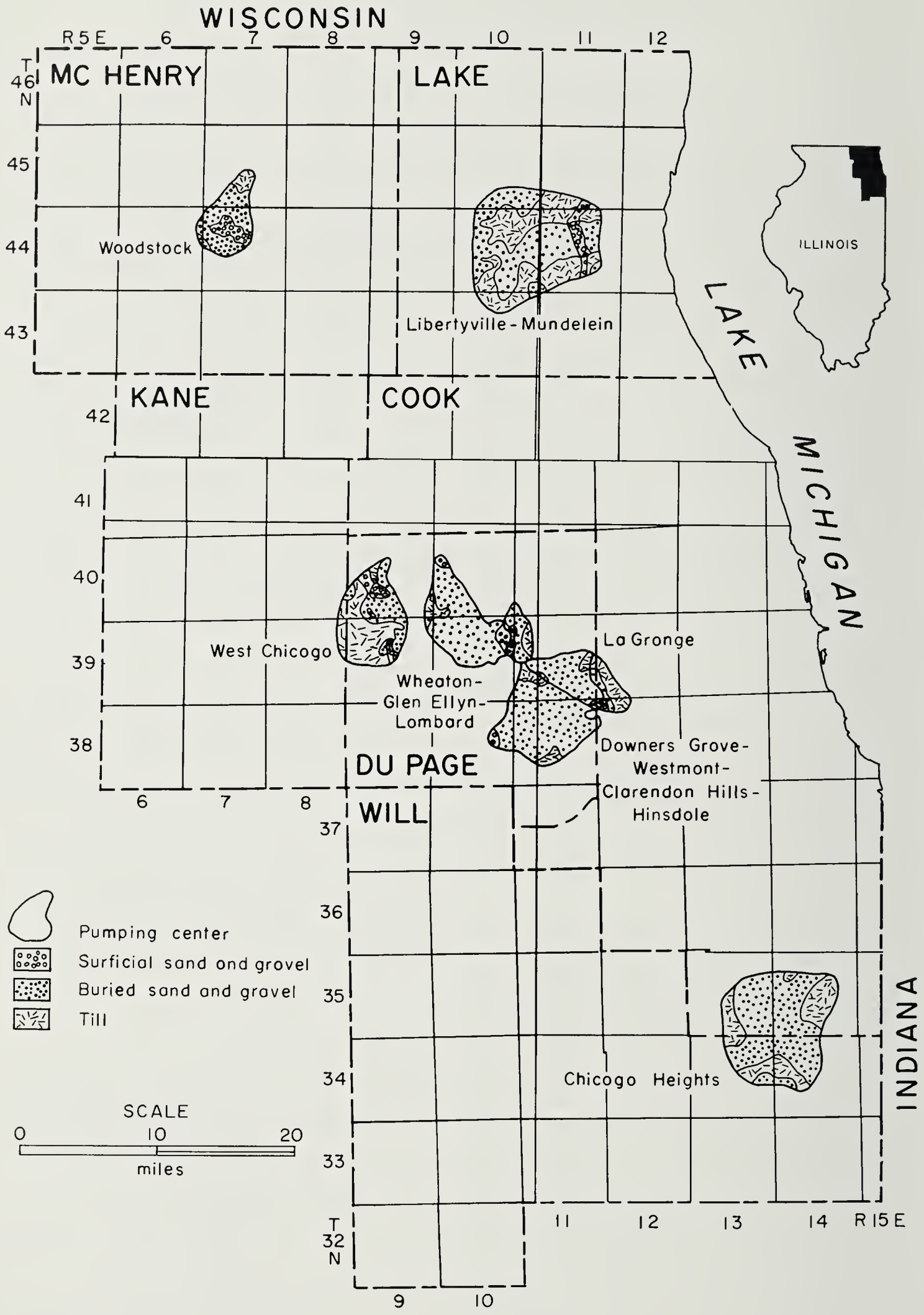

FIGURE 1. Distribution of glacial drift deposits in pumping centers in northeastern Illinois.

$\triangle h$ = difference between the head of the aquifer and the source bed above the confining bed (ft)

It can be applied to leakage through a confining layer of glacial till to evaluate quantitatively the effect of such a layer on ground-water recharge to underlying aquifers.

Geology

The shallow bedrock in northeastern Illinois consists mainly of rocks of the Maquoketa Group (Ordovician) and the Alexandrian and Niagaran Series (Silurian). Quaternary deposits, principally glacial drift, blanket the bedrock.

The Maquoketa Group underlies the glacial drift along the western margin of the study area. It consists of three formations, two shale formations and a middle formation that is argillaceous dolomite or limestone interbedded with shale. Dolomite is also present in the upper and lower shale formations, both of which are commonly fossiliferous and fine grained. The dolomitic middle formation is part of the shallow bedrock aquifer system.

Silurian deposits directly underlie the glacial drift in, approximately, the eastern three-fourths of the study region, which is where the seven pumping centers used in the tests are located. The Alexandrian Series (lower part of Silurian) consists of argillaceous to finely sandy, cherty, gray to light brown, finely crystalline dolomite; the overlying Niagaran Series consists of white to light gray, cherty, silty, finely crystalline to medium-crystalline dolomite. The total thickness of the Silurian deposits ranges from 0 to 465 feet, increasing toward the southeast. The Silurian is the principal water-yielding unit of the shallow aquifer system in northeastern Illinois.

The unconsolidated Quaternary deposits, overlying the Silurian and Maquoketa, resulted principally from the extensive continental glaciers and subsequent action by wind and running water; thus, most of the region is covered with thick drift. The drift varies in thickness from 0 to about 500 feet, depending on bedrock topography as well as the present land surface (Piskin and Bergstrom, 1967). In general, the drift thickens from south to north and from east to west; the thickest deposits, exceeding 250 feet, are restricted to the northern part of the study area.

The glacial drift consists principally of unsorted ice-laid rock debris (till), sorted meltwater deposits (glaciofluvial), and ancient lake-bed sediments (glaciolacustrine). The till found throughout the region is dense clayey silt to gravelly sand; it is most commonly fine grained. The till deposits are the principal confining layer for both the shallow bedrock and glacial drift aquifers. Glaciofluvial deposits of the area, in the form of terraces, outwash plains, and valley trains, are characteristically coarser in texture (sand and gravel) than the till and commonly exhibit cross-bedding. These sand and gravel deposits are the second major source of shallow ground water in the region. Glaciolacustrine deposits, generally the finest grained sediments, are found along Lake Michigan and in western Will County, and, like the till, serve as a confining layer.

In the seven pumping centers tested, till deposits occur at various levels within the outwash and valley train deposits. Locally, they rest on the underlying Silurian dolomite, where basal sand and gravel are absent. The areal relations of sand and gravel and till deposits for the seven pumping centers are shown in Figure 1. More detailed

geologic information on bedrock formations is given by Suter et al. (1959), Zeizel et al. (1962), and Hughes, Kraatz, and Landon (1966).

Method of Evaluation

The equation was used to determine vertical permeability of the confining beds at the seven pumping centers. In this report, the West Chicago pumping center, located in T. 39 and 40 N., R. 9 E., DuPage County, Illinois, is taken as an example. The area of influence of production wells was about 28.0 square miles in 1960 (Zeizel et al., 1962). The main aquifer at this center is the Silurian dolomite, which is recharged by vertical leakage through the overlying glacial drift. The drift itself is recharged by precipitation. Because water levels suggest that recharge from leakage increases with pumpage, recharge to the Silurian dolomite aquifer in the West Chicago pumping center was assumed to be equal to the pumpage, which was estimated to be 1.8 million gallons per day (mgd). Zeizel et al. (1962) estimated the rate of recharge to the Silurian dolomite in 1960 to be 64,000 gpd per square mile.

The map in Figure 2 shows the sand and gravel at the West Chicago site is at least 15 feet thick (Piskin, 1963). These deposits are divided into two groups, surficial deposits with less than 10 feet of fine-grained overburden, and buried deposits with more than 10 feet of fine-grained overburden, mainly till. Surficial and buried sand and gravel are assumed to be separated by till at least 10 feet thick. Surficial deposits, which attain a maximum thickness of 50 feet, cover an area of approximately 4 square miles; buried deposits, ranging from 15 to 100 feet thick occupy an area of about 10 square miles. Cross sections (Fig. 2) show that the present land surface approximates the topography of the bedrock surface, except in the north where a small valley in the Silurian dolomite has no present topographic expression.

At the West Chicago pumping center, confining beds of glacial till, which range in composition from yellow-gray-brown sandy silt through clayey silt and sand to

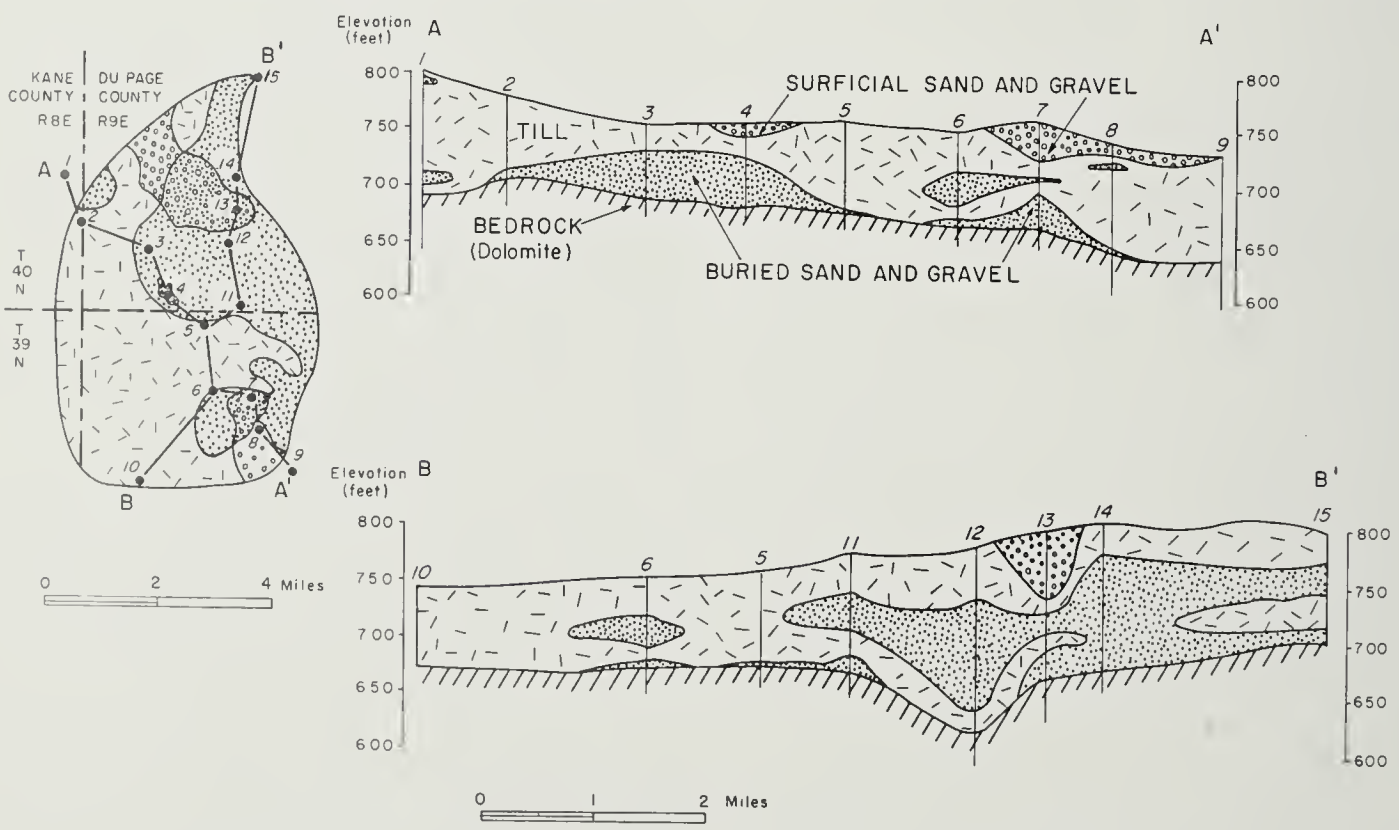

FIGURE 2. Geology of West Chicago pumping center.

slightly silty clayey sand, directly overlie the sand and gravel or, in some cases, Silurian dolomite (Fig. 2). The confining beds range from less than 25 to more than 75 feet thick (Fig. 3A), the thicker beds being located in the northeastern part of the pumping center. To represent the average thickness of the confining bed at this pumping center, the ratio of the area between the various isopachs to the total area was plotted against the thickness of the confining bed. The 50th percentile point of area (Fig. 3B) was then taken as the average thickness of the confining bed in the pumping center. The depth of the water table was assumed to be 10 feet (T. A. Prickett, personal communication, 1969). The saturated thickness of this bed may be roughly determined by subtracting the depth to the water table from the average thickness of the confining bed.

The vertical permeabilities of the confining beds in the West Chicago, Wheaton-Lombard-Glen Ellyn, and Downers Grove-Westmont-Hinsdale-Clarendon Hills pumping centers were calculated by using the equation with the values for saturated thickness inserted. Head loss ($\triangle h$) was estimated by computing the difference in elevation between the head of the aquifer and that of the source bed above the confining bed. The other hydrogeologic data for these centers were taken from Zeizel et al. (1962). Vertical permeabilities of the confining beds at the remaining four pumping centers (La Grange, Chicago Heights, Woodstock, and Libertyville-Mundelein) were determined from data taken from Prickett et al. (1964). These results were compared

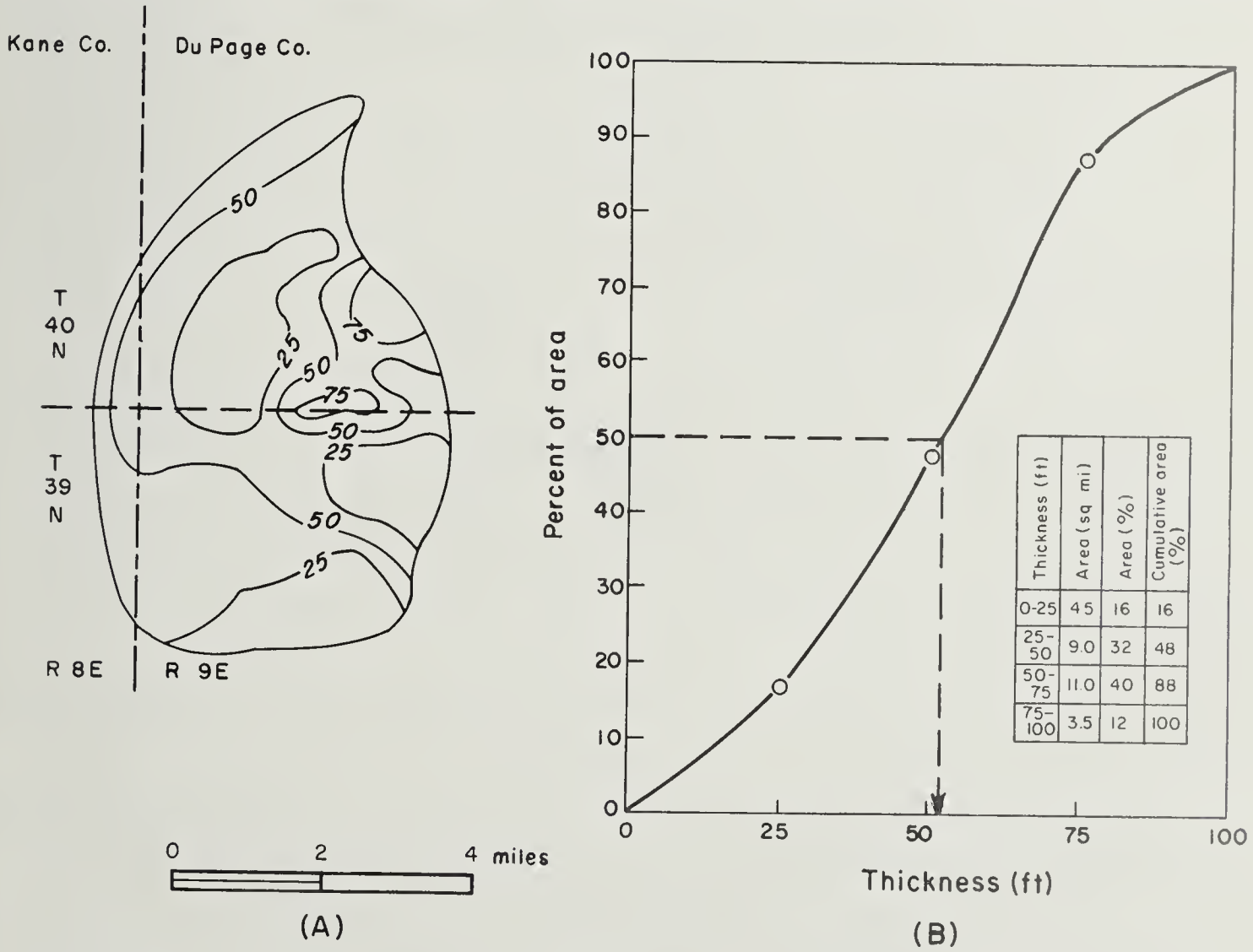

FIGURE 3. Thickness map (A) and graphical representation of average thickness (B) of confining beds in West Chicago pumping center.

to the vertical permeabilities for the four pumping centers obtained by Prickett from analysis of pump tests; the two set of data compare favorably.

To evaluate the effect of texture upon vertical permeability, grain-size distributions of the confining beds were obtained by hydrometer analyses of subsurface samples from 52 precisely-planned test holes drilled in the six-county area. These test holes, penetrating the entire thickness of glacial drift, were part of a study on water management coordinated by the Northeastern Illinois Metropolitan Area Planning Commission (Hackett and Hughes, 1965). Textural analyses of the confining beds were averaged and plotted in a triangular diagram (Fig. 4) to show the distribution of the sand-silt-clay ratios. Results were then compared with the ver-

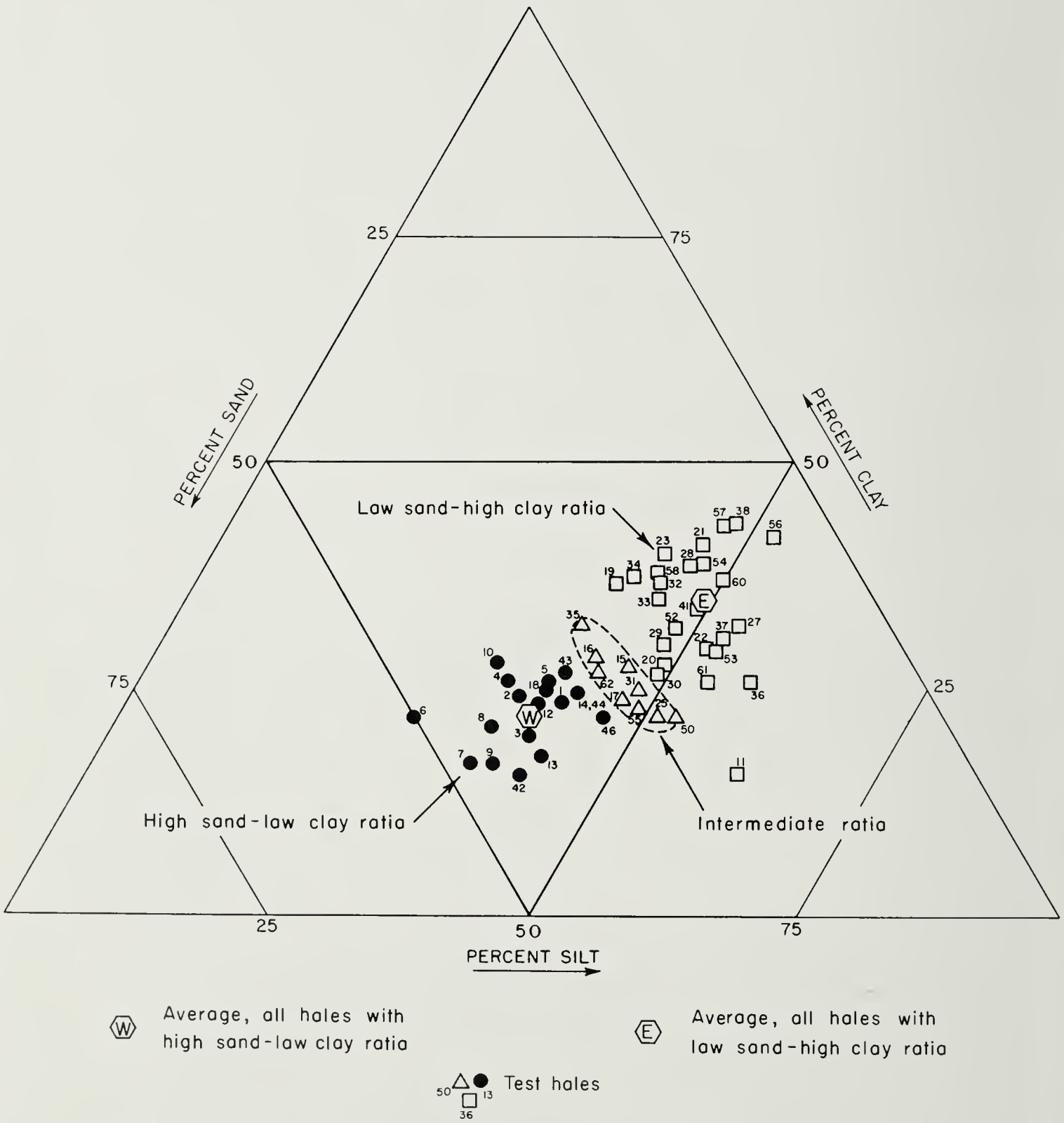

FIGURE 4. Average composition of confining beds penetrated in test holes.

tical permeabilities of the various confining beds in the seven pumping centers.

Results and Discussion

Table 1 shows the thickness and characteristics of the confining beds in the seven pumping centers; at these sites, total drift thickness ranges from 10 feet in the La Grange area to approximately 300 feet in the Woodstock and Libertyville-Mundelein areas. The table indicates that the average saturated thickness of the confining bed is higher in the Woodstock and Libertyville-Mundelein centers where the drift is thickest. Because the areas of thicker drift generally contain thicker deposits of confining till as well as sand and gravel, drift thickness directly influences the saturated thickness of the confining bed. These results are in agreement with previously published data on the saturated thickness of the confining bed in the Chicago Heights, La Grange, Libertyville-Mundelein, and Woodstock pumping centers (Prickett et al., 1964). The saturated thickness of the confining bed at the other three pumping centers (Table 1) was found to be reasonable (T. A. Prickett, personal communication, 1970).

Results of hydrometer analyses of the confining beds in the 52 test holes previously mentioned were obtained from standard grain-size ratios for sand (2.0 mm and 0.062 mm) silt (0.062 and 0.004 mm), and clay (< 0.004 mm). These figures indicate that, although the percentage of sand ranges from 6 to 50

Table 1. Thickness and Characteristics of Confining Beds in Pumping Centers

Pumping Center	Average Thickness (ft)	Assumed Saturated Thickness (ft)	Characteristics
Chicago Heights	44	34	Till, brown silty clay, clayey silt, and occasional gray silty clay with gravel and trace sand.
Downers Grove-Westmont-Hinsdale-Clarendon Hills	58	48	Till, gray silty clay with trace of sandy silt and brown gravel.
La Grange	45	35	Till, brown to gray silty clay and gray clayey sand with silt.
Libertyville-Mundelein	150	140	Till, gray clayey silt, silty clay with trace of sand and gravel; occasional gray clay and pebbles.
West Chicago	52	42	Till, yellow-gray-brown sandy silt, clayey silt, and sand to clayey sand with trace of silt.
Wheaton-Lombard-Glen Ellyn	45	35	Till, gray sandy silt, gray to brown clayey silt, and silty clay with occasional sand and gravel.
Woodstock	119	109	Till, gray to brown clayey sand with medium to coarse sandy gravel, and pinkish brown sandy silt with clayey gravel.

percent and that of clay from 16 to 49 percent, the amount of silt is more or less consistent, averaging 45 percent. These ratios are dependent on the character of local tills within the glacial drift.

The percentage of coarse materials is generally higher in the western than the eastern part of the area, and the reverse is true for the fine materials. This relation is apparent in Figure 4, which was compiled by plotting the average percentage composition of the confining bed at each test hole. Because of its relative consistency, the percentage of silt was not considered. On the basis these percentages, three main groups were identified. The first, which falls at the left side of the diagram (the western part of the study area), is characterized by high sand (32 to 50 percent) and low clay (16 to 28 percent) ratios; a second group at the far right (the eastern part of the area) is characterized by low sand (6 to 24 percent) and high clay (16 to 43 percent) ratios (Fig. 4). These two divisions are separated by a third group, which is characterized by intermediate ratios of sand (25 to 30 percent) and clay (22 to 32 percent). In addition, the average composition of all holes with high sand and low clay and low sand and high clay percentages within the two groups were averaged. These total averages are shown in Figure 4 by hexagons *W* and *E*, which represent the average sand-silt-clay ratios as 39:39:22 and 17:49:34, in west and east groupings, respectively.

Figure 5 illustrates the regional distribution of the 52 test holes where subsurface data were obtained for the confining beds. The holes with high sand and low clay ratios generally occupy the area west of the Fox River, while the the reverse is true east of the river. Holes with intermediate ratios are about equally distributed within the two groups. To compare variations in vertical permeability with textural distribution, the vertical permeability of the confining bed at each of the seven pumping centers was also included in Figure 5.

The vertical permeabilities obtained for the confining beds in the latter four pumping centers compares favorably with previously published data by Prickett et al. (1964). The vertical permeabilities in the various centers ranges from 0.3×10^{-2} gpd/ft^2 to 1.3×10^{-2} gpd/ft^2. The Woodstock pumping center, which is the only one located west of the Fox River, had the highest vertical permeability in the region. The rest of the pumping centers, located east of the Fox River, have comparatively low vertical permeabilities. These results appear to correspond with the regional distribution of sand-clay ratios within the confining beds at the various pumping centers. The vertical permeabilities and clay ratios of the confining beds in the seven pumping centers are plotted in Figure 6 with a linear regression line drawn through these points. This relationship suggests that the high sand and low clay percentages coincide with the area of high vertical permeability (west of the Fox River), while low sand and high clay percentages can be observed in the area with low vertical permeabilities (east of the Fox River).

Summary

The saturated thickness and vertical permeability of a glacial till overlying an aquifer has, under leaky artesian conditions, an effect on the recharge rate of the aquifer. Although this relation has long been inferred in this region, no previous experimental data have been obtained to support it. Data from subsurface sampling programs and from

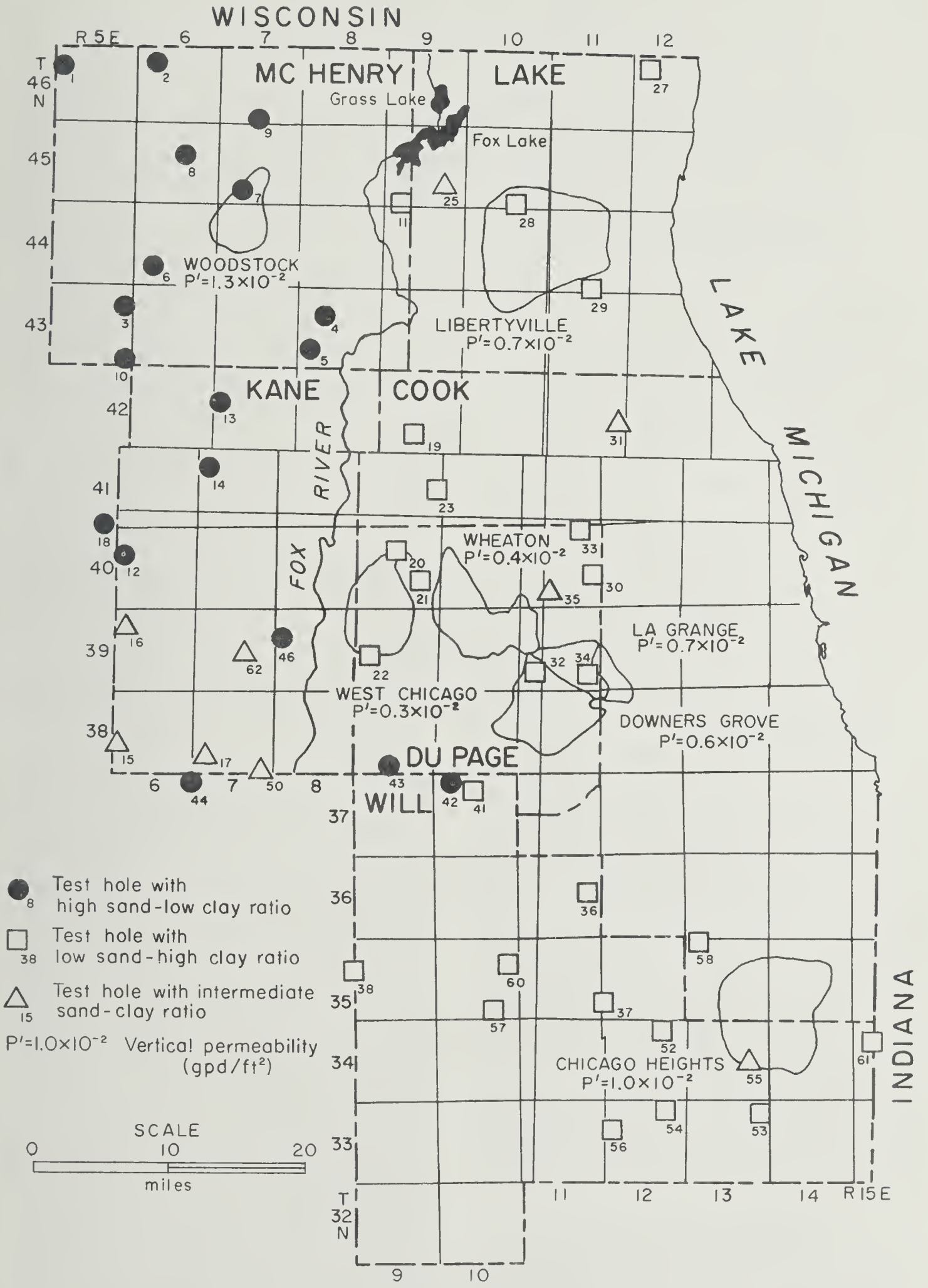

FIGURE 5. Distribution of test holes and pumping centers. Sand-silt-clay ratios for the test holes and vertical permeability of confining beds for the pumping centers.

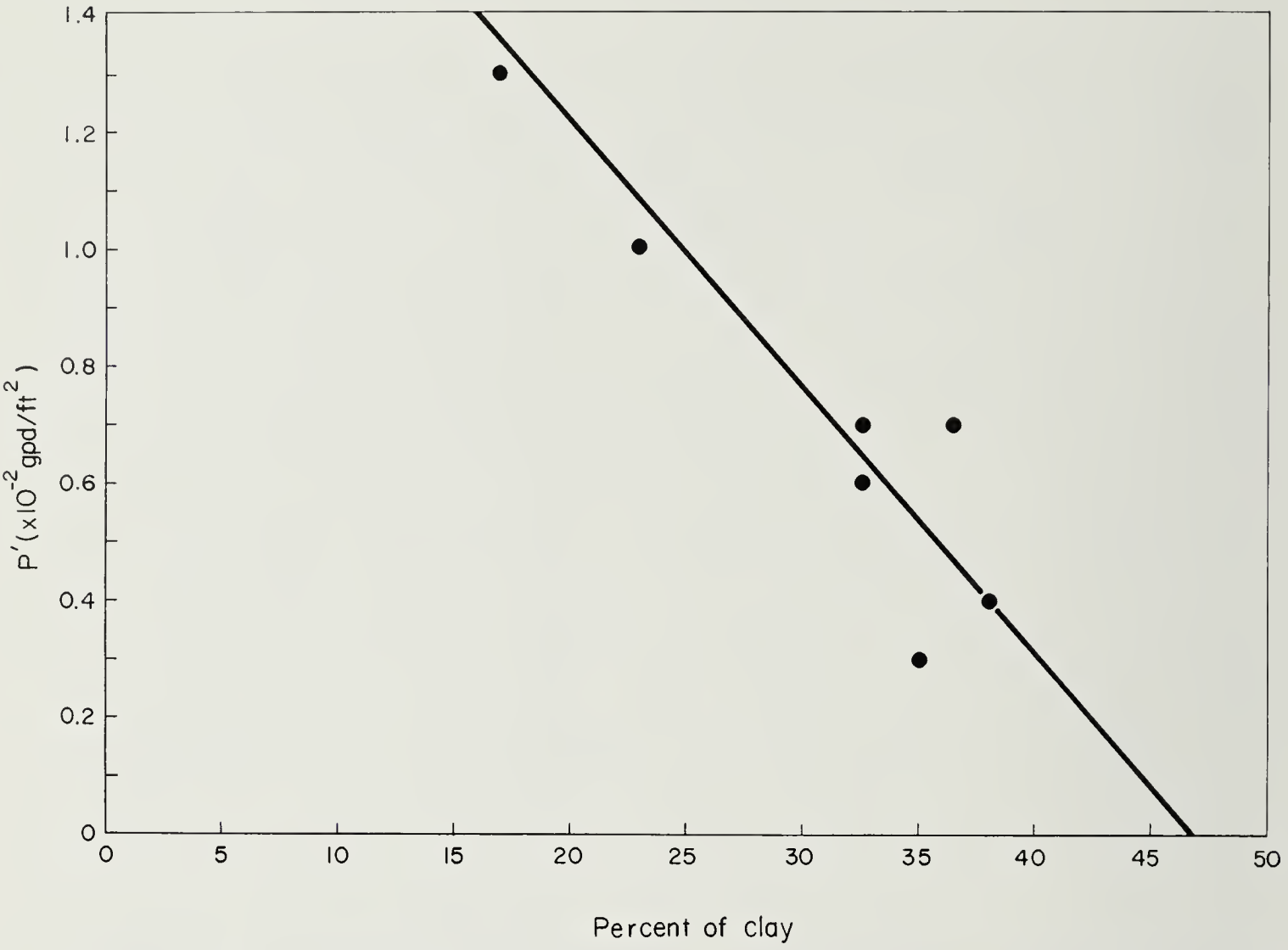

FIGURE 6. Comparison of vertical permeability with clay ratio of confining beds in 7 pumping centers.

cone analyses of selected pumping centers, both used in this study, are generally reliable. However, the vertical permeability differences among the seven pumping centers have a limited range, and greater variations might be expected in other areas. The relation between sand-clay ratios and vertical permeabilities can be better established when additional pumping centers, especially west of the Fox River, are considered. Supporting studies from other areas would prove helpful.

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THE PILEATE PORE FUNGI OF ILLINOIS

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ABSTRACT.—A survey was made to determine the species of pileate pore fungi which occur in Illinois. Studies of literature and herbarium specimens show that at least 92 species can be found in the state. Of these, 22 species have not previously been listed for Illinois.

The Polyporaceae, or pore fungi, are those Basidiomycetes with a hymenium composed of pores within which basidia are borne. The Boletes, which are typically fleshy, are excluded from this family. This study was undertaken to determine the species of pileate polypores that are native to the state of Illinois. Specimens were examined from several herbaria in the state, including those of Eastern Illinois University, Illinois State University, the University of Illinois, and the Field Museum of Natural History. A few specimens from Southern Illinois University were also studied.

In addition to actual identification of specimens, a survey of previous literature concerning Illinois polypores was made. Two important works which list Illinois polypores are Moffatt (1909) and Overholts (1953). Other listings are given in McDougall (1917 and 1919), and Graham (1944).

In the following list, those species previously cited for the state will be followed by the name of the author who first reported them. Those species of which specimens have not been examined will be followed by "sec." (secundum) and the earliest author. If a species has not previously been reported from the state, it will be preceded by an asterisk and a reference for the collection from which specimens were examined will be given.

Daedalea aesculi (Schw. ex Fr.) Murr.
Overholts (as *Daedalea ambigua*)
Daedalea confragosa Bolt. ex Fries
Moffatt
Daedalea farinacea (Fries) Overh.
Sec. Overholts
**Daedalea quercina* L. ex Fries
University of Illinois collection
Daedalea unicolor Bull. ex Fries
Moffatt
Favolus alveolaris (D.C. ex Fries) Quel.
Moffatt
**Favolus rhipidium* (Berk.) Sacc.
University of Illinois collection
**Fomes annosus* (Fries) Cooke University of Illinois collection
Fomes applanatus (Pers. ex Wallr.) Gill.
Moffatt
Fomes conchatus (Pers. ex Fries) Gill.
Overholts
Fomes everhartii (Ell. & Gall.) Von Schrenk & Spaulding
Moffatt
**Fomes fomentarius* (L. ex Fries) Kickx
Field Museum of Natural History collection
Fomes fraxineus (Bull. ex Fries) Cooke
Sec. Moffatt
Fomes fraxinophilus (Peck.) Sacc.
Moffatt
Fomes fulvus (Scop. ex Fries) Gill.
Moffatt
Fomes igniarius (L. ex Fries) Kickx
Moffatt
**Fomes johnsonianus* (Murr.) Lowe
University of Illinois collection
Fomes lobatus (Schw.) Cooke
Overholts
Fomes ohiensis (Berk.) Murr.
Overholts
Fomes pini (Thore ex Fries) Karst.
Owens
Fomes populinus (Schum. ex Fries) Cooke
Moffatt (as *Fomes connatus*)
Fomes ribis (Schum. ex Fries) Gill.
Moffatt
**Fomes rimosus* (Berk.) Cooke
University of Illinois collection
Fomes scutellatus (Schw.) Cooke
Moffatt
Fomes viticola (Schw.) Lowe
Sec. Overholts (as *Fomes tenuis*)
Lenzites betulina (L. ex Fries) Fries
Overholts
**Lenzites sepiaria* (Wulf. ex Fries) Fries
University of Illinois collection
Lenzites trabea (Pers. ex Fries) Fries
Overholts

**Polyporus abietinus* Dicks. ex Fries
Field Museum of Natural History collection
Polyporus adustus Willd. ex Fries
Moffatt
**Polyporus albellus* Peck
University of Illinois collection
Polyporus arcularius Batsch ex Fries
Moffatt
Polyporus berkeleyi Fries
Moffatt
**Polyporus betulinus* Bull. ex Fries
University of Illinois collection
Polyporus biennis (Bull. ex Fries) Fries
Sec. Overholts
Polyporus biformis Fries
Moffatt (as *Polystictus pergamenus*)
Polyporus brumalis Pers. ex Fries
Moffatt
Polyporus chioneus Fries
Sec. Moffatt
Polyporus cinnabarinus Jacq. ex Fries
Moffatt
Polyporus cinnamomeus Jacq. ex Fries
Moffatt
Polyporus compactus Overh.
Sec. Overholts
Polyporus conchifer (Schw.) Fries
Moffatt
Polyporus cristatus Pers. ex Fries
Moffatt (as *Polyporus poripes*)
Polyporus croceus Pers. ex Fries
Overholts
**Polyporus curtisii* Berk.
University of Illinois collection
Polyporus cuticularis Bull. ex Fries
Sec. Overholts
**Polyporus delectans* Peck
University of Illinois collection
Polyporus dichrous Fries
Moffatt
Polyporus distortus Schw.
Sec. Moffatt
Polyporus dryadeus Pers. ex Fries
McDougall
Polyporus dryophilus Berk.
Sec. Overholts
**Polyporus elegans* Bull. ex Trog
Field Museum of Natural History collection
**Polyporus focicola* Berk. & Curt.
Eastern Illinois University collection
Polyporus frondosus Dicks. ex Fries
Moffatt
Polyporus fumosus Pers. ex Fries
Moffatt
Polyporus galactinus Berk.
Sec. Moffatt
Polyporus giganteus Pers. ex Fries
McDougall
Polyporus gilvus (Schw.) Fries
Moffatt
Polyporus glomeratus Peck
Sec. Overholts
Polyporus graveolens (Schw.) Fries
Sec. Overholts
Polyporus hirsutus Wulf. ex Fries
Moffatt
**Polyporus licnoides* Mont.
Field Museum of Natural History collection
Polyporus lucidus Leys. ex Fries
Moffatt (as *Fomes lucidus*)
Polyporus molliusculus Berk. & Curt.
Moffatt (as *Polystictus biformis*)
Polyporus mutabilis Berk. & Curt.
Sec. Overholts
Polyporus pocula (Schw.) Berk. & Curt.
University of Illinois collection
**Polyporus perennis* L. ex Fries
Field Museum of Natural History collection
Polyporus picipes Fries
Moffatt
Polyporus radiatus Sow. ex Fries
Sec. Overholts
Polyporus radicatus Schw.
Moffatt
Polyporus resinosus Schrad. ex Fries
Moffatt
Polyporus robiniophilus (Murr.) Lloyd
Moffatt
Polyporus rutilans (Pers.) Fries
Moffatt
Polyporus sanguineus L. ex Fries
Sec. Overholts
Polyporus schweinitzii Fries
Moffatt
Polyporus semipileatus Peck
Sec. Overholts
Polyporus spraguei Berk. & Curt.
Overholts
Polyporus squamosus Micheli ex Fries
Sec. Overholts
Polyporus sulphureus Bull. ex Fries
Moffatt
**Polyporus tephroleucus* Fries
Field Museum of Natural History collection
Polyporus tulipiferae (Schw.) Overh.
Overholts
**Polyporus umbellatus* Pers. ex Fries
Collection of author (received from K. Andrew West of Southern Illinois University)
**Polyporus unicolor* Fries
University of Illinois collection
Polyporus versicolor L. ex Fries
Moffatt
**Trametes americana* Overh.
Field Museum of Natural History collection (as *Fomes odoratus*)
Trametes hispida Bagl.
Moffatt (as *Trametes peckii*)
Trametes malicola Berk. & Curt.
Sec. Overholts
Trametes mollis (Sommerf.) Fries
Sec. Overholts
Trametes rigida Berk. & Mont.
Moffatt

Trametes sepium Berk.
Overholts
Trametes serialis Fries
Sec. Overholts
**Trametes trogii* Berk.
University of Illinois collection

Acknowledgements

The author wishes to thank Dr. Wesley C. Whiteside for his help and encouragement during the early stages of this study. Special thanks are due Dr. Donald P. Rogers for his guidance during the final stages and his excellent help with taxonomical problems and identifications. Thanks are also due the many people who lent specimens for study, especially Dr. Anthony E. Liberta, Dr. Patricio Ponce de Leon, and Mr. K. Andrew West.

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A CENSUS OF MOULD SPORES IN THE ATMOSPHERE

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ABSTRACT.—Using Aurora, Illinois as the research area this study determines the ecology of atmospheric moulds by the culture plate method. 2,480 colonies have been counted, made up as follows: *Alternaria*, 992; *Penicillium*, 562; *Aspergillus*, 258; *Hormodendrum*, 223; *Fusarium*, 186; *Mucor*, 67; *Helminthosporium*, 63; *Yeast*, 60; *Pleospora*, 21; *Epicoccum*, 13; miscellaneous, 105. In addition to the above genera, less common air borne spores were found of the genera *Acremonium*, *Botryosporium*, *Mortierella*, *Phoma*, *Monilia*, *Chaetomium*, *Macrosporium*, *Trichoderma* and *Pullaria*.

This paper is concerned with ecological considerations of viable air borne fungus spores in Aurora, Illinois. The literature abounds with references indicating that mould spores are of especial economic significance, owing chiefly to their disease-inciting proclivities.

Alternaria solani cause tuber rot in potatoes (Falsom and Bonde, 1925),*Fusarium* cause wilting of cabbage (Armstrong and Armstrong, 1952), and tomatoes (Bohn and Tucker, 1940), *Aspergillus niger* cause crown rot of peanuts (Gibson 1953 a and b), cotton boll rot (Ray, 1946), black rot of onions (Venkatarayan and Delvi, 1951), spoilage of dates (Bliss 1946, Almandel 1961), grape rot (Gupta 1956), seedling blight (Leukel and Martin, 1943), bole rot (Wallace, 1952; Lock, 1962), garlic rot (Mathur and Mathur, 1958), stem rot (Natour and Miller, 1960), root rot (Alvarez and Diaz, 1949) and many other diseases (Christenson 1962, Durbin 1959).

In man asthma has been shown to be caused by *Aspergillus* (Bernton, 1930) by *Hormodendrum* (Cobe, 1932) by yeast (Taub, 1932) and by *Alternaria* by several workers (Hopkins, et al. 1930; Underwood, 1938 and 1941; Harris, 1939; Chobot, et al. 1940; and Durham, 1937). Conjunctivitis has been shown caused by *Alternaria* and *Hormodendrum* (Simon, 1938). *Aspergillus* also has been shown to cause respiratory infection (Hertzog, et al., 1949; Orie, et al., 1960), cardiovascular lesions (Merchant, et al. 1958; Hadorn, 1960), skin lesions (Frank and Alton, 1933; Sartory & Sartory, 1945), eye infections (Fine, 1962) and generalized infections (Cawely, 1947, Grcevic and Matthews, 1959).

Pady (1962) and others (Kramer et al., 1963) conclude that the knowledge of numbers and types of viable air spora is greatly needed, yet present spore sampling techniques give no information on the viability of the spore load. Furthermore some genera, particularly those like *Penicillum* and *Aspergillus*, are identifiable only by culture. This study employs nutrient plates to determine the ecology of viable air spora.

MATERIALS AND METHODS

During June and July, 1968, Petri dishes were exposed at several locations. The culture media used in this investigation were as follows:

1. Sabouraud Dextrose Agar
   TM-MFG slants received from Hyland Div. Travenol Laboratories Los Angeles, California, USA
   Lot 6285E8
   pH - 5.6
2. Mycology Agar
   TM-MFG slants received from Hyland Div. Travenol Laboratories Los Angeles, California, USA
   List #56-200 Lot 6200-D9
3. Potato Dextrose Agar
   Potatoes.....300 gm
   Dextrose..... 10 gm
   Agar..... 15 gm
   H_2O.....to make 1 liter
   Lactic Acid.....to make pH 4.5

4. Water Agar
   Agar..... 25 gm
   H_2O.....to make 1 liter
   Lactic Acid.....to make pH 3.5

Media No. 1 and 2 were tried at the outset of the experiment. Since medium No. 1 was found to produce higher rates of growth and larger numbers of colonies, medium No. 1 was used throughout June. There was no appreciable difference in the range of organisms yielded by medium No. 1 and medium No. 3, yet medium No. 3 was found to produce higher rates of growth. Therefore, medium No. 3 was used throughout the remainder of the exposure trials. Medium No. 4 was found to be best suited to accept transfer of fast growing colonies.

After exposure the plates were sealed with cellulose tape to prevent excessive drying and contamination. The cultures were then incubated in a dark chamber at room temperatures which varied from 65-80°F. The initial incubation period ranged from five to twelve days. Each different colony was transferred by flamed needle loop to a sterile plate and incubated for further observation.

In an effort to provide an ecological survey of the air borne fungi, it appeared wholly essential to classify the localized fungi according to recognized criteria. The majority of the genera were identified at 100X magnification with the use of an illustrated key (Barnett, 1960). In questionable cases, a higher magnification was used for examination of finer, structural details, and frequently bits of colony were removed with a needle loop and examined under a cover slip as a moist preparation. This technique frequently disclosed spores when they could not be seen previously.

A daily survey was undertaken for a month so as to increase the breadth of the study for the total effectiveness of obtaining conclusions of significance and repeatability. The exposure station was located in a position not immediately flanked by a taller structure, on a flat roof in a building in Aurora, Illinois at an elevation of 20 feet, 764 feet above sea level. Beginning August 1, 1968, four 90 mm Petri dishes were exposed consecutively for fifteen minutes daily between 7:00 p.m. and 8:00 p.m. for a period of thirty days (excluding August 3rd and 4th). Temperature was recorded at time of exposure and reported together with daily high and low values.

Results

The most abundant, identifiable air borne spores belong to the genera *Alternaria, Penicillium, Aspergillus, Hormodendrum* (*Cladosporium*), *Fusarium, Mucor,* and *Helminthosporium, Pleospora* and *Epicoccum* (Figures 1, 2, 3).

Of the 2480 colonies included in the survey, 2325 were placed in these nine genera (Figure 4). In addition to the above genera, less common air borne spores were found of the genera *Acremonium, Botryosporium, Mortierella, Phoma, Monilia, Chaetomium, Macrosporium, Trichoderma* and *Pullularia.*

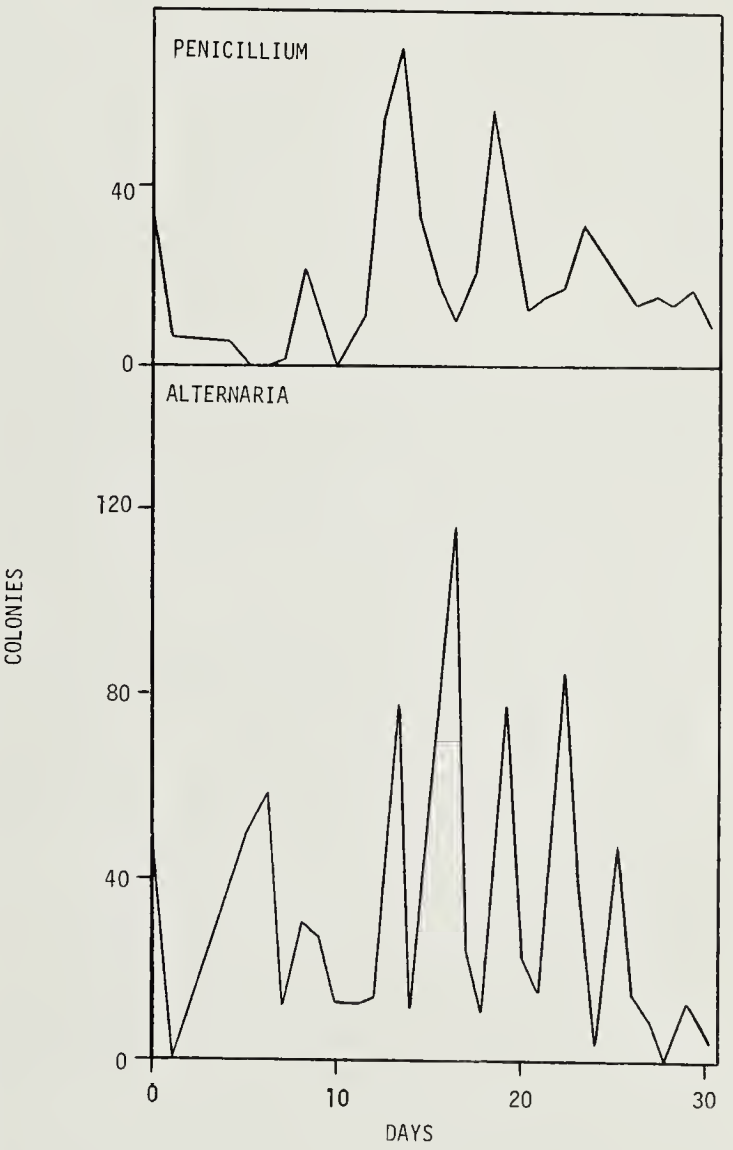

FIGURE 1. Fungus spore colonies at Aurora, Illinois. Each record represents the number of colonies recorded from 4, 90mm Petri plates exposed consecutively for 15 minutes daily from August 1, 1968 through August 31, 1968.

Discussion

Since many fungus spores are capable of inducing respiratory illness, it would be interesting to postulate how many spores a person might

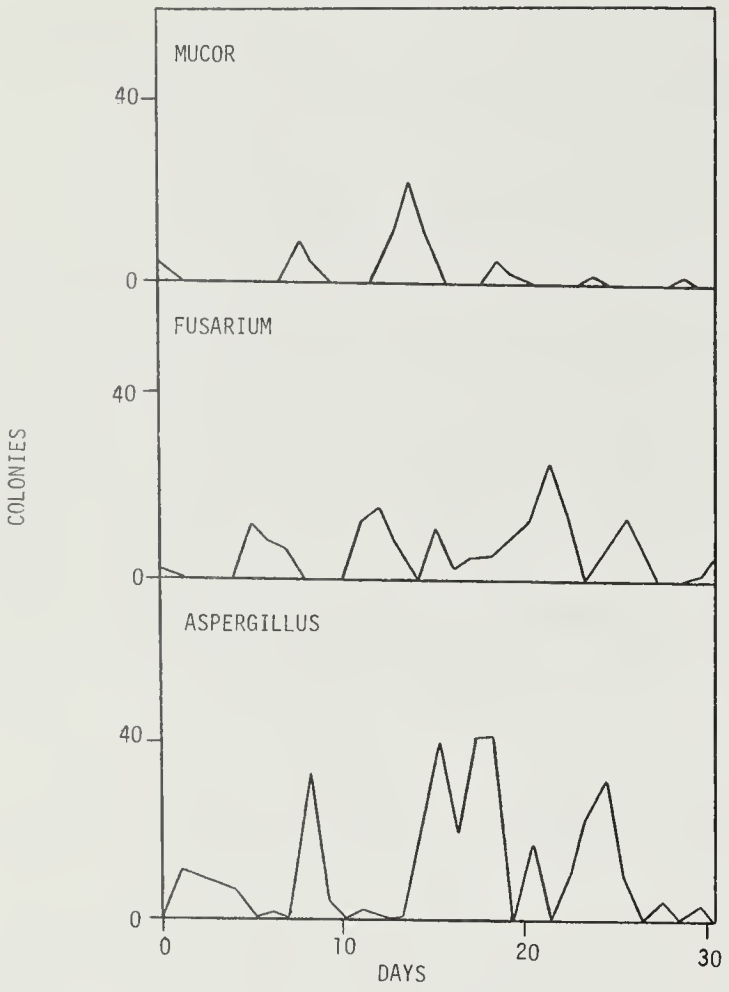

FIGURE 2. Fungus spore colonies at Aurora, Illinois. Each record represents the number of colonies recorded from 4, 90mm Petri plates exposed consecutively for 15 minutes daily from August 1, 1968 through August 31, 1968.

inhale during a specific day. For example let us take *Alternaria* at Aurora, Illinois on August 17, 1968. By consulting our data (Table 1) for *Alternaria* at Aurora on the date specified we find 116 colonies per Petri plate (90 mm) per hour, or at least 37 spores per cm. per 24 hours. Assuming each colony was started by at least one spore, (however, several spores may have been clumped and this number may be much greater), we can now obtain the number of spores per cubic yard of air by multiplying by 14.90, the conversion factor for *Alternaria* (Durham, 1946). This gives 551 spores per cubic yard of air. Since an average adult male has a lung capacity (tidal air) of approximately 500 cubic centimeters, and breathes between sixteen and eighteen times per minute, he would inhale approximately 12,240,000 cubic centimeters (12.24 cubic centimeters or 16 cubic yards) of air in a 24-hour period. Since there are 551 spores in each cubic yard of air, he would inhale approximately 8816 *Alternaria* spores in a 24-hour period.

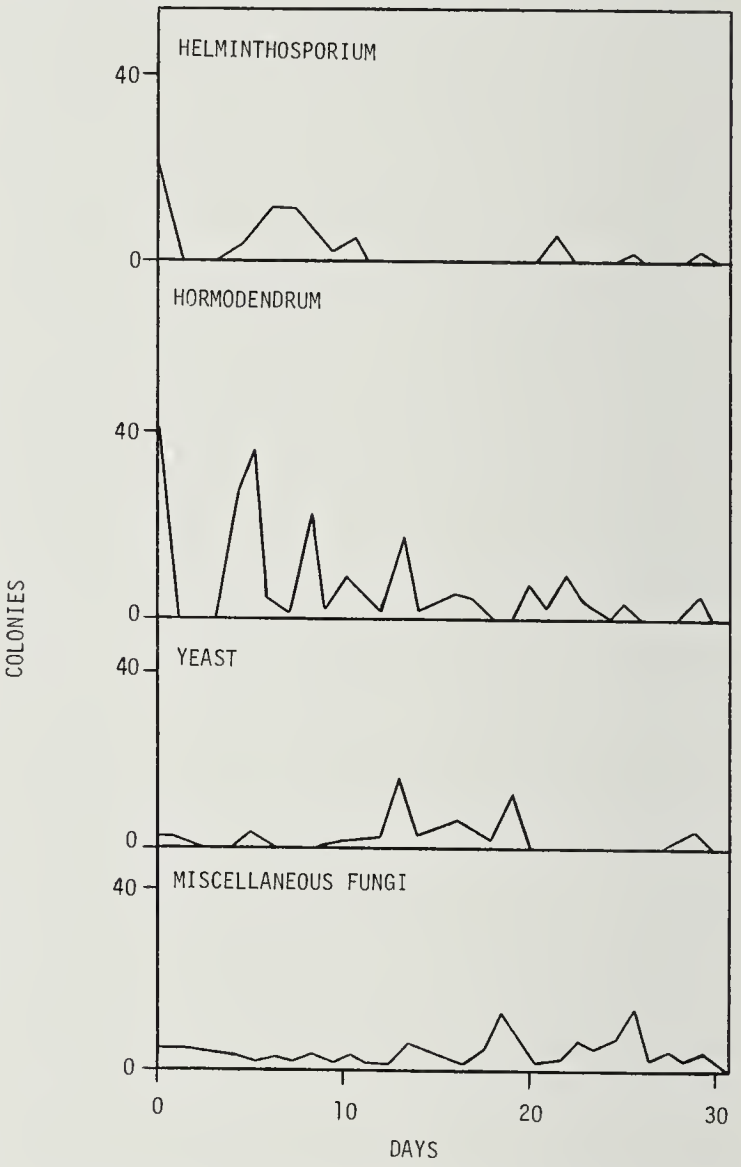

FIGURE 3. Fungus spore colonies at Aurora, Illinois. Each record represents the number of colonies recorded from 4, 90mm Petri plates exposed consecutively for 15 minutes daily from August 1, 1968 through August 31, 1968.

Basic weather data were obtained through Dr. Clarence F. Smith, Professor of Physics, Aurora College, and an attempt was made to correlate the prevalence of fungus spore colonies with weather data. It was found that the initial rainfall was heavily laden with fungus spores. The data show that the number of genera increases with rain cloud altitudes. (Table 3). It was also observed that the perimeters of pits formed by raindrops

TABLE 1. Fungus spore colonies at Aurora, Illinois. Each record represents the number of colonies recorded from 4, 90 mm Petri plates exposed consecutively for 15 minutes daily from August 1, 1968 through August 31, 1968.

Alternaria	*Aspergillus*	*Epicoccum*	*Fusarium*	*Helmintho-sporium*	*Hormodendrum*	*Mucor*	*Penicillium*	*Pleospora*	Yeast	Misc. Fungi	Daily Total Count	Date
47	0	0	3	17	42	2	31	0	2	4	148	8/1
2	10	0	2	0	0	0	7	0	2	4	27	8/2
NO RECORD TAKEN												8/3
NO RECORD TAKEN												8/4
39	6	0	0	4	27	0	6	1	0	2	85	8/5
48	0	1	13	0	38	0	1	0	3	1	105	8/6
59	2	0	8	12	4	0	0	0	0	3	88	8/7
12	0	0	7	12	0	9	2	0	0	1	43	8/8
31	30	0	0	0	23	5	21	2	0	3	115	8/9
27	4	0	0	0	0	0	10	0	0	1	47	8/10
12	0	0	0	5	9	0	0	2	1	1	30	8/11
12	2	0	13	0	6	0	9	1	1	6	50	8/12
13	0	2	16	0	3	10	55	0	2	4	105	8/13
77	0	0	7	0	17	21	69	0	15	3	209	8/14
10	19	0	0	0	3	9	32	0	2	1	76	8/15
64	37	0	12	0	4	0	20	0	4	4	145	8/16
116	17	5	2	3	8	0	10	7	6	12	176	8/17
23	0	0	5	0	5	0	20	0	4	6	68	8/18
9	40	2	6	0	0	6	56	0	1	1	121	8/19
73	0	0	3	0	0	2	32	3	12	2	127	8/20
21	16	0	14	0	9	0	12	0	0	5	77	8/21
14	0	0	27	7	2	0	16	0	0	4	70	8/22
82	9	0	13	0	11	0	17	0	0	6	138	8/23
39	21	0	0	0	4	2	32	0	0	13	111	8/24
2	31	0	7	0	0	0	26	0	0	1	67	8/25
46	8	0	15	1	3	0	9	0	0	4	86	8/26
14	0	0	6	0	0	0	13	5	0	3	41	8/27
9	4	3	0	0	0	0	16	0	0	3	35	8/28
0	0	0	0	0	0	1	14	0	2	2	19	8/29
13	2	0	2	2	5	0	17	0	3	4	48	8/30
8	0	0	5	0	0	0	9	0	0	1	23	8/31
922	258	13	186	63	223	67	562	21	60	105	2480	TOTAL
37.18	10.40	0.52	7.50	2.54	8.99	2.70	22.67	0.85	2.42	4.23	= 100%	

FIGURE 4. Fungus spore colonies at Aurora, Illinois. Each record represents the number of colonies recorded from 4, 90mm Petri plates exposed consecutively for 15 minutes daily from August 1, 1968 through August 31, 1968.

were covered by colonies of fungi. This suggests that a raindrop collects spores as it falls through air. Although the colonies were too numerous to be counted, observations of the culture plates showed greater numbers of colonies when they were derived from raindrops originating at high rather than from low altitudes.

In general, the number of fungus spore colonies was highest during the beginning of the rain showers but were few at the end. Even though the air tends to be washed free of fungus spores during a prolonged rain, there is no assurance that a distant mass of air with its attendant high concentration of spores will not invade the area soon after the storm has passed.

Hamilton (1959) recorded the temperature at which different spore types were in the air in maximum numbers. She reported the optimum temperatures within a 4°F range for each spore category, and constructed graphs showing the effect of temperature on spore counts. This effect was not readily observed in this investigation. For example, on August 17th, the highest count of *Alternaria* was recorded (Table 1) at temperatures ranging from 68-66°F (Table 2). On August 23rd, the next highest count of *Alternaria* was recorded at temperatures ranging from 88-89°F. In fact, exact correlation between temperatures and spore incidence is not readily evident for any genus in this investigation (Figures 1-6). Perhaps local effluvia differ and thus the irre-

TABLE 2. Data collected from culture plates exposed during rain showers on the dates given.

August 10th (Precipitation 0.86″) clouds altitude 500 ft.		*August 17th* (Precipitation 3.83″) clouds altitude 21,000 ft.	
Alternaria	27	*Alternaria*	116
Aspergillus	4	*Aspergillus*	17
Penicillium	10	*Epicoccum*	5
Unidentified	1	*Fusarium*	2
Indistinguishable	over 400	*Helminthosporium*	3
		Hormodendrum	8
		Penicillium	10
		Pleospora	7
		Yeast like	6
		Unidentified	12
		Indistinguishable	over 400

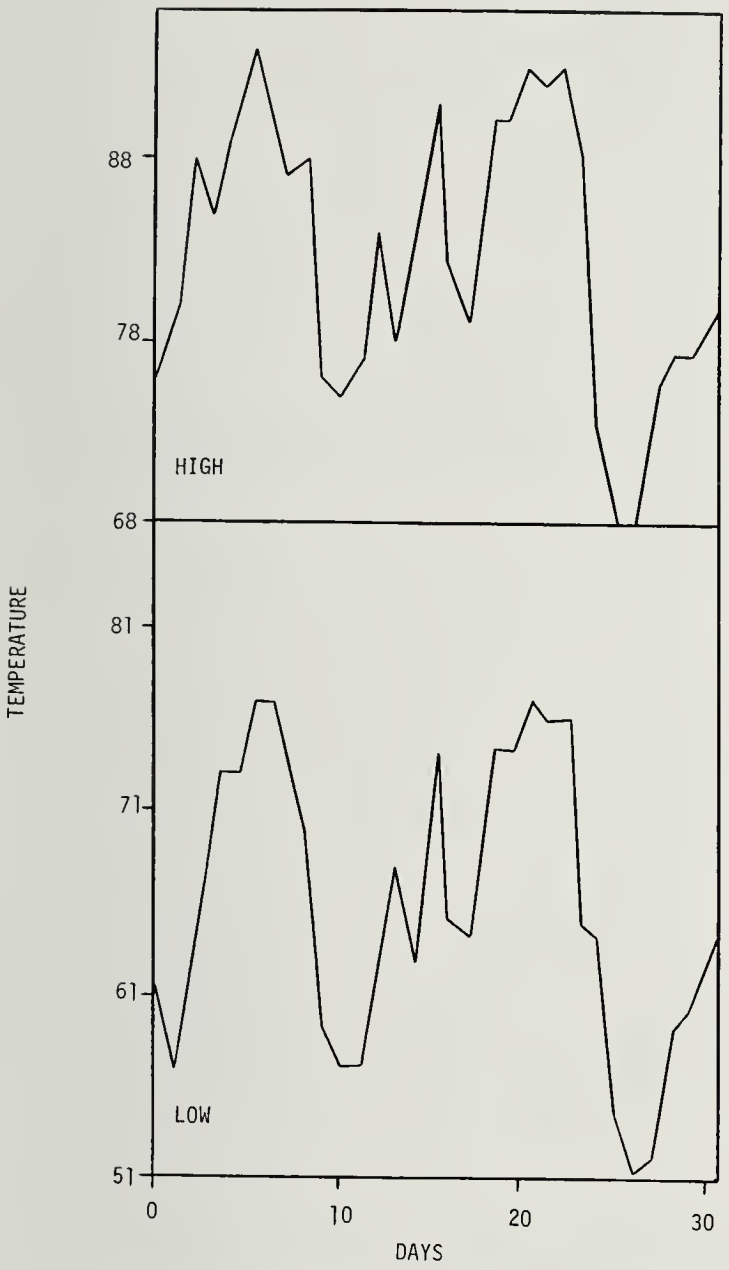

FIGURE 5. High and low temperatures recorded at Aurora, Illinois, daily from August 1, 1968 through August 31, 1968.

concilable disparity between these findings and those of Hamilton.

It was observed (Table 3) that mowing of grass produces a great and immediate local increase in fungi spore count. This is evidence that human activity and local flora may play a part in affecting atmospheric spore concentration. Lacey (1962) has shown that local flora may influence air spora.

The study has produced information which is representative of the viable air borne fungus spore content of the air (Figures 1-4). It

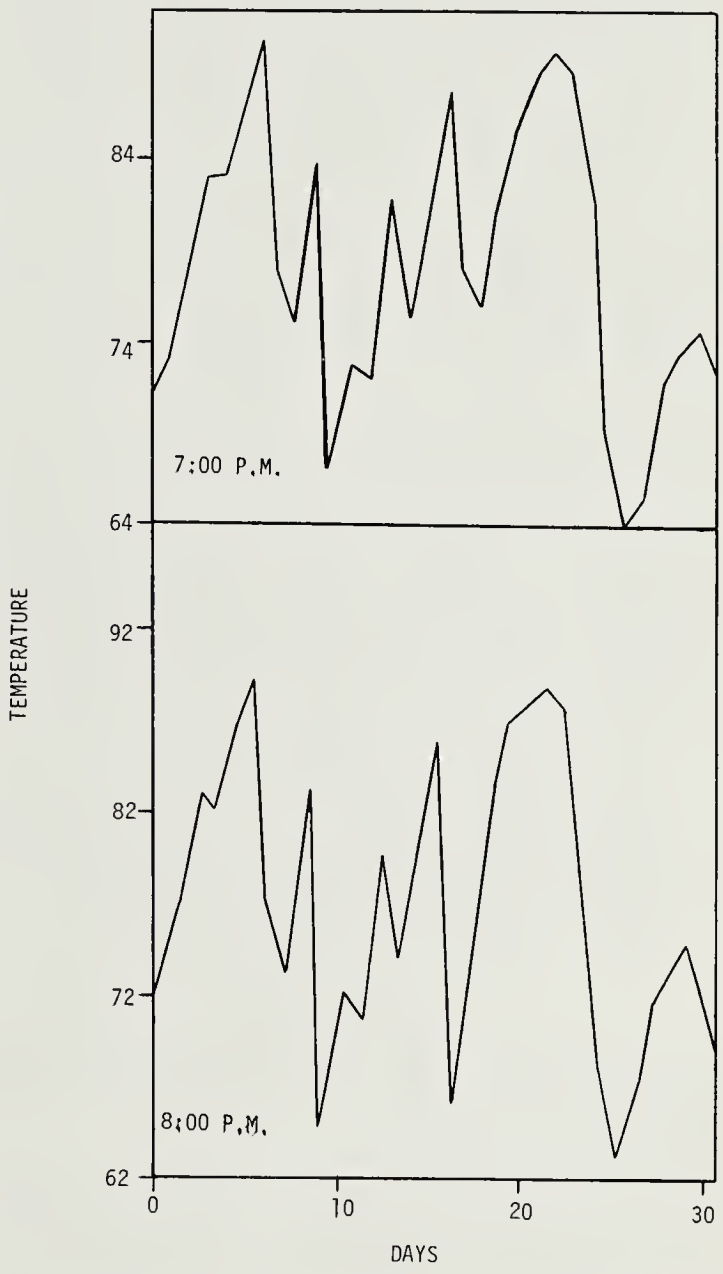

FIGURE 6. 7:00 P.M. and 8:00 P.M. temperatures recorded at Aurora, Illinois, daily from August 1, 1968 through August 31, 1968.

TABLE 3. Data collected from culture plates exposed one foot above the ground in the center of a 1/4 acre lawn before and after mowing.

August 30th Before mowing. (One plate exposed 15 min.)		*August 30th* After mowing. (One plate exposed 15 min.)	
Alternaria	7	*Alternaria*	34
Aspergillus	2	*Aspergillus*	11
Hormodendrum	3	*Hormodendrum*	51
Fusarium	1	*Helminthosporium*	5
Unknown	7	*Fusarium*	15
		Penicillium	21
		Unknown	2

seems very likely that natural selection has tended to limit the spores transported by air. Most of the air borne fungi identified in this investigation are those that have special adaptations for aerial dissemination. Usually they are somewhat thick walled or are able to resist dessication, are prolific sporulators, are commonly found in nature, and have some of the widest host ranges.

Conclusions

The results of the nutrient plate study reveal that certain viable fungi are present in the air throughout at least a 3-month period but vary in quantity. It is evident that the fungus spore content of the atmosphere at any given time may originate from unknown or local sources. The fungus spore shower records point out that the source may be from unknown altitudes.

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INHIBITION OF LEUKOCYTE INDUCED GERMINATION AND TOXIN RELEASE FROM *CLOSTRIDIUM BOTULINUM* TYPE A SPORES BY CHLOROCRESOL.

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ABSTRACT.—Chlorocresol (0.44 mM) inhibits initiation of germination and subsequent toxin release from spores of *Clostridium botulinum* type A Strain 33A in an *in vitro* guinea pig-polymorphonuclear leukocyte culture as well as in trypticase-peptone broth. In both systems without chlorocresol, labeled *C. botulinum* spores released previously bound ^{45}Ca at 6-8 hrs indicating that normal germination had occurred. Release of spore-bound botulinum toxin correlated well with spore germination, however, toxin release was totally inhibited for at least 48 hrs by 0.44 mM chlorocresol. These experiments support the idea that germination of spores of *C. botulinum* is an essential step before spores can release spore-bound toxin, thus, becoming pathogenic and that inhibition of germination may be useful in preventing this type of pathogenesis.

The *in vivo* fate of *C. botulinum* spores upon intramuscular (Keppie, 1951) and intraperitoneal (i.p.) (Booth, *et al.* 1970, Grecz and Lin, 1968, Grecz *et al.* 1967) injections have been studied with respect to pathogenicity of these spores. The results to date indicate that in order to release spore-bound toxin with fatal consequences the spores of *C. botulinum* type A must first germinate. The changes which accompany germination and outgrowth includes - among others - appearance of stainability, release of calcium, and loss of heat resistance (Murrell, 1961, Hansen, *et al.* 1970); studies of these changes in relation to pathogenesis of *C. botulinum* spores are summarized below.

Staining of peritoneal exudates from guinea pigs injected i.p. with 10^9 *C. botulinum* spores revealed macrophages and polymorphonuclear (PMN) leukocytes with phagocytized spores in various stages of germination (Booth, *et al.* 1971). Further studies on isolated guinea pig PMN leukocyte-*C. botulinum* spore systems utilizing ^{45}Ca release from labeled spores as indication of germination revealed that spore germination occurred at 6-8 hrs (Suzuki, *et al.* 1971a). Heat resistant (80C, 15 min) spores were converted to heat-sensitive cells at 4-8 hrs in an *in vitro* leukocyte *C. botulinum* spore system (Suzuki, *et al.* 1970), i.e. at a time correlating with ^{45}Ca release. In the same report, using mice i.p. assay, botulinal toxin was released into the media also at 4-8 hrs.

Thus, germination appears to be an essential step for pathogenesis of spores of *C. botulinum*. Therefore, it was of practical and theoretical importance to examine germination inhibitors for control of this process. Finding an inhibitor (chlorocresol) which does not affect PMN leukocyte viability and function provided us with an important tool to study the mechanism of pathogenesis by *C. botulinum* spores as described in the present paper.

MATERIALS AND METHODS

C. botulinum spores:

A culture of *C. botulinum* type A strain 33A was aseptically inoculated into a medium containing 5% Trypticase (BBL), 0.5% peptone (Difco), 0.1% sodium thioglycolate (V/V) (TP broth) and 20 microcuries of ^{45}Ca (Tracer Lab. Waltham, Mass.). ^{45}Ca labeled spores were harvested in a Sorval continu-

ous flow centrifuge (4500 X g, 45 min.) and cleaned with trypsin and lysozyme by the method of Grecz, *et al.* (1962).

Experimental Animals:

Hartley strain male guinea pigs were raised for 6 generations in a thermostatically controlled room at the Illinois Institute of Technology. They were contained in metal cages with a solid floor and were watered and fed *ad libitum* with Rockland guinea pig diet with weekly supplements of lettuce and greens. Guinea pigs attained a weight of 600+ grams before used.

White Swiss mice were raised for 10 generations under similar conditions and were watered and fed *ad libitum* with Rockland Rat and Mouse diet. All mice weighed 25 grams before i.p. botulinal toxin assays were performed.

PMN Leukocytes:

A suspension of 85-90% polymorphonuclear (PMN) leukocytes were obtained from guinea pig peritoneal exudates by a modified method of Sbarra and Karnovsky (1959) and explained in detail in these *Communications* (Suzuki, *et al.* 1970).

In vitro System:

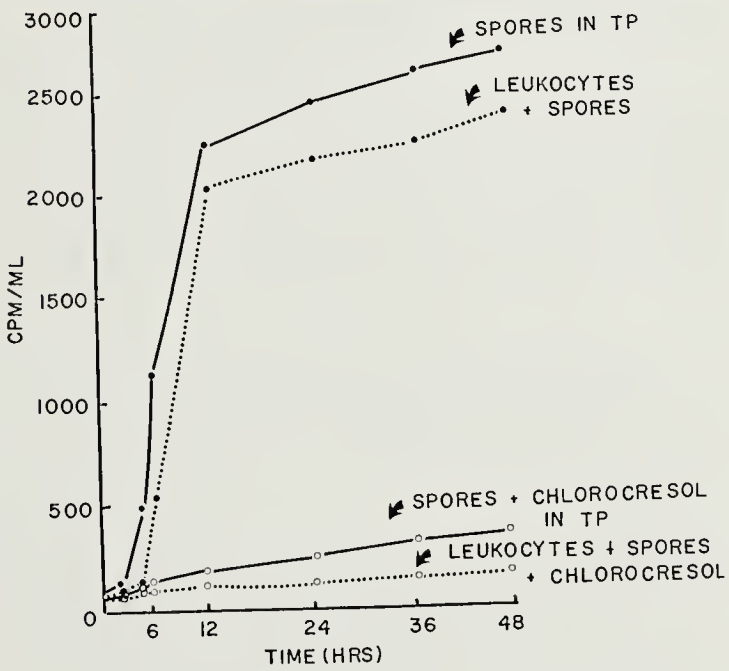
One ml of 1 x 10^8 PMN leukocytes/ml was added to a siliconized screw-cap tube containing 4 ml Krebs-Ringer Phosphate Medium, pH 7.4 (KRPM) and 3 ml non-immune guinea pig serum (Animal Blood Center, Syracuse, N.Y.) and allowed to equilibrate 15 min at 37C in a slowly shaking water bath. A similar system has been described for phagocytic index determinations (Suzuki, *et al.* 1971). One ml of 1 x 10^9 ^{45}Ca labeled *C. botulinum* type A spores and 1 ml 4.4 mM chlorocresol (p-chloro-m-cresol, Fisher) were then added to the guinea pig PMN leukocyte suspension and zero time marked. Chlorocresol is an established inhibitor of spore germination (Sierra, 1970, Parker and Bradley, 1968). At time intervals (0,2,4,6,12,24,36, and 48 hrs), a 1.1 ml sample was pipetted and filtered through a Millipore Swinney (Millipore Filter Corp., Bedford, Mass.) containing a 0.22 micron filter. One-tenth ml of the filtrate was placed in a glass scintillation vial (Amersham Seale, Des Plaines, Ill.), 10 ml Bray's solution (Bray, 1960) added, and kept at 14C until radioactivity was counted. The remaining filtrate (1 ml) was assayed i.p. into mice for presence of botulinal toxin. Control mice were passively immunized with botulinal type A antitoxin. An identical *in vitro* system in TP broth without PMN leukocytes and botulinal toxin assay were carried out concurrently and 0.22 micron filtrates treated as above.

Radioactivity:

Scintillation vials were counted at 14C for 10 min in a Beckman LS-200 liquid scintillation Counter using a P-32 window.

Results

Figure 1 illustrates ^{45}Ca release appearing in 0.22 micron filtrates



[a]Average of 5 experiments.
Background has been subtracted.

FIGURE 1. Effect of Chlorocresol on Released ^{45}Ca in Filtrate (0.22*u*). From Incubation of 1 X 10^8/ml labeled *C. botulinum* type A spores and 1 X 10^7/ml PMN Leukocytes at 37C.[a]

when 1 x 10^9 labeled *C. botulinum* type A spores are incubated with 1 x 10^8 guinea pig PMN leukocytes or TP broth both in the presence and absence of 0.44 mM chlorocresol. Labeled *C. botulinum* type A spores in TP broth or in an in vitro PMN leukocyte culture exhibited normal germination patterns substantiating a recent report (Suzuki, *et al.* 1971). Chlorocresol, final concentration of 0.44 mM, introduced into either of the above systems, inhibits germination of *C. botulinum* spores.

Release of botulinal type A toxin into cell free extracts as determined by mice i.p. assay in presence of 0.44 mM chlorocresol and in control systems are summarized in Table 1. From 0-4 hrs of spore incubation in all conditions, lethal amounts of botulinal toxin could not be detected. At 6-48 hrs without chlorocresol added to TP broth or leukocyte cultures, *C. botulinum* spores did invariably release lethal amount of botulinal toxin. However, chlorocresol in either system demonstrated a remarkable inhibition of toxin release into cell free 0.22 micron filtrates.

Trypan blue dye exclusion tests on PMN leukocytes bathed in 0.44 mM chlorocresol demonstrated negligible loss in cell viability from control PMN leukocytes. Phagocytic indices were determined using radioisotope labeling by the method of Suzuki, *et al.* (1971b). Values for chlorocresol-treated PMN leukocytes were within normal limits, i.e. 61-64% spores being engulfed.

Discussion

The effect of chlorocresol on bacterial spore germination has been documented (Sierra, 1970; Parker and Bradley, 1968) and is substantiated in the present communication. However, the use of chlorocresol in reducing or completely inhibiting leukocyte induced bacterial spore germination is novel. Furthermore, in establishing blockage of *C. botulinum* type A spore germination, the requirement for the pathogenesis of these spores to first germinate and then release toxin can be supported.

Using the release of ^{45}Ca from labeled *C. botulinum* type A spores as evidence of germination, it was observed that germination did indeed occur between 6-8 hrs in TP broth or *in vitro* in guinea pig PMN leukocyte cultures. Botulinal toxin was found to be released from both systems, also at 6-8 hrs, suggesting either: 1) toxin is released synchronously from spores as they germinate, or 2) toxin is released from germinated spores and vegetative

Table 1. Death of i.p. injected mice demonstrating effect of chlorocresol on Release of Type A botulinal toxin in 0.22 Micron Filtrates from *C. botulinum* type A spores when incubated under the indicated conditions.

Time of incubation at 37C Hrs a) b)	Number of mice expired of a total of 5 injected with cell free filtrates			
	Without Chlorocresol		With 0.44 mM Chlorocresol	
	10^9 spores in TP-broth c)	10^9 spores + 10^8 leukocytes	19^9 spores in TP-broth c)	10^9 spores + 10^8 leukocytes
0-4	0	0	0	0
6-48	5	5	0	0

a/5 mice injected i.p. with 0.22 micron cell free filtrates taken at each of the following time intervals: 0,2,4,6,12,24,36 and 48 hrs.

b/All mice protected with anti-botulinal type A toxin survived

c/TP = 5% Trypticase, 0.5% peptone, 0.1% sodium thioglycollate broth

cells after being degraded by leukocytes or autolysis in TP broth.

The first suggestion conflicts with reports of Tang and Grecz (1968) that toxin was transferred from heat-resistant spores to heat-sensitive cells and not released into the surrounding medium (phosphate buffer) during this conversion. Therefore, the second suggestion seems more tenable at this point. Preliminary experiments by our laboratory, utilizing PMN leukocyte metabolic inhibitors further support this view (Suzuki, *et al.* 1971). Relationships of leukocyte metabolism to *Clostridium botulinum* pathogenesis. (Manuscript in preparation).

Lethal amounts of botulinal toxin were released into the surrounding media at times (6-8 hrs.) correlating with or subsequent to *C. botulinum* spore germination. Chlorocresol prevented the release of lethal amounts of botulinal toxin while inhibiting spore germination. Thus, the data strongly suggests that *C. botulinum* type A spores must germinate before becoming pathogenic, and that inhibition of germination may be useful in preventing this type of pathogenesis.

Acknowledgement

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ECOLOGICAL STUDY OF A HILLSIDE MARSH IN EAST-CENTRAL ILLINOIS

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ABSTRACT.—The marshes studied are located about 5 miles east of Charleston, Coles County, Illinois. They are divided into five areas based on the topography, shading, moisture, and grazing and form habitats in which many plants occur that are rare to east-central Illinois. Nineteen species of woody plants occur in the marshes while the herbaceous vegetation consists of 70 species, of which 3 are fern or fern-allies, 27 are monocots, and 40 are dicots. Two distinct herbaceous communities exist in the marsh areas depending upon the amount of shading. The first is an *Impatiens biflora* dominated community in shaded areas, while one of more open areas consists of *Glyceria striata, Pilea fontana, Agrostis alba* and *Carex lurida.*

Five miles to the east of Charleston, Coles County, Illinois, exist a series of small marshes which are unique in that they occur on the side of a hill. These hillside marshes are the result of seepage from a 2 m layer of gravel between two layers of clay. The upper clay layer is about 7 cm thick and allows some water penetration through it, while the lower layer is much thicker and does not allow water to seep through. The water accumulates in the band of gravel and is carried to the outlet areas which form the seepline of the marshes. The resulting marshes form habitats in which many plants occur that are rare to east-central Illinois.

This unique area was first studied by Phipps and Speer (1958), who revealed a number of new plant records for Coles County, Illinois. Parker, Rayhill and Ebinger (1970) also studied this marsh complex and reported 56 new plant records. The presence of this type of flora indicated that a more intensive study of the area was necessary to determine the relationships between these plants and the habitat. In addition, a thorough study was warranted due to the possible destruction of the marshes by the proposed Lincoln Reservoir. When the Reservoir is completed, the normal pool level will be at an elevation of 629 feet above sea level, 30 feet above the level of the marshes.

METHOD OF STUDY

This study was conducted during the growing seasons of 1967 and 1968. At the beginning of the first growing season 20, one meter rectangular plots were randomly located throughout the marshes. Every two weeks during the growing season the species found in these plots were identified and counted. From this data the density per square meter was determined for each species.

An abundance study of the herbaceous plants was undertaken during the growing season of 1968 (Acocks, 1953). A mean distance was determined after a series of measurements were made between individuals of the same species and its abundance class determined by using the following scale:

Symbol	Meaning	Distance
VA	Very Abundant	3 inches apart
A	Abundant	6 inches apart
VC	Very Common	1 foot apart
C	Common	1.5 feet apart
VF	Very Frequent	2 feet apart
F	Frequent	3 feet apart

F –		6 feet apart
FF	Fairly Frequent	12 feet apart
FO	Fairly Occasional	30 feet apart
O	Occasional	50 feet apart

The letter "L" is used after the above letters to indicate local abundance.

A diameter class study of the woody species found in the marsh areas was undertaken during the summer of 1968. These diameter classes were based on diameter at breast height (d.b.h.) and consisted of seedlings (less than 1 inch d.b.h.), saplings (1-4 inches d.b.h.) and trees (more than 4 inches d.b.h.).

The taxonomic nomenclature used throughout this paper follows that of Jones (1963).

Results and Discussion

The marshes are located in a small valley which enters the Embarrass River a short distance south of where Illinois Route 16 crosses the Embarrass River (NE1/4, SW1/4, Section 4, T12N, R10E). The valley floor is at an elevation of 590 feet and the ridges average 655 feet above sea level. The hillsides are relatively steep, with the slope ranging from 40 to 60 degrees. All of the marshes considered in this study are found on the northwest-facing hillside and slope to the west from a seepline that is about 10 feet above the valley floor. The soil of the marshes is mainly peat, which in some places reaches a depth of 1-1/4 m.

The vegetation immediately surrounding the marshes was studied to determine the ecological conditions and cover types in which the marshes are located (Figure 1). From the lower edge of areas A, B, and C to the creek is a grazed, lowland woods which has an overall variation in topography of about 2 m. *Juglans nigra*, *Quercus macrocarpa*, and *Carya cordiformis* are the dominant overstory trees here while *Crataegus mollis* and *C. crusgalli* dominate the understory. From the lower edge of areas D and E to the creek is an ungrazed, lowland woods which has an overall variation in topography of about 2 m and is relatively wet. The dense overstory consists of *Juglans nigra*, *Platanus occidentalis*, and *Acer saccharum*. The understory is dominated by *Carpinus caroliniana*, *Cercis canadensis*, and *Ostrya virginiana*. From the upper edge of areas A, B, and C to the summit of the hill is a heavily grazed, open woods. This area is relatively dry and is dominated by *Quercus alba*, *Q. velutina*, and *Q. rubra*. The thorny species (*Crataegus* spp. and *Malus ioensis*) dominate the understory. From the upper edge of areas D and E to the summit of the hill is an ungrazed, mesic woods. The vegetation here is more mature than in any other region surrounding the marshes. The dominant species of this hillside are *Quercus alba*, *Q. velutina*, *Q. rubra*, and *Acer saccharum* while the understory is dominated by *Ostrya virginiana*, *Cornus florida*, and *A. saccharum*.

A total of 19 species of woody plants are found in the marshes, but none has a d.b.h. above 4 inches. Table 1 lists the actual number of seedlings and saplings found in each of the marsh areas studied. The herbaceous vegetation is also not extremely diverse and only 70 species are present. Of these, 3 are fern or fern-allies, 27 are monocots and 40 are dicots. The commonly encountered herbaceous species are listed in Tables 2 and 3.

Two major herbaceous communities exist in the marshes. These result from the amount of shading due to the overstory trees and vary to some extent in composition depending upon local habitat conditions. In the parts of the marshes

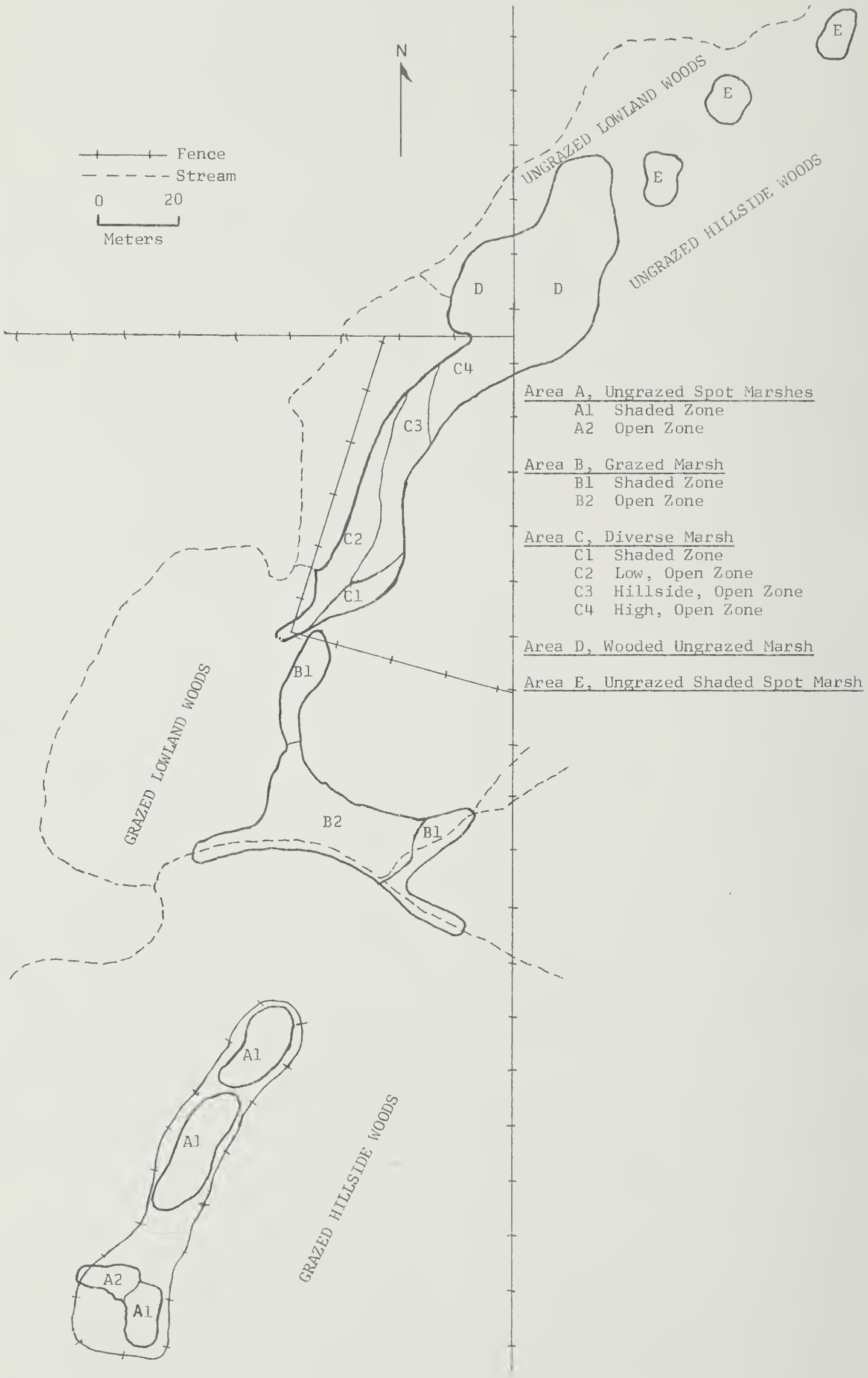

FIGURE 1. Map showing the marsh areas studied.

TABLE 1. Total number of seedlings and saplings of the various species of woody plants found in the zones of the marsh areas studied.

	Area A				Area B				Area C								Area D		Area E	
	(A1) Shaded Zone		(A2) Open Zone		(B1) Shaded Zone		(B2) Open Zone		(C1) Shaded Zone		(C2) Low, Open Zone		(C3) Hillside, Open Zone		(C4) High, Open Zone		Wooded, Ungrazed Marsh		Shaded, Ungrazed Spot Marshes	
	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling
Salix discolor	24	23	..	..	..	..	25	4	..	..	5	1	50	33	15	3	6	64	9	..
Salix rigida	7	2	..	..	..	..	1	..	..	..	..	..	3	1	..	..	8884	..	4	..
Carpinus caroliniana	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	14	3	1	..
Ulmus americana	4	..	..	..	..	..	1	..	..	..	..	1	..	1	..	..	6	4	..	..
Ulmus rubra	3	..	..	..	..	..	..	..	..	..	..	..	1	2	..	..	....	..	..	..
Gleditsia triacanthos	3	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	..	..	..
Juglans nigra	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
Rhus vernix	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	6	..	..
Others	8	6	..	..	..	..	..	..	..	..	..	3	30	14	..	..	....	..	..	..
TOTALS	50	34	..	..	..	..	27	4	..	..	5	5	84	53	15	3	8911	77	14	..

TABLE 2. Abundance classes and density per square meter for the dominant species of the densely shaded areas of the hillside marshes. For explanation of abundance class symbols see the *Method of Study* section of this paper.

Species	Abundance Classes by Areas				Density per square meter
	Area A1	Area B1	Area C1	Area E	
Impatiens biflora	VA	VA	A	A	17.0
Glyceria striata	C	VC	F –	F	4.8
Carex lurida	FF	VF	VC	CL	0.4
Cardamine bulbosa	FF	C	FF	VF	2.0
Pilea fontana	...	AL	VA	FF	0.2
Caltha palustris	F	F	...	F	2.0
Solidago patula	F –	FF	FF	F	4.0
Aster lateriflorus	FF	F	F –	FF	1.6
Oxypolis rigidior	O	...	F –	FF	1.0
Equisetum arvense	VF	FF	...	O	0.2
Cinna arundinacea	O	FF	F –	FF	...
Others	...	...	...	...	4.4
TOTAL	...	...	...	...	37.6

that are densely shaded, *Impatiens biflora* is the dominant species encountered (Table 2). In all of the shaded zones of areas A, B, C and E this species varies in abundance classes from abundant to very abundant. Numerous seedlings of this species occur in the shaded parts of the marsh early in the growing season, but most do not reach maturity. *Impatiens biflora* averages 17 individuals per sq m which accounts for nearly half of the density observed in the plots of the shaded zones. The commonly encountered associated species are *Glyceria striata, Carex lurida, Cardamine bulbosa,* and *Pilea fontana.* In most instances these species are scattered, rarely exceeding an abundance class of frequent. The only exception being *Pilea fontana* which replaces *Impatiens biflora* in a few small areas.

At the edge of the shaded parts of the marshes a very narrow transition zone exists where *Impatiens biflora* rapidly decreases in abundance while *Glyceria striata* and *Carex lurida* greatly increase, becoming very frequent to abundant. Also, *Agrostis alba* and *Juncus dudleyi,* which did not occur in the shaded parts of the marsh, become common to very abundant. *Pilea fontana* also becomes more abundant, but always as small plants, less than 3 inches tall, that persist under the larger herbaceous plants. This second community is considered to be of an open overstory type and is seldom shaded through the day (Table 3). It is dominated by *Glyceria striata, Pilea fontana, Agrostis alba* and *Carex lurida* which collectively make up about one-half of the total density per sq m and are classed as very abundant to very frequent.

The hillside marshes are divided into five areas based on the topography, shading, moisture, and grazing. Starting with the southernmost marsh, the various marsh areas are listed below (Figure 1).

Area A.— Ungrazed, Spot Marshes: This group of three marshes was fenced in 1956 and has not been grazed since that time. Two distinct zones exist here, depending

TABLE 3. Abundance classes and density per square meter for the dominant species of the open areas of the hillside marshes. For explanation of abundance class symbols see the *Method of Study* section of this paper.

Species	Abundance Classes by Area					Density per square meter
	Area A2	Area B2	Area C2	Area C3	Area C4	
Glyceria striata	VC	VC	A	A	A	16.5
Pilea fontana	....	VA	VA	VA	VCL	32.3
Agrostis alba	VA	C	C	VC	C	9.6
Carex lurida	VFL	A	VC	A	A	7.1
Juncus dudleyi	....	A	VC	C	VC	5.1
Equisetum arvense	....	....	C	VC	VC	5.3
Selaginella apoda	....	VC	C	FF	FF	4.5
Eleocharis erythropoda	....	A	A	....	....	13.6
Eupatorium perfoliatum	F–	F	FF	F	F	3.6
Solidago patula	F	F	F	VF	VF	2.9
Pedicularis lanceolata	VF	F–	FF	F	VF	1.1
Caltha palustris	....	O	VC	FF	A	2.4
Aster lateriflorus	F	F–	FF	....	FF	2.0
Chelone glabra	AL	O	F–	F	F–	1.3
Lobelia siphilitica	F–	F–	FF	F–	FF	1.2
Scirpus atrovirens	C	VC	VFL	VFL	VFL	.6
Oxypolis rigidior	VF	F–	F–	F–	F	.7
Carex vulpinoidea	FL	F	CL	FL	CL	.5
Others	....	....	....	....	....	26.5
TOTAL	....	....	....	....	....	136.8

on the degree of shading due to trees surrounding the marshes. The shaded zone is dominated by the *Impatiens biflora* community while *Salix discolor* is the dominant woody species (Table 1). The presence of woody species is probably due to the lack of grazing in this zone for the last 14 years. A small section of the southern-most spot marsh is in full sunlight (Table 3, Area A2). The herbaceous vegetation of this zone is dominated by *Glyceria striata, Agrostis alba,* and a third species of grass, *Leersia oryzoides* which did not occur in the other open marsh areas.

Area B.—Grazed Marsh: Thirty meters to the north of Area A is a boot-shaped marsh consisting of 0.17 acres which has been grazed for at least the past 20 years. This marsh is extremely wet and standing water is found in the depressions most of the year. Its northern and extreme eastern edge are heavily shaded by surrounding trees. This shaded zone is dominated by *Impatiens biflora* while *Pilea fontana* and *Glyceria striata* are important associated species (Table 2, Area B1). The remainder of this marsh area is in full sunlight for most of the day and is dominated by the *Glyceria striata, Pilea fontana, Agrostis alba* and *Carex lurida* community (Table 3, Area B2). Also, *Scirpus validus* is very abundant here but did not occur in the other marsh areas. All of the woody plants observed in the grazed marsh occurred here (Table 1).

Area C.—Diverse Marsh: Three meters to the north of Area B is the marsh studied by Phipps and Speer (1958). It consists of 0.20 acres and has not been grazed since March of 1967, when it was fenced. The topography varies from a flat area to a hillside with a slope of about

45 degrees and has an overall fall of about 4 m from the seepline to the lowest point.

The typical shaded zone vegetation, dominated by *Impatiens biflora*, exists only in the extreme southern corner of this marsh (Table 2, Area C1) while the remainder of this marsh is dominated by the *Glyceria striata, Pilea fontana, Agrostis alba, Carex lurida* community (Table 3, Area C2, C3 and C4). Three distinct zones exist in the open parts of this diverse marsh depending upon topography and moisture and, as a result, minor variations in this dominant plant community occur. These are a Low, Open Zone, a Hillside, Open Zone and a High, Open Zone (Figure 1).

The Low, Open Zone is about 22 m long, averages 3 m wide, and occurs in a depression at the base of the hill in which water from the hillside seep accumulates. The water in the depression is about 2 dm deep and remains as a pool for the entire year. Besides the species typically found in the open area community, *Eleocharis erythropoda, Bidens cernua, Carex festucacea*, and *Sagittaria latifolia* are abundant to very common in this zone.

The Hillside, Open Zone extends from the seepline to the Low, Open Zone and has a fall of about 4 m from the seepline to the base of the hillside with a slope of about 45 degrees. It is quite wet but without standing water, is very soft and muddy near the seepline, and is in full sunlight for most of the day. More woody individuals and species occur here than in any other part of the diverse marsh because grazing was impossible owing to the steep slope (Table 1).

The High, Open Zone of the diverse marsh area lies between the seepline and the upper edge of the Hillside, Open Zone, is in full sunlight for most of the day and has an overall fall of about 1.5 m. The area is 20 m long, 7 m wide, has a northwest exposure, and is very wet with standing water in depressions throughout the year. Besides the typical open areas species, *Carex vulpinoidea* and *C. stipata* are common while *Caltha palustris* is abundant throughout this zone. Few woody plants exist here (Table 1), although *Salix discolor* is represented by a few individuals near the fence that separate this zone from Area D.

Area D.—Wooded, Ungrazed Marsh: This marsh is an extension of the High, Open Zone of Area C. It consists of 0.33 acres and averages 45 m long and 20 m wide. It has about a 5-degree slope to the northwest and an overall fall of about 2 m from the seepline to an outlet depression that drains into the creek. Phipps and Speer (1958) reported that this area had not been grazed in 1957, and it appears that no grazing has taken place since that time.

Saplings of *Salix discolor* are very common on the slope above the outlet depression, while *Salix rigida* with 8,884 seedlings dominate this depression at the base of the slope. *Carpinus caroliniana* and *Ulmus americana* are commonly found near the seepline where the willows are fairly sparse and some shading occurs. *Rhus vernix* exists as a single clump on the slope associated with *Salix discolor*. A few specimens of *Sambucus canadensis* and *Ribes americanum* are found in the outlet depression at the base of the marsh (Table 1).

The herbaceous vegetation of this area is extremely diverse, and because of the degree of shading, appears to be intermediate between the two common communities occurring in the rest of the marsh areas. Also, some species are found here and in no other part of the

marshes. These include *Iris shrevei, Carex hyalinolepis, Apios americana* and *Lysimachia ciliata.*

Area E.—Ungrazed, Shaded Spot Marshes: Northeast of Area D are three small spot marshes which are similar in topography to the spot marshes of Area A. These marshes, which average 100 sq m in size, slope to the northwest with about a 5-degree incline. They are extremely wet with standing water in the depressions throughout the year. The herbaceous vegetation of this area is very similar to that of the shaded zone of Area A (Table 2).

Conclusions

As a result of variation in topography, available moisture, light penetration, and grazing, certain trends can be observed in the marsh areas studied. Many indications suggest that grazing has been very effective in controlling the invasion of woody plants into the marshes. This can be determined by comparing the various marsh areas which have been grazed to varying extents during the past 14 years. The Spot Marshes (Area A) and the Wooded (Area D) have not been grazed for at least 14 years (Phipps and Speer, 1958), and in both areas numerous woody plants occur. In contrast, the Grazed Marsh (Area B) and the Diverse Marsh (Area C) have been subjected to extensive grazing for at least the past 20 years. In both, few woody plants exist, except in areas that are not accessible to cattle.

Distinct herbaceous communities are present in the marsh areas studied. The shaded zones of Areas A, B, C, and E are similar in ecological condition and associated plant species. In these zones, which are shaded for most of the day, *Impatiens biflora* dominates and is classed as abundant to very abundant. Few other species occur in this wet, shaded situation, and all are in low abundance classes.

The open zones of the marsh areas studied are similar ecologically and the associated species are similar though they vary in abundance. The dominant species here are *Glyceria striata, Pilea fontana, Agrostis alba* and *Carex lurida.* The open nature and the similarity of plants that occur here tend to place these zones in the same habitat type.

If part of the area continues to be protected from grazing and if the seep continues to produce an abundant amount of water, it is very possible that this unique community will persist. Under these conditions most of the plants will survive and continue to flourish. One exception may be *Rhus vernix,* which is now limited to one clump and does not appear to be reproducing.

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THE EFFECT OF DIMETHYL SULFOXIDE ON HUMAN ERYTHROCYTE MEMBRANE

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ABSTRACT.—*Isolated human erythrocyte membranes were submitted to sonication in the presence and absence of dimethyl sulfoxide. Solubilized protein fractions containing high sialic acid and low lipid content were obtained. The possible role of DMSO as a cryoprotective agent in a membrane system was discussed.*

The human erythrocyte membrane has been the subject of intensive research because of its ready availability and ease of isolation. Knowledge of the precise arrangement of its components may open the way for treatment of blood diseases such as hereditary elliptocytosis and hereditary spherocytosis, which are suspected to be caused by membrane defects. The structure of erythrocyte membranes may also be common to that of other biological membranes. It is therefore hoped that other diseases may be explainable in terms of membrane activity.

Adequate methods of solubilization of the membrane components must be developed before the structure of the membrane can be elucidated. Several attempts to develop suitable procedures have been made (Maddy, 1970). These include organic solvent extractions (butanol, 2-chloroethanol, phenol, aqueous pyridine and pentanol), detergent solubilization (cholate, deoxycholate, sodium dodecyl sulfate and Triton X100), as well as hydrogen bond-breaking reagents (urea and guanidine hydrochloride). However, these reagents either lack specificity in solubilizing a particular component, forming complexes with the membrane components, or denature proteins. Therefore, it is important to explore the potential of other reagents for the specific removal of membrane components. For example, dimethyl sulfoxide (DMSO) has been known for its usefulness in the solubilization of glycogen (Whistler and DeMiller, 1962) and selective extraction of lipopolysaccharide from the outer membrane of Gram-negative bacteria (Adams, 1967).

It would be of interest to determine the chemical nature of the components extractable from human erythrocyte membranes by dimethyl sulfoxide. This report represents a study of the extraction procedures and the chemical analyses of the extracts as well as the residues.

MATERIALS AND METHODS

Hemoglobin-free human erythrocyte membranes were isolated from outdated blood (Peoria Red Cross, Peoria, Illinois) according to the procedure of Dodge, *et al.*, 1963. The cells were washed three times in isotonic 310 ideal milliosmolar phosphate buffer, pH 7.4, lysed overnight in hypotonic 20 ideal milliosmolar phosphate buffer and washed with hypotonic buffer until free of hemoglobin.

After dialysis and lyophilization, a 200 mg sample of membrane was added to 50 ml of 0.1 M phosphate buffer, pH 7.4, and stirred to homogeniety for 1-2 hours. An equal volume (50 ml) of DMSO was slowly added, stirred to homogeniety for 1 hour, and sonicated (Branson Sonicator Model W140D) in 50 ml aliquots at 4° C at 60 watts for 6 minutes. Centrifugation was performed at 20,000 x g for 20 minutes at 4° C after the sonicates were combined. The supernate was de-

canted, and the precipitate was reconstituted for 2-3 hours in 25 ml of 0.1 M phosphate buffer, pH 7.4. An additional portion of DMSO (25 ml) was slowly added to the suspension; sonication and centrifution were executed as before. The two combined supernates (Fraction S) and the reconstituted precipitate (Fraction P) were dialyzed against distilled water for one week and lyophilized.

A control sample was treated exactly as described above, except an equal volume of 0.1 M phosphate buffer, pH 7.4, was added to the buffer-membrane suspension in place of the equal volume of DMSO. The supernate and precipitate of this treatment were recovered as NS and NP, respectively.

Protein (Lowry, *et al.*, 1959), hexosamine (Rondle and Morgan, 1955), hexoses (Koehler, 1952), sialic acid (Warren, 1959), and phosphorous (Bartlett, 1959) were determined. Total lipids were extracted with chloroform:methanol (2:1 v/v) in a Soxhlet apparatus and determined gravimetrically.

Results and Discussion

Dimethyl sulfoxide (DMSO), an aprotic solvent, is used in biological systems to protect cells against freezing and radiation damage (Farrant, 1965; Chang and Simon, 1968). One of the hypotheses to explain the cryoprotective and radio-protective action of DMSO assumes that this solvent prevents changes in the cell's lipoprotein membranes and stabilizes lipoprotein complexes (Keysary and Kohn, 1970). Five to ten percent DMSO is widely used as an additive for the protection of animal cells during freezing storage. However, when continuously present, these concentrations might be toxic. It seems therefore paradoxical that a toxic substance should be protective. A similar situation occurs in the bacterial endotoxin systems, which are toxic over high concentration and protective at low concentrations. Furthermore, it is puzzling that a reagent known to extract carbohydrates (Whistler and DeMiller, 1962; Adams, 1967) would serve as a stabilizing agent for the membrane system which is known to consist of protein, lipids and carbohydrates (Bakerman and Wasemiller, 1967).

In experiments reported here, a high concentration of DMSO (50%) in a buffered solution was used to extract human erythrocyte membranes. DMSO extraction along with sonic treatment was found to solubilize 28.7% while sonic treatment alone solubilized 23.6%. In both cases, the ratio of the amount of residue to amount solubilized was approximately 2:1 (Table 1 and 2). Since the presence of DMSO did not affect the relative ratio of residues to solubilized material, it appears that sonication alone was responsible for the extraction efficiency. In order to determine the chemical nature of the fractions, chemical analyses were performed (Table 1 and 2). In both cases significant amounts of total carbohydrates (15%), especially sialic acids (5%) was found in the solubilized fractions. On the other hand, there was a slightly larger amount of total free lipids in the residues (60%) than in the solubilized fractions (45%). There was no significant difference in the amount of proteins (43%) in the residues which have a similar protein composition as the starting intact membranes. The sialic acid-rich fractions (Fractions S and NS) resemble those prepared by aqueous pyridine extraction (Blumenfeld, 1968) and by pronase treatment (Ohkuma and Furuhata, 1969) of human erythrocyte membranes. From these studies, it seems that there are two types

TABLE 1. Chemical Composition of Human Erythrocyte Membrane Fractions After Sonication Treatment in the Presence of Dimethyl Sulfoxide

Fractions	S	P	Intact Membrane	
			Experimental	Literature
Yield (%)	28.7	49.6	0.7608*	....
Protein (%)	34.9	42.2	46.1	55.0**
Total Lipid (%)	50.0	61.0	37.2	35.0**
Phosphorous (%)	1.40	1.29	0.90	1.10***
Total Carbohydrate (%)	15.19	11.28	9.49	10.0**
Hexosamine (%)	5.00	3.92	3.00	
Sialic Acid (%)	5.35	2.02	2.57	
Hexoses (%)	4.84	4.34	3.92	

*Number of grams from 600 ml blood
**Bakerman, *et al.*, 1967
***Lauf and Poulik, 1968

TABLE 2. Chemical Composition of Human Erythrocyte Membrane Fractions After Sonication

Fractions	NS	NP	Intact Membrane	
			Experimental	Literature
Yield (%)	23.6	49.8	0.7608*	....
Protein (%)	33.0	44.1	46.1	55.0**
Total Lipid (%)	45.0	60.0	37.2	35.0**
Phosphorous (%)	1.46	1.08	0.90	1.10***
Total Carbohydrate (%)	15.89	9.32	9.49	10.0**
Hexosamine (%)	4.90	3.82	3.00	
Sialic Acid (%)	5.10	1.70	2.57	
Hexoses (%)	5.89	3.80	3.92	

*Number of grams from 600 ml blood
**Bakerman, *et al.*, 1967
***Lauf and Poulik, 1968

of proteins in the erythrocyte membrane, one is high in sialic acid and low in lipid; the other low in sialic acid and high in lipid. In both the aqueous pyridine and pronase preparations, the fractions rich in sialic acids bear antigenic determinants of the MN blood group system. It is not known if the sialic acid-rich fractions isolated by DMSO treatment would share this immunological property.

It is surprising that DMSO failed to extract much free lipid. It would be expected that sonication alone in an aqueous medium would be less effective and DMSO, being an aprotic organic solvent, more effective in removing lipid materials. On the other hand, this unique property of DMSO may explain the possible stabilization effect on the lipoprotein complexes of the membrane.

It has been reported (Rosenberg and McIntosh, 1968) that sonication does not alter the molecular membrane structure. The solubilization effect by sonication was suggested to be caused by the disintegration of the total membrane system and reconstitution to small unit-membrane pieces which did not sediment under high centrifugation force. These solubilized unit-membranes were shown to have similar chemical composition as the

intact membranes. Our results are not consistent with their findings. Similar to the treatment in the presence of DMSO, sonication alone was able to release a sugar-rich moiety (Fraction NS). The exact nature of the solubilized material in the presence and absence of DMSO may not be known until their homogeneity is established by column fractionation or polyacrylamide disc electrophoresis. These solubilized fractions may well be a complex (or complexes) of lipids, proteins, glycoproteins and glycolipids. One thing is certain, however, that there is an easily releasable sialic acid-rich moiety in the human erythrocyte membrane.

Although DMSO extraction did not provide a clear-cut selectivity for any particular components of the erythrocyte membrane, the inability of DMSO to remove lipid to any great extent does not contradict with the suggestion that membrane lipids may be the site or sites for freezing injury to cellular systems (Livne, 1969) which could be protected by low concentrations of DMSO.

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LAKE SHORE EROSION

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ABSTRACT.—Erosion of lake shores by boat traffic is a serious problem which needs to be given more attention. This erosion contributes to the filling in of reservoirs. A gabion wall is recommended.

Present concern with ecological problems tends to obscure natural geological processes which have been proceeding since the beginning of time and which man is accelerating. This is apparent when a lake is constructed, for any impoundment represents a block to Nature's orderly process. The arresting of water flow behind a dam means that the sediment load carried by moving water cannot be held and is deposited on the floor of the lake. This process is largely overlooked because it is unseen and, depending upon the size of the area contributing runoff, not observed until considerable storage volume has been lost to sediment.

Although most of the deposited sediment has been transported from the total watershed, a portion consists of material eroded from headlands in the lake that have been under attack of wave-generated winds and ice pressure, like the slope shown in Figure 1. When sufficient sediment is eroded to make a protective beach, the rate of headland erosion is greatly reduced.

FIGURE 1. Lake headland eroded by wave action.

However, the process results in the loss of considerable land, generally high-value promentary land. Usually, land owners try to protect exposed erodable areas with seawalls, such as the low barrier pictured in Figure 2. Unless they are well constructed, they require almost constant upkeep at great expense.

Man contributes markedly to this type of erosion through the uncontrolled use of power boats on lakes. This is especially noticeable on narrow lakes where wave-generated waves reach the shore with maximum crests and undiminished force. Even with lake police protection, man-made lakes continue to be damaged by fast boat traffic throughout the months of heavy recreational use. One has only to observe the recreational use of municipally owned and operated lakes to know that boating enthusiasts frequently overstep the rules of safe operation. Especially is this true for water skiers. In making 180 degree turns it is frequently necessary for the boat to make a circular course that almost intersects the shore. Waves generated by this maneuver cause considerable erosion.

Normal wave action generated by boats cruising 100 yards from shore reaches the shore with a dynamic pressure of less than 5 pounds per square foot. A speeding boat pulling a skier at 20 miles per hour as shown in Figure 3 and at a distance of 20 yards from shore can send shoreward waves that exert pressures on the shore of 500 to 1000 pounds per square foot. Obviously, the answer to this kind of

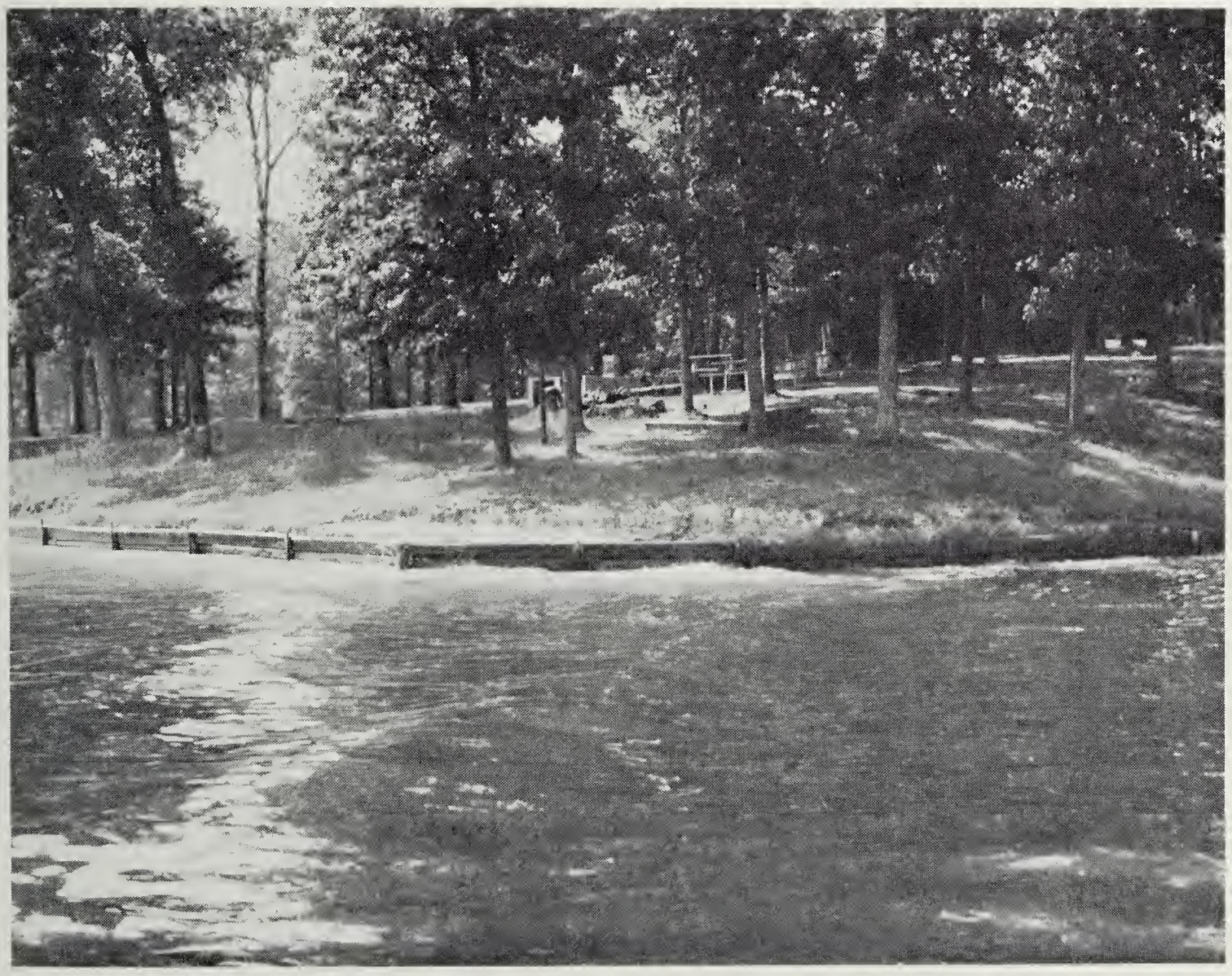

FIGURE 2. Low wood barrier wall.

erosion is the enforcement of stricter rules for boat operation. Since dynamic pressure varies as the cube of the wave height, erosion can be reduced considerably if waves have more distance to travel before they intersect the exposed shore and break with less force.

In view of the destruction of lake shore lines that could be attributable to boat traffic, it is strange that there are not uniform codes controlling all lake boat traffic similar to the motor vehicle laws. The tendency has been for a situation to develop to a critical stage and then investigate the history of older lakes and adopt some of rules formulated for their protection. Some general practices can be learned in this way.

Rules and Regulations

Article XXIII of the Rules and Regulations of the Illinois Department of Conservation states that it is unlawful for any person to use an outboard motor on any state-owned lake under its jurisdiction that has less than 60 acres of water. On the larger state-owned lakes, the limitation applies to use of an outboard motor of a size larger than 10 horsepower. These regulations are established in accordance with Section 4 of the Fish Code of Illinois, and persons violating provisions are subject to the penalties provided by the Code. The laws supplementing these rules are to be found in Chapter 56 of the Illinois Revised Statutes.

Rules governing boat traffic on municipally owned lakes vary greatly. Lake Bloomington, a 600-acre lake owned by the City of Bloomington, has a limit of 25 horsepower for motor boat operation. Lake Sara, a 735-acre lake built by the Effingham Water Authority, has an upper limit of 75 horsepower for its boat traffic. Lake Springfield, which covers an area of 5000 acres, has no horsepower

FIGURE 3. Pleasure boat producing waves.

limit, with several boats having motors in excess of 300 horsepower. However, hydroplanes are not allowed, and no boat may speed in excess of 35 miles per hour.

License Fees

There is a charge for operation of any boat on practically all Illinois lakes. The State Department of Conservation charges fees varying from $12.50 per season for power boats to $25.00 for pontoon boats.

Approximately 3100 boats are licensed to operate on Lake Springfield. There are regularly 2200 locally-owned boats with licenses, and the remainder come from great distances, often beyond the state's borders. Annual fees vary from $2.00 for a rowboat less than 16 feet in length and having no motor to $10.00 for a boat that is powered by a motor in the range of 51 to 74 horsepower.

At Lake Decatur, which has an area of slightly more than 2400 acres, the schedule of fees blankets all outboard motors under 15 horsepower at $7.50 annually, and $10.00 for larger sizes. Boats with inboard motors are charged $12.50 if the size is under 175 horsepower and $15.00 for more powerful engines. Sailboats are all charged $7.50.

Such fees are collected by city clerks, and the funds are used to help defray the cost of policing the lakes. Violations are punishable with fines, and these also help toward the same costs.

Erosion Barrier Construction

Generally, shore property along Illinois lakes is not provided with protective devices until erosion has progressed considerably. The disturbed owner will then observe the protective devices already installed on other shore properties and will copy them without regard to applicability to a specific situation. Figure 4 is typical of improper planning to curtail bank erosion.

At Lake Sara, wooden seawalls are popular. They are composed of wood posts 6 feet long driven vertically into the shoreline and protruding above spillway crest elevation one or two feet. Wood planks, each 2 by 6-inches by 12 feet, are nailed horizontally to the posts. The wall is also supported from the shore side with

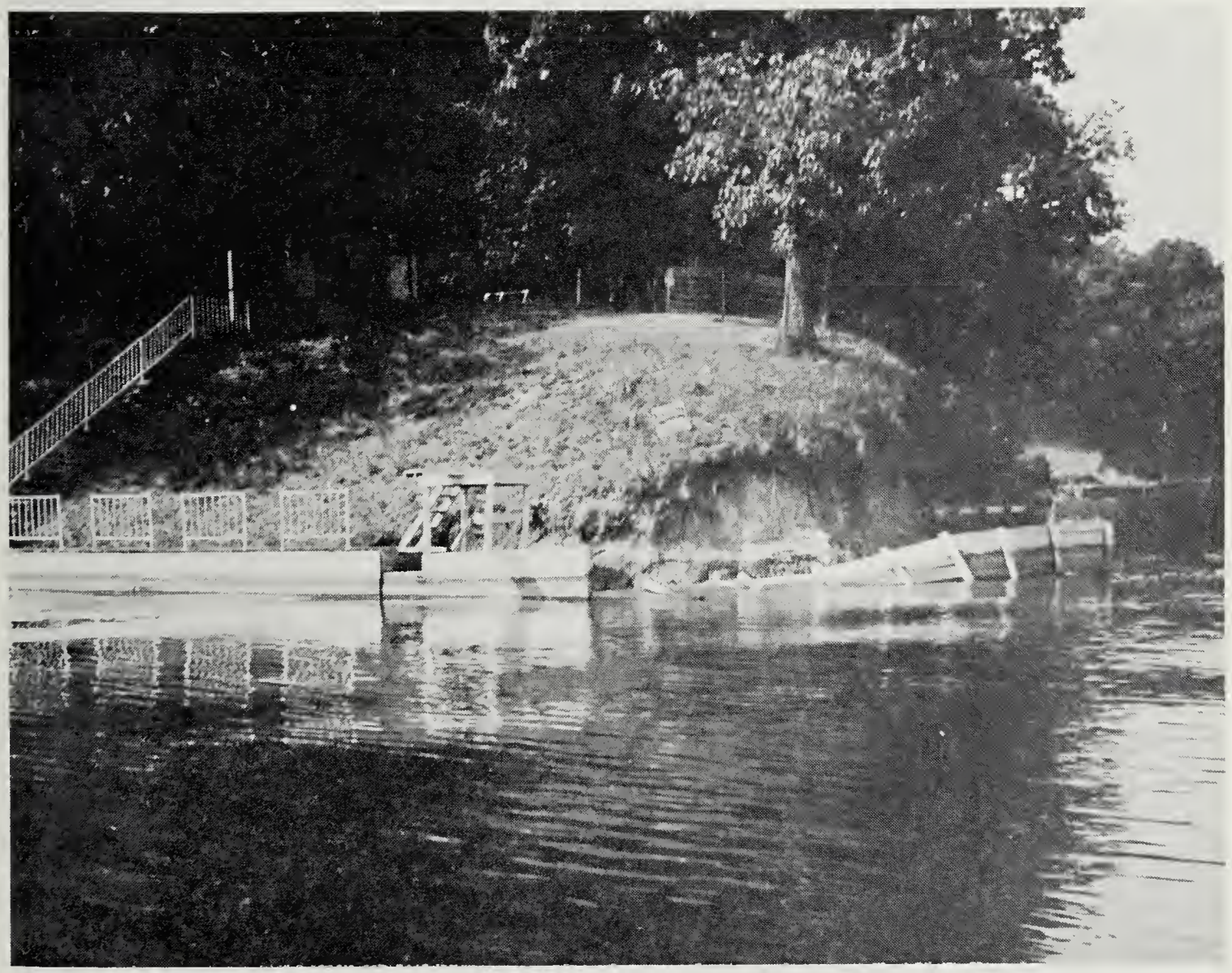

FIGURE 4. Inadequate barrier fails to stop bank erosion.

coarse filler material such as bricks, rocks and cement blocks. Most owners have had relatively good experience with this type of construction, but any unusual high water tends to lift the structure out of the water as shown in Figure 5.

One erosion barrier that has had a fair measure of success consists of a continuous concrete wall approximately 6 inches wide and extending from the ground vertically to a height 6 inches above spillway crest elevation. The wall is strengthened with reinforcing rods and supported on the shore side with rocks, bricks, and broken concrete. Where rods have not been tied together, failure has occurred in the wall at the discontinuity, as shown in Figure 6a. A similar wall may be strengthened at regular intervals with corrugated metal area-way projections 2 feet deep and 3 feet in diameter. They are connected to the main wall with trellis-type wire and filled with concrete. The semicircular shape of the way-walls tends to absorb much of the wave energy. The wall shown in Figure 6b has withstood three years of battering.

Property owners have experienced only limited success with protective barriers installed along their water lines, and in many cases failure has occurred within a year. Since permanent walls, such as driven sheet piling, are expensive, it is imperative that effective yet less costly devices be investigated.

One form of protection that should prove beneficial is the gabion. This is a steel wire mesh box made of a complete and continuous fabric, as shown in Figure 7, and filled with stones or rocks. It may be constructed in varying sizes and shapes, although in practice the horizontal width should be at least three feet. The height can be fractions of the width and the length up to four multiples of the width. The wire mesh, having a minimum size of U.S. Steel Wire Gage No. 11, should be galvanized with a minimum zinc coating of 0.80 oz./per square foot of wire surface. The area of the mesh openings should not exceed 8 square inches, and the maximum linear dimension of the opening not exceed 4.5 inches. Strength, elasticity, and flexibility are necessary requirements of gabions so they should be divided into compartments with dia-

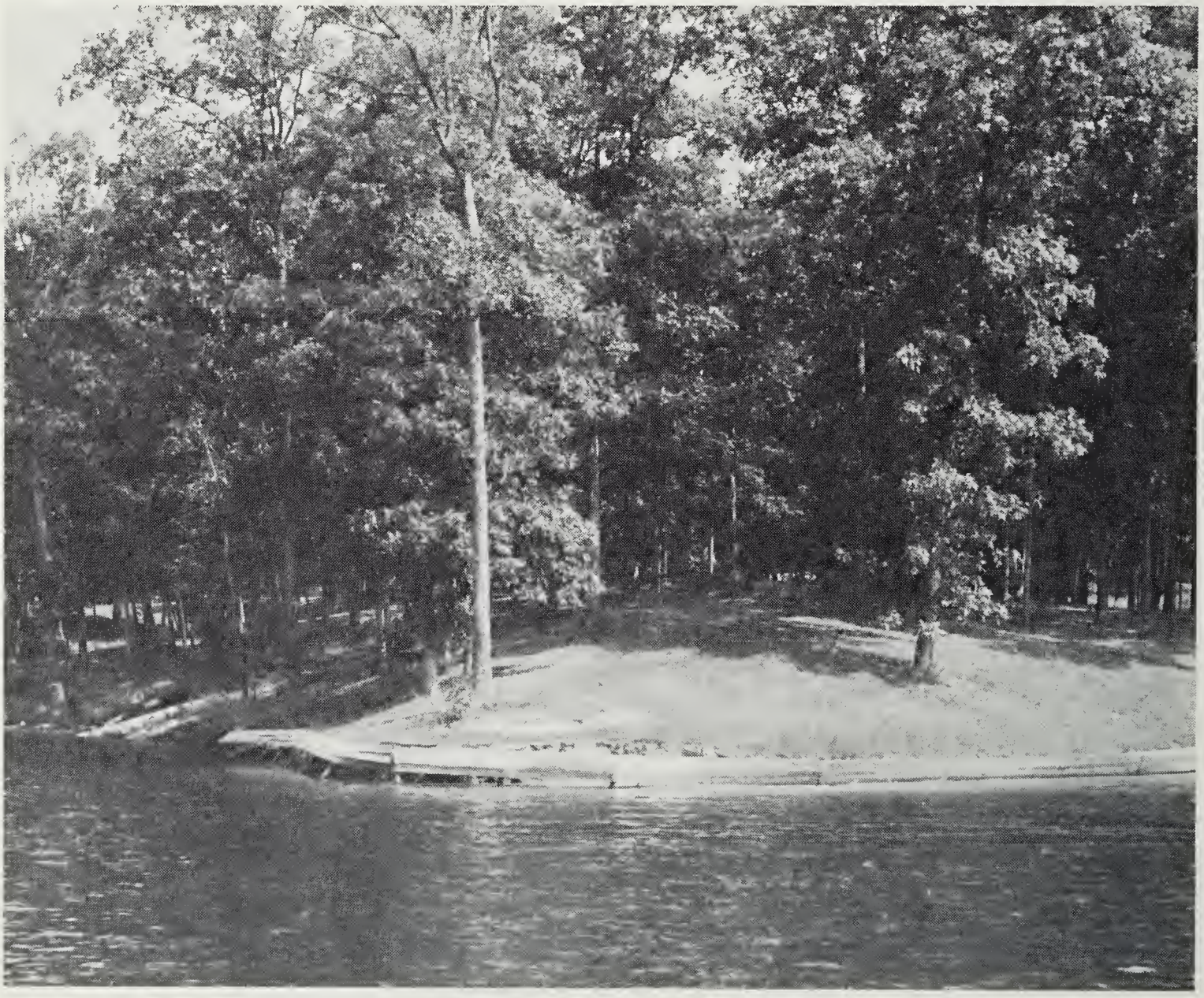

FIGURE 5. Wood barrier uprooted by high water.

phragms of the same mesh and gage as the main body. The smaller cells diminish the internal movement of the rock fill. Single unit construction is necessary so that edges of the base, lid, ends, and sides have the same strength and flexibility as the body of mesh.

Cost of erosion Barriers

There is considerable variation in the prices owners have reported for their barrier installations. The wooden post variety with horizontal boards costs about $40 a lineal foot when installed by a contractor. Many do-it-yourselfers have built such walls for about $14 a lineal foot.

Contractors have installed gabion walls at a cost of $69 per running foot. Figure 8 shows a typical gabion wall. A sheet steel type of wall driven to a depth of ten feet costs about $1800 per lineal foot. While this is the best protection, especially where salt water and wave action is great, the gabion has attributes that make it preferable for preventing shore erosion at small lakes. The rock-filled walls are porous and therefore not as subject to hydrostatic pressure deformations, especially concrete ones. As they fill with silt they support plant life and blend in with the natural surroundings. The gabion type of structure protects the headland or "toe" of the shoreline from undermining, which causes most bank failures due to wave action.

Conclusions

Shoreline erosion is a continuing process, even after adequate beaches have developed. However, the average lot owner knows that he will lose some of his water-line property land to wave erosion and generally makes an attempt to protect his shoreline. Unfortunately, the present make-shift schemes used by most property owners do not begin to do an adequate job of protecting lake shore property lines, and costs for this work are expanding. There is need for more adequate information on workable shore protective devices. Cost of experimental devices could be met by the state through bills proposed by legislators for experimental barriers built at specific locations. A state department, such as the Department of Conservation, could then make the results available to all shore property owners.

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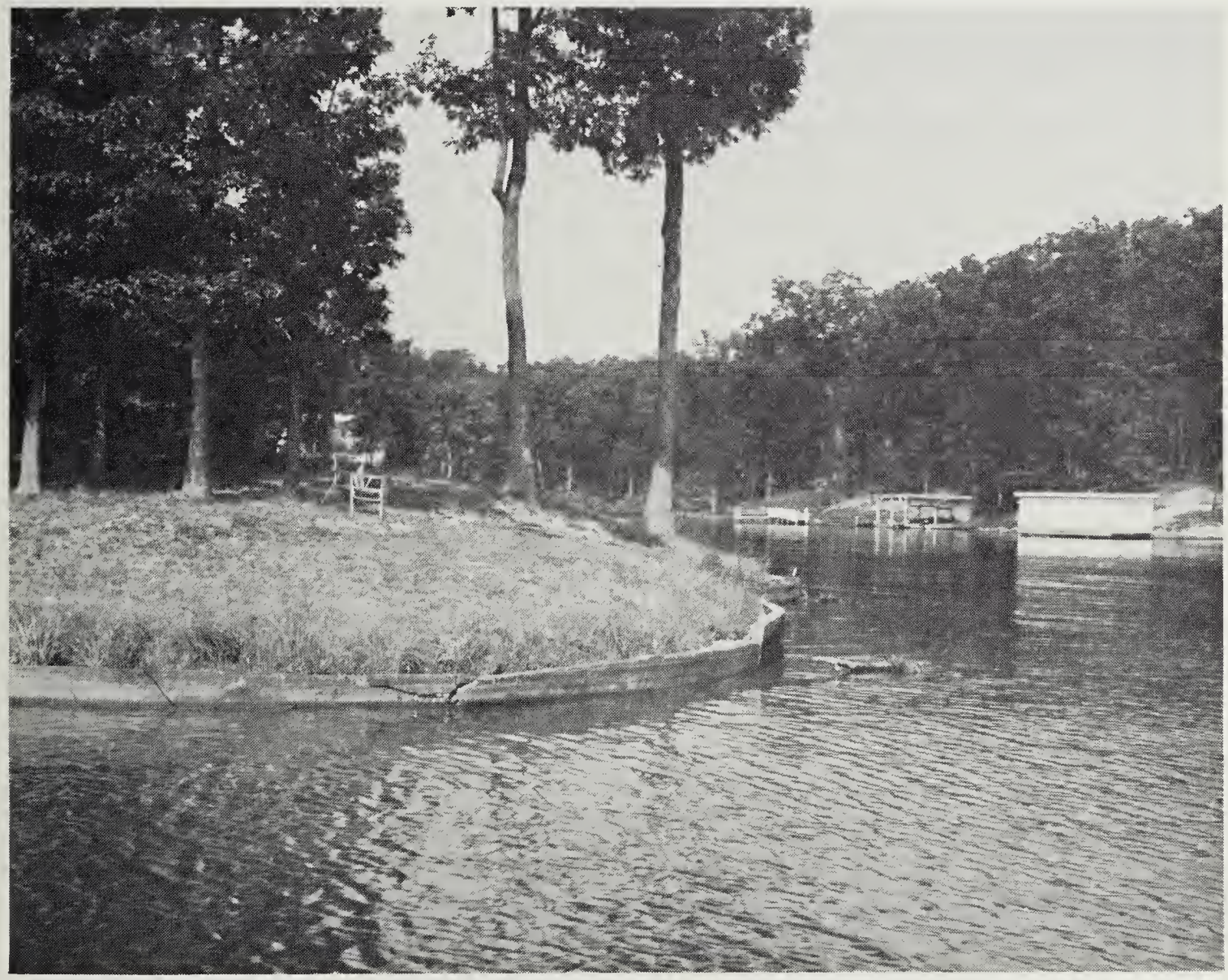

FIGURE 6a. Wall failure where reinforcing rods were omitted.

FIGURE 6b. Corrugated area-way projections provide strength to erosion barrier.

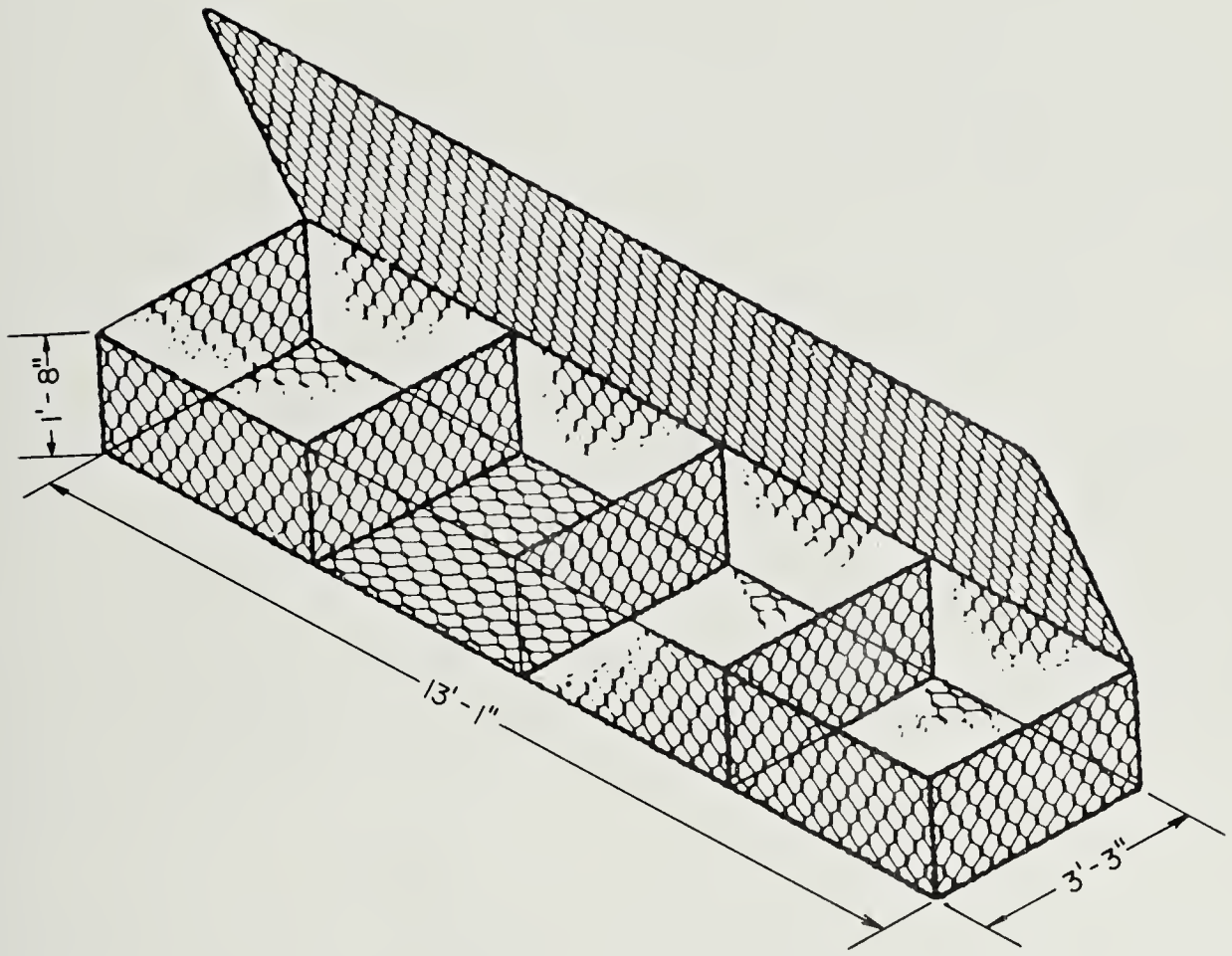

FIGURE 7. Gabion fabricated from heavy steel wire mesh.

FIGURE 8. This gabion wall protects lake-front property.

LOCAL EFFECTS OF THE NEW ACS CURRICULUM

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ABSTRACT.—The questionnaire method was used to study high school background of and the effects of course prerequisites and content upon students in two successive fall terms of the freshman chemistry class. The data were analysed with means and correlation coefficients, and statistical interpretations thereof. Well-known effects upon student performance were more relevant than the criticisms of a vocal minority.

In Fall, 1965, the Department of Chemistry of Southern Illinois University, Carbondale, changed their undergraduate curriculum (Southern Illinois University (1965)), in accordance with changes suggested in standards for accreditation of bachelor degree programs by the Committee on Professional Training of the ACS (American Chemical Society (1965)). The changes were the culmination of efforts by members of the ACS, along with highly qualified secondary school chemistry teachers, to develop two modern secondary school curricular plans (CHEMS, 1960; CBA, 1964). The ACS (1960) strongly stressed that a perspective science major should be prepared in one of these new curricular studies; thus, by 1965 the supposition that entering college science majors were better prepared seemed valid.

The new SIU freshman curriculum assumed that only a student without secondary school preparation was deficient and could enter for credit the first course of the major sequence. Student complaints, the majority of which centered upon this selection process, were of continuing concern. Therefore, the purpose of this research was to ascertain the suitability of the selection method and, in addition, to ascertain certain student attitudes which were developed by the inauguration of the new program.

THE QUESTIONNAIRE

A questionnaire was sent to every student whose name appeared on the final class listing of the Fall, 1965 and Fall, 1966 second course classes. No mailing was made to Winter classes whose student population differed markedly, *viz*, no high school background, general science converts, and Fall failures. The first year mailing was made in the subsequent Winter quarter to a campus address. The returns which came over a prolonged time period included many rejections for insufficient address therefore necessitating many repeat mailings. Returns from the second year mailing, made to a home address during the holiday period, came quickly with only two mail rejections, but with a smaller percentage return (70% *vs.* 63%). Total mailings were 402 and 398, respectively, with no follow-up mailing because of the high initial responses.

The questionnaire was divided into three parts: factual information concerning the student and his background; subjective opinions related to background, instructors, and instructional materials; and subjective opinions related to performance level. The final part was to be answered only by those students who received a grade below C. The second year mailing differed in two respects: an added question categorizing, if possible, the student's high school course as CBA, CHEMS, or other, and the addi-

tion of Biology as a choice of collegiate major. Other factors such as secondary school physics and mathematics which have been shown to influence collegiate chemistry grade performance (Hadley, *et al.* (1953)) were not investigated.

In the treatment of data, each response in each item was given a numerical value and all responses for one student placed on a single punched card. A missing data correlation program (Wright (1962)) was run on an IBM 1620 digital computer with 40K storage and a PR 025 monitor. This program yields the mean of each variable, the standard deviation of each variable, and the bivariate (Pearson) correlation coefficient (Baten (1938)) for each variable pair.

Sample Profile

The profile of the average student, constructed from an analysis of variable means, for each class is presented in Table 1. Response spread may be found from one standard deviation of each item. The difference between the means in Table 1 are all significant at more than the 1% level (Baten (1938)). A few items are compared to replies obtained in a one-year survey taken by the Department of Chemistry (1965). Briefly the means indicate that the two student samples are very similar but with the more recent class containing a somewhat higher quality (academically) student from a more populated county (geographically, more central) of the state. This improvement in academic quality is to be expected from the national trend of rising admission standards.

Comparison to a sample profile of the entire freshman class (Chamberlein (1965, 1966)) indicates that the average chemistry student is more likely to be a male, to major in a science-related field, to be above average in academic quality, and to come from a small secondary school in the immediate geographic region of the university. The mean high school chemistry background reflects the time-lag in curriculum change in these smaller, regional secondary schools and would indicate a high probability that the

Table 1. A Profile of the Average Introductory Chemistry Student

Item	1965-6	1966-7
Sex (% Males)	89 61*	85 58*
Age (Years)	19.3	19.3
College Credit Earned (Quarter Hours)	34.2	41.0
Percent with Academic Scholarship	28	32
Percent Working	40	44
Ill. High School Metropolitan Area (In Kilopeople)	40.5 72.1*	54.2 72.9*
Size of High School	196	212
High School Rank (Percentile)	68.2 60.0*	73.5 65.2*
Years of High School Chemistry	1.15 1.18**	1.02
High School Chemistry Average (A = 5.00)	3.80	3.86
Attendance in other non-High Chemistry (Percent)	30	30
Grade Average in Introductory College Chemistry (A = 5.00)	2.57	2.87

*Entire Freshman Class
**Independent Survey of Introductory Chemistry Class

average freshman chemistry student might be deficient in modern high school chemistry. The slightly higher 1965 mean is explained by an abnormal influx of Oriental students as a result of a revised immigration statute. The mean credit hours earned indicate a sizeable number of non-first term freshman and those with previous collegiate chemistry creating a heterogeneity contrary to a goal sought by the curriculum changes.

Correlation Ranking

Table 2 presents the correlation coefficients between the student's grade performance and each factor affecting grade performance. The table is divided into objective and subjective sections and, within each section, the factors are ordered according to decreasing importance. Individual items are grouped according to approximate level of significance, *e.g.* 0.16 is significant at the 1% level and 0.12 at the 5% level (Baten (1938)) for these sample sizes.

The results show that the most important factor affecting the student's collegiate grade was his grade performance in high school chemistry. Less than 10% of the students responding claimed to have had either CBA or CHEMS which may be the reason for the relatively minor role of high school size and location. Overall the type of background is not as important as the amount of background, and the background factors are not as important as the academic quality of the student. Table 2 also indicates that the better student felt better prepared but neither hours of study nor text opinion had any significant effect on the student's grade performance. The students tended to report a low number of hours of study (3 - 7) relative to the rule of thumb (two hours study per credit hour, *i.e.*, 10) quoted to the students. Uniformly, all students reported an unfavorable impression of the textbook.

Table 3 (in the same format as Table 2) which correlates the student's opinion of his background to various factors again illustrates the most important factor is final grade performance. Maturity factors are significant at the 1% level indicating an increasing ability to judge

Table 2. Correlation Coefficient Ranking of Factors Affecting the Student's Grade Performance

Item	1965-6	1966-7
Objective Items		
High School Chemistry Average	.402	.410
High School Class Rank	.333	.384
Collegiate Major	.235	.166
Years of High School Chemistry	.234	.222
Illinois High School Location	.133	.251
Age	.158	.090
Size of High School	.149	.082
Year in College	.113	.099
Prior Years of College Chemistry	.095	.026
Sex	.044	.065
Type of Instructor	.089	.007
Type of High School Course	....	.005
Subjective Items		
Background	.354	.379
Opinion of Instructor	.262	.223
Opinion of Graduate Assistant	.228	.101
Hours of Study Per Week	.126	.091
Opinion of Text	.065	.110

background. The better prepared student studies a lesser number of hours which creates an inherent bias in that coefficient.

The Below Average Group

In both years, the poorly performing student felt slightly superior, in intellectual ability, to his peers. This tendency manifested itself to a greater degree in the more recent class whose overall academic quality was superior. Contrary to the directions, many selected multiple reasons in appraising their poor performance with not enough study as the principal cause. Lack of interest and/or poor background were choices within one standard deviation of the mean. Consequently, in a random sample (110 and 80, respectively) of poor students, poor background does not emerge as their primary reason for poor performance. Poor collegiate instruction was not significant.

The 1965 class best correlated poor background to the nature of the material given by a particular lecture instructor (for the purpose of judging correlation coefficients, a subjective judgment was made on the amount of theoretical material used by each lecturer in the freshman course). That is, the poorer students in the sections which emphasized theoretical material felt their lack of background to a greater degree than the poorer students in other sections. The significance was approximately at the 1% level. For the 1966 class, the correlation coefficient (.116) was nonsignificant for the sample size thus precluding the same conclusion.

Table 3. Correlation Coefficient Ranking of Factors Affecting the Student's Opinion of Background

Item	1965-6	1966-7
Objective Items		
Grade in College Chemistry	.354	.379
Age	.212	.157
Year in College	.174	.176
Prior Years of College Chemistry	.171	.137
Illinois High School Location	.193	.068
Years of High School Chemistry	.108	.116
High School Chemistry Average	.162	.063
Size of High School	.086	.066
Sex	.073	.059
Collegiate Major	.047	.053
High School Class Rank	.027	.053
Type of High School Course	....	.005
Subjective Items		
Hours of Study Per Week	.189	.262
Opinion of Graduate Assistant	.143	.006
Opinion of Instructor	.009	.152
Opinion of Text	.049	.060

Summary

This study shows that the student's academic quality is far more important than any other single factor. The degree of importance is great enough to override limitations imposed by a possible non-normal distribution in the student sample with respect to secondary school background. Collegiate chemistry departments need not be greatly concerned over the type of background because the amount of secondary school chemistry is more important than the type of such chemistry.

Student sampling indicated that the most vociferous comments originated from a minority grouping.

Acknowledgement

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THE STABILIZATION OF A GULLY BY NATURAL FOREST SUCCESSION

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ABSTRACT.—In an abandoned pasture in Rockvale Township of Ogle County, Illinois, there is an old gully formed during a period of cultivation between 1835 and 1900. It is about 1300 feet long, varies in width from five to 100 feet and ranges to a depth of 15 feet. A photograph of 1904 shows a few scattered clumps of small trees in the gully. Today it is obscured by many mature trees and abundant undergrowth. A survey of gully trees in 1958 revealed box elder to be the most abundant, with black walnut, red elm, and American elm following. In 1969, red elm and black walnut shared dominance, with hackberry, black oak, box elder, black cherry and American elm next. Most of the older box elder were dead. The American elm had increased substantially in total number, but one-half were dead of Dutch elm disease. Most red elm were unaffected by the disease. The number of tree species increased from 13 to 21 during the years 1958 to 1969. Many new species of forest-related herbaceous plants had appeared. It is evident that in a period of about 70 years the gully has become stabilized and revegetated with numerous forest species and is developing rapidly into a mature deciduous forest condition.

Few ecological studies of secondary succession give any attention to the development of natural vegetation in gullies. Most are concerned with abandoned agricultural land. In some studies the recovery of a gully may be mentioned incidentally.

Oosting (1942) reported that, in the Piedmont, natural vegetation of gullies is very slow if head erosion continues. Eventually, the typical herbs and legumes of the old field appear. Marks (1942) also reported slow recovery of gullies in Wisconsin, with local weeds followed by perennial grasses and herbs. Early woody vegetation consisted of *Rubus, Ribes,* and *Prunus virginiana* with elder (*Sambucus*) and black locust (*Robinia*) developing on the upper gully slopes. In a gully thicket in Michigan, Brewer et al. (1969) found black locust a dominant tree associated with various shrubs, vines, and herbs.

Since no detailed analysis of the vegetation of a stabilized gully has been found in the literature, the author will attempt to show what has happened in recent decades in a northern Illinois setting.

DESCRIPTION OF STUDY AREA

A detailed account of the study site has previously been reported (Bullington, 1970). Only a brief description will be included here. The main features are shown in Figure 1.

An old pasture of approximately 30 acres is located on property known as The Stronghold, owned by the Presbyterian Camping Association of Northern Illinois. The field is in the center of section 33 of Rockvale Township (T 24 N., R 10 E.), Ogle County, Illinois. The site is on a hillside to the west of the Rock River Valley extending westward from Illinois Highway 2, north of the town of Oregon.

The gully is the dominant feature of the old pasture, extending from the center to the eastern edge, a distance of about 1300 feet, and dropping from an elevation of about 780 feet at the west end to 710 at the east. At the gully head it rapidly deepens to about 15 feet below the adjacent ground level and varies in width from a few to about 30 feet. As it progresses to less steep terrain, it becomes quite shallow and broadens to about 100 feet.

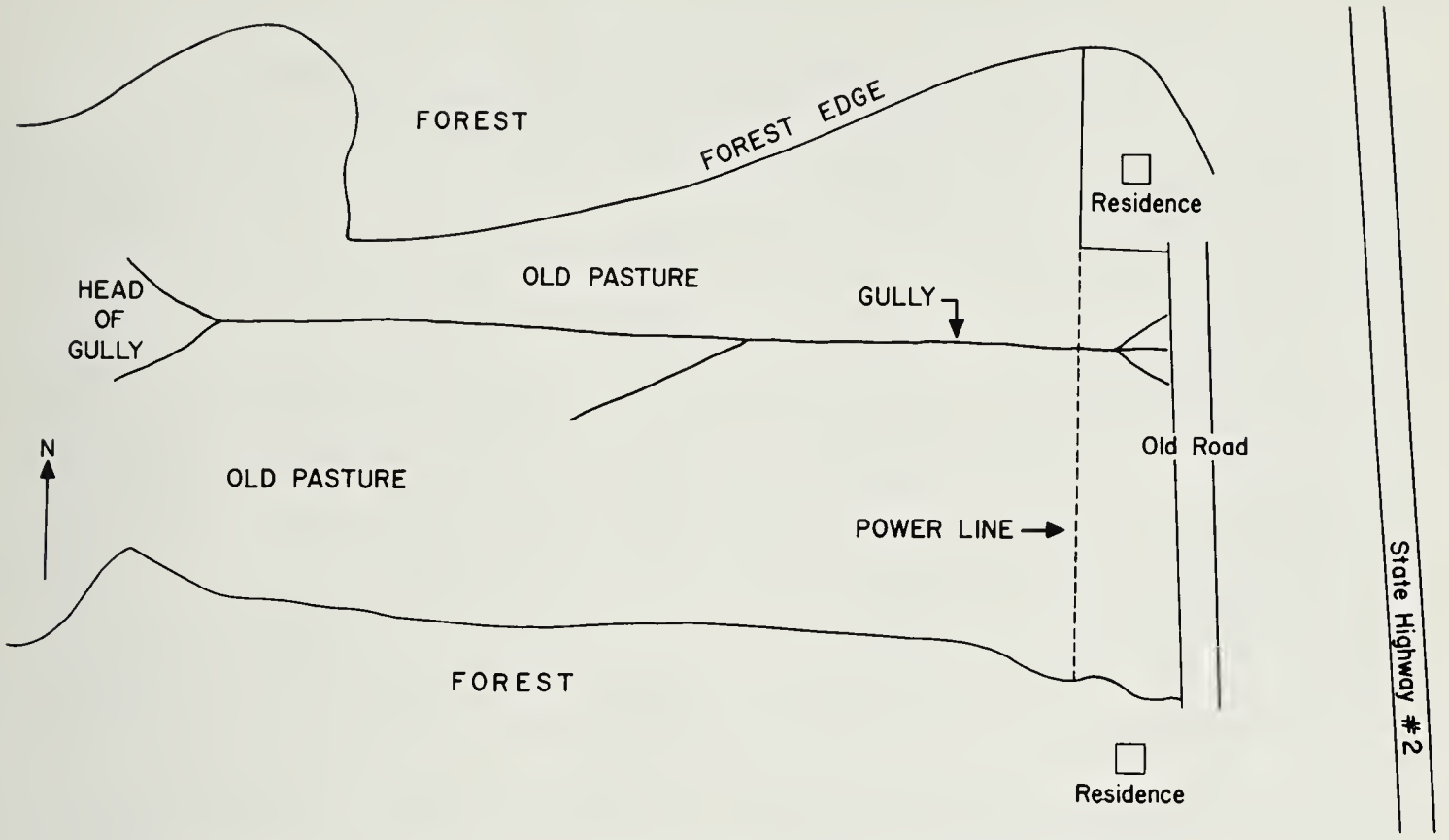

FIGURE 1. The study area at The Stronghold, Oregon, Illinois.

Portions of the upper part are cut down to bedrock of Platteville limestone, while the lower part has considerable areas of deposited silt and debris. At various places, the gully has been partially filled with old fence wire, farm machinery, and other debris. Little visible erosion has occurred in the past 20 years except for some shallow channels cut in the sediments of the lower part of the gully.

The hillside field was cleared of forest and cultivated by pioneers starting soon after 1835. Decades of cultivation resulted in extensive erosion of the field, with removal of all topsoil and the formation of the large central gully with a few small branches. Sometime before 1900, the field was seeded to bluegrass (and perhaps other grasses and legumes) and used for pasture until 1948. No domestic livestock has disturbed the field since. The entire field has undergone rapid secondary succession since 1948, with distinct competition between forest and prairie vegetation (Bullington, 1970).

A photo published in 1904 shows the gully quite clearly with a few clumps of small trees in limited areas. By the time of an aerial photo of 1939, there was a distinct cover of trees present over most of the gully.

PROCEDURE AND OBSERVATIONS

The author's first observation of the gully vegetation was in 1958 (Table 1). In all there were 551 trees counted, with box elder (*Acer negundo*) accounting for 295, or 53.5%. The likely seed source was box elders along the old roadway at the foot of the gully, a few of which were present in the 1904 photo. They are uncommon in the forest both to the north and the south of the field. Everywhere in the field, however, box elder is prominent in the new growth. Evidently the seeds can be dispersed a considerable distance, and the seedlings develop readily in an open area. The box elder is a common pioneer tree.

The 1958 survey showed that walnut (*Juglans nigra*) was next in

TABLE 1. Trees found in the gully of Strong field by reconnaissance survey in summer of 1958.

Species	Number	Percent of Total
Acer negundo	295	53.5
Juglans nigra	95	17.2
Ulmus rubra	35	6.4
Ptelea trifoliata	23	4.2
Ulmus americana	18	3.3
Populus grandidentata	17	3.1
Malus ioensis	16	2.9
Carya ovata	15	2.7
Crataegus mollis	11	2.0
Prunus serotina	10	1.8
Quercus velutina	9	1.6
Celtis occidentalis	6	1.1
Prunus americana	1	.2
Total	551	100.0

abundance with 95 trees, or 17.2%. Shagbark hickory (*Carya ovata*) and black oak (*Quercus velutina*) were represented by a small number of trees. Both could have been introduced from the mature forest about 200 feet to the north by the activity of squirrels.

Increment borer cores were taken of the larger trees in the gully in 1959 and 1963 (Table 2). Cores were collected and studied from walnut, box elder, American and red elms (*Ulmus americana* and *U. rubra*), black oak, large-toothed aspen (*Populus grandidentata*), and black cherry (*Prunus serotina*). The trees ranged in estimated age from 25 to 40 years. It was determined that they became established in the years between 1914 and 1933, a period when the field was used for pasturing and hay meadow. It is not known whether the trees present in the 1904 photo were still alive when the check on age was made.

Between 1958 and 1969, the gully was visited frequently at various seasons. The presence of new species of plants was often noted but no statistical information was gathered. During the eleven-year period the gully vegetation matured and took on a more forest-like aspect. It was obvious that new forest species of herbs and woody plants were becoming established.

In October of 1969 a detailed survey of the trees of the gully was undertaken. Because there was no uniformity in distribution of species, a complete count of all trees was made. Starting at the upper west end, ten-feet wide strips were marked off with string. Not only were the trees of the gully included, but also those to a distance of ten feet on each side of the banks. This was roughly the point of division between the adjacent old-field vegetation and that of the eroded gully.

Trees were tallied by species and by size classes. Size was measured in inches of diameter at four and a half feet above the ground (dbh). Five size classes were arbitrarily selected (under 1 inch, 1-6 inches, 6-9 inches, 9-12 inches, and over 12 inches). Separate records were kept of the west one-half and the east one-half because of differences in both terrain and species.

Because of considerable mortality among a few species, dead trees were recorded. Numerous American elms were dead from the Dutch elm disease. This was not present in the earlier survey of 1958. Many

TABLE 2. Age estimates of gully trees by increment borer samples at 4-1/2 feet height.

Year Taken in October	Species	Estimated Age at 4-1/2 Feet	Estimated Date of Establishment
1959	*Juglans nigra*	40	1914
1959	*Ulmus americana*	30	1924
1959	*Acer negundo*	30	1924
1963	*Quercus velutina*	40	1918
1963	*Ulmus americana*	35	1923
1963	*Juglans nigra*	30-35	1923-28
1963	*Ulmus americana*	30	1928
1963	*Populus grandidentata*	30	1928
1963	*Quercus velutina*	30	1928
1963	*Acer negundo*	25-30	1928-33
1963	*Ulmus americana*	25-30	1928-33
1963	*Prunus serotina*	25	1933
1963	*Ulmus rubra*	25	1933

box elders were also dead, presumably from shading and age.

The results of the 1969 survey are given in Table 3. Thirty percent of the total were under one inch dbh. Over 53% were between one and six inches. New trees were much more abundant in the west half of the gully, where 17 species were in the smallest size category and 15 in the second group. In the east half, there were 11 and 10 species in the two smallest size groups. The west half of the gully has more of the appearance of a forest, with a well-developed crown and open understory. The east half, much broader and with more silt deposits, is quite open, with more scattered trees and a heavy ground cover of brambles and vines which are almost impenetrable in places. Seedling trees have a better chance of surviving in the west half.

The total number of species in 1969 was 21. Only 13 were recorded in 1958. All but one of the new species of 1969 were represented by young trees under 6 inches dbh. The exception was white oak (*Quercus alba*) with two trees between 6 and 12 inches. They were located along the margin where they were not counted in 1958.

Abundant new reproduction has occurred with red elm, black oak, hackberry (*Celtis occidentalis*), and the black cherry in the west end only. There are mature trees of these species for seed sources in the west end but not in the east end, except for one red elm. On the other hand, the black walnut is reproducing heavily in both areas. Mature trees are abundant throughout the gully, but there are more in the east end where walnut reproduction is the heaviest. It is likely that a portion of the lower east end of the gully will eventually develop into a dominant black walnut forest.

In order to compare reproduction in the gully with that in a mature forest on the north side of the old field, only a distance of 300 feet from the gully at the east end, and 100 feet at the west end, a count was made of the trees in a marginal 50-feet strip running parallel with the gully. The north forest strip is 1000 feet long and is the fence-row margin of an extensive mature upland oak forest, probably of the type that was cleared from the old-field area in the 1830's. The north strip was assumed to be close enough to have had significance as an early seed source for the gully.

TABLE 3. Number of trees in gully by size classes (Oct. 1969).

Size Classes*	-1		1-6		6-9		9-12		12+		Total	%
Location**	W	E	W	E	W	E	W	E	W	E		
Species												
Acer negundo (living)	7	9	27	27	2	2		3		2	79	7.3
Acer negundo (dead)			*9*	*31*	*4*	*18*	*5*	*1*		*3*	*71*	
Acer saccharum	1	1									2	.2
Carya ovata	12		48		6						66	6.1
Catalpa speciosa			1								1	.1
Celtis occidentalis	27	30	34	16	5		1				113	10.4
Crataegus mollis	3	1	1	1							6	.6
Fraxinus americana	1		3	1							5	.5
Juglans nigra	37	46	39	75	7	7	9	13	2	9	244	22.4
Juniperus virginiana	8		2								10	1.0
Malus ioensis	17		1	5							23	2.1
Populus grandidentata	2		22		1		5				30	2.8
Prunus americana	2										2	.2
Prunus serotina	19	3	32	10	9		3		1		77	7.1
Prunus virginiana	15	4									19	1.7
Ptelea trifoliata		2									2	.2
Quercus alba					1		1				2	.2
Quercus macrocarpa	1		1								2	.2
Quercus velutina	34	2	17	7	9		10		4		83	7.6
Ulmus americana (living)	3	7	15	26	2	6	3	1		1	64	5.9
Ulmus americana (dead)		*1*	*21*	*11*	*21*	*1*	*3*		*2*		*60*	
Ulmus rubra (living)	69	4	136	31	7	1	8		1		257	23.6
Ulmus rubra (dead)							*1*		*1*		*2*	
Viburnum lentago		1									1	.1
Sub-totals (Living)	258	110	379	199	49	16	40	17	8	12	1088	100.3
Totals by Size Class	368		578		65		57		20		1088	
Percent of All Trees (Living only)	33.8		53.1		6.0		5.3		1.8		100	

*dbh in inches

**W = west half of gully, on upper slope
E = east half of gully, on lower slope

Note: Dead trees of three species are not included in table totals. Numbers not totalled are italicized.

TABLE 4. Trees of gully in order of abundance in 1969 compared with 1958 and with rank in adjacent areas.

No. in Gully 1969	Species	Rank Gully 1969	Rank Gully 1958	Rank in Old Field		Rank in Forest 1969	Rank in Old Road 1970
				1959	1970		
257	*Ulmus rubra*	1	3	3	2	3	4
244	*Juglans nigra*	2	2	2	1	8	2
113	*Celtis occidentalis*	3	12			5	3
83	*Quercus velutina*	4	11	5	4	2	
79	*Acer negundo*	5	1	1	4	9	1
77	*Prunus serotina*	6	10	10	11	7	4
66	*Carya ovata*	7	8	9	10	1	8
64	*Ulmus americana*	8	5	7	9		4
30	*Populus grandidentata*	9	6	11	7	6	
23	*Malus ioensis*	10	7	6	3		9
19	*Prunus virginiana*	11					7
10	*Juniperus virginiana*	12					
6	*Crataegus mollis*	13	9	4	8		
5	*Fraxinus americana*	14					
2	*Acer saccharum*	15					
2	*Quercus macrocarpa*						
2	*Quercus alba*				12	4	
2	*Prunus americana*		13	7	6		
2	*Ptelea trifoliata*		4				
1	*Viburnum lentago*						
1	*Catalpa speciosa*	21					
1088	Total Species Present	21	13	11	12	9	9

The same size classes were used in the forest count as in the gully. The survey was also made in October of 1969. The details of this survey will be reported in another paper. Brief mention will be made here of pertinent information. Black oaks and the shagbark hickory were discovered to be the dominant trees of this forest. Most of the recent reproduction was hickory. There were few walnuts.

Table 4 compares the various areas studied in terms of rank order based on total numbers of each species. There were only nine species in the 50-feet strip at the edge of this forest. This pattern continues through the forest. Table 4 also compares the gully trees with those of the adjacent old field to the north recorded in two quadrat surveys in 1959 and 1970. This narrow strip of former pasture separates the gully from the forest. It varies from 100 to 300 feet in width, with the narrow end to the west. Its principal species were the black walnut and red elm. The strip had 11 species in 1959 and 12 in 1970.

Also compared with the gully is a 420-feet strip of abandoned road running at right angles to the east end of the gully. This old gravel road has been undisturbed since 1922 except for a mowed path down the center. Large trees now arch overhead. The old road right-of-way is approximately 60 feet wide. A count of the road trees in 1970 showed that box elder was the most abundant. Except for the absence of the black oak, the top five species of the roadway were the same group as the top six of the gully, although the rank order was different (see Table 4).

No detailed analysis of shrubs and vines has been made, although they are now a prominent part of the ground cover in portions of the

gully. The 14 species identified are listed in Appendix I.

As the forest of the gully matures, there is a steady invasion of herbaceous plants, particularly the spring ephemerals. The 50 species identified to date are listed in Appendix II.

Species common to disturbed areas, such as stinging nettle (*Urtica dioica*) and black snakeroot (*Sanicula gregaria*) have been common since the first observations were made. Species of rich woods, such as rattlesnake fern (*Botrychium virginianum*), purple trillium (*Trillium recurvatum*), may apple (*Podophyllum peltatum*), and Solomon's Seal (*Polygonatum biflorum*) have entered recently.

Of special interest are the two orchids found in the gully, each in the type of habitat described for it in Gray's *Manual of Botany* (Fernald, 1950). The broad-leaved twayblade (*Liparis lilifolia*) was found in 1970 in the more open, drier, upper part of the gully. It had been established nearby for several years in a young thicket of trees in the old field.

The showy orchis (*Orchis spectabilis*) was first reported about 1958 in a moist wooded area across the old roadway from the east end of the gully. By 1970 it was abundant in the more mature central portions of the gully. Fell (1955) reported the two orchids growing together in a woods in adjacent Winnebago County.

Most herbaceous species listed in Appendix II are in the type of habitat and associated with the typical tree species reported by Swink (1969) for northern Illinois counties.

Discussion

The eleven-year period from 1958 to 1969 was a time of rapid change and development of the gully vegetation. The area had progressed from an open gully in 1900, still subject to erosion, to a fairly stable deciduous forest situation in about seven decades.

Some distinct changes have occurred in the species of trees. A few were disappearing. The wafer ash (*Ptelea trifoliata*), number four in abundance in 1958, dwindled to two specimens in 1969. Heavy shading no doubt accounted for its demise. It is still common in the open parts of the old field.

Box elder, a common pioneer tree dominant in the old field and also in the gully in 1958, was rapidly dying. Most of the box elders over six inches dbh were dead. The smaller specimens recorded were mostly sprouts from the base of dead or dying trees.

The American elm is dying from the Dutch elm disease, with about one-half of the trees dead. It is decreasing in rank, going from 5th to 8th, but the actual percentage of American elm trees is increasing (3.3% to 5.9%). There is still active reproduction of this species.

Red elm is reproducing vigorously, especially in the west end of the gully. It has moved from third rank with 6.4% to first place in 1969 with 23.6% of the total of 1088 live trees in the gully. Only two large trees were found dead. Apparently at this midpoint in succession, red elm is an important species. So far, it does not seem very susceptible to the disease that is decimating the American elm.

Downy haw (*Crataegus mollis*) is not surviving well in the shade of the gully. The few remaining specimens are quite small. Wild crab apple (*Malus ioensis*), a common pioneer associated with the haw, is still a common understory tree in the gully. However, it ranked in tenth place there in 1969 compared to a third place rank in the adjacent field. It was absent from the adja-

cent mature forest sample. As shading increases, the haw and crab apple will probably disappear from the gully.

The increase in some tree species is quite marked. Hackberry has moved from near the bottom of the 1958 list to third place in 1969, with 10.4% of the total trees. There is very heavy reproduction of the hackberry. However, the saplings are being damaged or killed at a rapid rate by winter girdling by rabbits.

Black oak has assumed a prominent place in recent years. It is codominant with hickory in the nearby forest and shows signs of assuming this same place in the gully, at least in the west half. Hickory is also increasing in prominence. There is active reproduction of both species.

Black walnut, actively reproducing and accounting for 22.4% of the total trees in 1969, is a rather surprising contender for future dominance of the gully forest. It is rather infrequent in the mature forest. However, it is known that mature trees have been selectively logged from the forest in the past. Black walnut is known to have an inhibiting effect upon other species. Perhaps it is the presence of walnut that is hastening the death of box elder. The two grow in the same habitat. Another species that is increasing in abundance is the black cherry. A few mature trees provide the abundant seeds for proliferation of this tree.

The improvement of the gully environment as a habitat for forest trees is indicated by the rapid increase in total species to 21. No other area sampled has more than a dozen species. Some of the recently invading species are probably random or accidental seedings of no significance. Catalpa (*Catalpa speciosa*) is not likely to multiply, and the two specimens of burr oak (*Quercus macrocarpa*) may be the only ones that enter. Red cedar (*Juniperus virginiana*) has been spread from open-grown trees in the old field. It will not do well in the shade. The newly reported choke cherry (*Prunus virginiana*) may have been overlooked in 1958. It will not be more than an understory tree.

Two new species are of special interest, even though their numbers are small. Two sugar maple (*Acer saccharum*) saplings have appeared. This tree of the climax deciduous forest is abundant in a forest valley only a few hundred yards south of the gully. Also from the forest valley have come at least five young white ash (*Fraxinus americana*). Four specimens have passed the 1 inch dbh mark and show promise of future development.

It should be noted that nine of the ten most abundant trees in the gully are also among the most common invaders of the adjacent bluegrass old field. The exception is hackberry, which is absent from the field. Two species common as pioneers in the old field, downy haw and wild plum (*Prunus americana*) are rare in the gully. As noted before, shading is probably the limiting factor.

Conclusion

In six or seven decades, an open gully has become stabilized by natural vegetation and has progressed to a mid-successional stage of deciduous forest development. There is active reproduction of numerous tree species. Many herbaceous plants, typical of rich deciduous woods, have become established under the mature trees. There is evidence that the rate of development is increasing. A few more decades may result in a near-climax

deciduous forest in a stabilized condition.

The observations of this study have implications for gully management in deciduous forest areas. Planting an exotic species is a common practice in the management of waste areas such as gullies. It is suggested that such places be permitted to develop naturally through the processes of secondary succession, especially if good seed sources are in the vicinity.

Two circumstances that have existed in the Strong old-field situation are no doubt of considerable importance. The eroded field surrounding the gully was planted to grass, thus stabilizing the soil of the area and reducing runoff of water. At a later time, livestock were excluded from the area. With these aids in management, natural processes have succeeded.

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Appendix I. Shrubs and Vines of Gully

Berberis Thunbergii DC	Japanese barberry
Lonicera prolifera (Kirchn.) Rehd.	grape honeysuckle
Lonicera sp.	honeysuckle (ornamental)
Parthenocissus quinquefolia Planch.	Virginia creeper
Rhamnus cathartica L.	common buckthorn
Rhus glabra L.	smooth sumac
Rhus radicans L.	poison ivy
Ribes missouriense Nutt.	wild gooseberry
Rubus allegheniensis Porter	blackberry
Rubus occidentalis L.	black raspberry
Sambucus canadensis L.	common elderberry
Smilax tamnoides L. *hispida* (Muhl.) Fern.	bristly greenbrier
Viburnum trilobum Marsh.	highbush cranberry
Vitis riparia Michx.	riverbank grape

Appendix II. Herbaceous Plants of Gully

Note: The identification of the herbaceous plants of the gully is an on-going project. Some of the plants listed are tentatively identified and require examination at other times of the year than when seen, especially at flowering time. No attempt has been made to sort out the species of certain genera, as *Aster* and *Solidago*. On each visit to the gully, new species are discovered and no doubt there will be new ones found in the future. Some plants have undoubtedly been missed. As the environment changes, there will be new invaders for future discovery.

Agrimonia gryposepala Wallr.	tall agrimony
Ambrosia artemisiifolia L.	common ragweed
Ambrosia trifida L.	great ragweed
Aquilegia canadensis L.	wild columbine
Arctium minus (Hill) Bernh.	common burdock
Arenaria laterifolia L.	blunt-leaved sandwort
Arisaema triphyllum (L.) Schott	Jack-in-the-pulpit
Aster sp.	aster
Athyrium sp.	lady fern
Barbarea vulgaris R. Br.	yellow rocket
Botrychium virginianum (L.) Sw.	rattlesnake fern
Brassica nigra (L.) Koch	black mustard
Cacalia atriplicifolia L.	pale Indian-plantain
Carex pennsylvanica Lam.	sedge
Desmodium glutinosum (Muhl.) Wood (?)	tick-trefoil
Dodecatheon meadia L.	shooting-star
Eupatorium perfoliatum L. (?)	common boneset
Eupatorium purpureum L.	Joe-pye-weed
Fragaria virginiana Duchesne	wild strawberry
Galium aparine L.	bedstraw
Galium circaezans Michx.	wild licorice
Galium trifidum L.	small bedstraw
Geum canadense Jacq.	white avens
Geranium maculatum L.	wild cranesbill
Helianthus sp.	sunflower
Hydrophyllum virginianum L.	Virginia waterleaf
Impatiens sp.	Jewelweed
Liparis lilifolia Richard	broad-leaved twayblade
Mertensia virginica (L.) Pers.	Virginia bluebells
Monarda fistulosa L.	wild bergamot
Orchis spectabilis L.	showy orchis
Osmorhiza Claytoni (Michx.) C. B. Clarke	hairy sweet cicely
Pastinaca sativa L.	parsnip
Plantago major L.	common plantain
Podophyllum peltatum L.	mayapple
Polygonatum biflorum (Walt) Ell.	Solomon's seal
Ranunculus abortivus L.	kidneyleaf buttercup
Ranunculus hispidus Michx. (?)	bristly buttercup
Rudbeckia laciniata L.	golden-glow
Sanicula gregaria Bickn.	black snakeroot
Scrophularia marilandica L.	late figwort
Senecio aureus L. (?)	golden ragwort
Smilacina racemosa (L.) Desf.	false spikenard
Smilacina stellata (L.) Desf.	starry false Solomon's seal
Smilax lasioneura Hook.	carrion flower
Solidago sp.	goldenrod
Taraxacum officinale Weber	common dandelion
Thalictrum dasycarpum Fisch. & Lall.	purple meadow-rue
Trillium recurvatum Beck	purple trillium
Triosteum perfoliatum L.	wild coffee
Urtica dioica L.	stinging nettle
Viola pennsylvanica Michx.	smooth yellow violet
Viola papilionacea Pursh	common blue violet

A NEW LOCALITY AND A NEW HOST RECORD FOR *TRICHODINA DISCOIDEA* DAVIS, 1947 IN SOUTHERN ILLINOIS

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ABSTRACT.—*Trichodina discoidea* is reported from *Lepomis cyanellus* in Jackson County Illinois. This report marks the occurrence of this peritrich on a new host and in a new geographic area. *T. discoidea* was also found on *L. macrochirus* and *Ictalurus punctatus* in Jackson County.

Davis (1947) named *T. discoidea* from material collected at Kearneysville, West Virginia. At that location *T. discoidea* was found on the gills of the bluegill, *Lepomis macrochirus*, the black crappie, *Pomoxis sparoides*, and the rock bass, *Ambloplites rupestris*. Davis also noted the occurrence of *T. discoidea* on the gills of the channel catfish, *Ictalurus punctatus* taken from the Mississippi River near Fairport, Iowa.

From July-December, 1970, fish from a commercial hatchery near Gorham, Jackson County, Illinois were collected by seining and transported to the laboratory alive for examination. Captive fish were segregated into species and carried in 19 l. plastic bags containing 3-4 l. of oxygenated well water. All specimens were examined for ectoparasites within 36 hr. Before examination the fish were double pithed, the opercula, fins, tail, and body were placed in separate dishes of distilled water, and were observed under stereomicroscope at 30-60X. Trichodinids were prepared for silver impregnation by smearing the host material on a clean glass slide. The slides were air dried and stained after the method of Lom (1970). Infected gills were also sectioned and stained with hematoxylin and eosin after Humason (1967). Identification and biometric characteristics followed the criteria of Lom (1958). Microscopy was with a Ziess photomicroscope.

During the study the occurrence and incidence of *T. discoidea* was noted on 17.6% of 17 *L. macrochirus*, 18.1% of 22 *L. cyanellus*, and 21.0% of 93 *I. punctatus*. The parasite occurred only on the gills of these hosts. This is the first report of *T. discoidea* from southern Illinois, and the first time the parasite has been reported from the green sunfish, *L. cyanellus*.

T. discoidea has now been reported from four centrarchids as well as the channel catfish which would seem to indicate a rather broad host specificity. Presently additional studies into the parasite's host specificity, host induced variation, and biology are underway.

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A NEW DISTRIBUTION RECORD FOR *LYCOPODIUM FLABELLIFORME* IN ILLINOIS

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On September 20, 1970, a clubmoss, *Lycopodium flabelliforme* (Fern.) Blanch. was collected near Ducanville (Sect. 25, T6N, R12W) in Crawford County, Illinois. This appears to be the third county in the state in which a naturally occurring population of this species has been found. The first native station was found in Pope County near Lusk Creek (Jones & Fuller, 1955) and the second in Ogle County near Muir Creek (Mohlenbrock, 1967). The Crawford County specimen is about 100 miles north of the Lusk Creek station and over 200 miles south of the Ogle County station. In Indiana, Deam (1940) records this clubmoss for 7 counties. The nearest site is in Martin County, about 50 miles east of this recent Illinois find. Three adventive stations of this species have also been reported from northern Illinois by Mohlenbrock (1967).

The habitat in Crawford County for the *Lycopodium flabelliforme* population is a lowland terrace thicket of second growth woods near a branch of Brushy Creek. The small clump of clubmoss is growing at the base of a 15-foot tall red oak. The dominant trees of the area, which are all under 20 ft. tall, are river birch and sugar maple and to a lesser extent black cherry, sassafras, sycamore, shingle oak, and red oak. The understory shrubs consist mostly of *Campsis radicans* (L.) Seem. and *Rubus* spp. The herbaceous plants of the immediate area consist of *Asplenium platyneuron* (L.) Oakes, *Onoclea sensibilis* L., *Cinna arundinacea* L., *Muhlenbergia sobolifera* (Muhl.) Trin., *Glyceria striata* (Lam.) Hitchc., *Panicum latifolium* L., *Agrimonia parvifiora* Ait., *Galium aparine* L., *Prunella vulgaris* L., *Aster pilosus* Willd., *Eupatorium perfoliatum* L., *Eupatorium rugosum* Houtt., and *Solidago caesia* L.

A small portion of one plant (Phillippe #68) is preserved in the herbarium of Eastern Illinois University.

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AN ALBINO DE KAY'S SNAKE (*STORERIA DEKAYI WRIGHTORUM* TRAPIDO) FROM CENTRAL ILLINOIS

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ABSTRACT.—The second record of an albino De Kay's snake from Illinois.

An albino snake, *Storeria dekayi wrightorum* Trapido, collected 1.5 miles west of Lexington, McLean County, Illinois, was brought to me in June of 1969. This animal lived until August 27, 1969, when it died from unknown causes. It was preserved and placed in my personal collection (J.H.T. 33).

Two other albino De Kay's snakes have been reported, one from Illinois (Hensely, 1959). The first albino De Kay's snake reported from Illinois, although not preserved, is described by Smith (1961) as having been cream colored with pink dorsal spots. The albino reported here is pink with pinkish brown dorsal spots and pink eyes and tongue.

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I would like to thank Mrs. Barbara Gardener and family who collected the snake and were kind enough to bring it to me.

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HARLOW BURGESS MILLS
1906 - 1971

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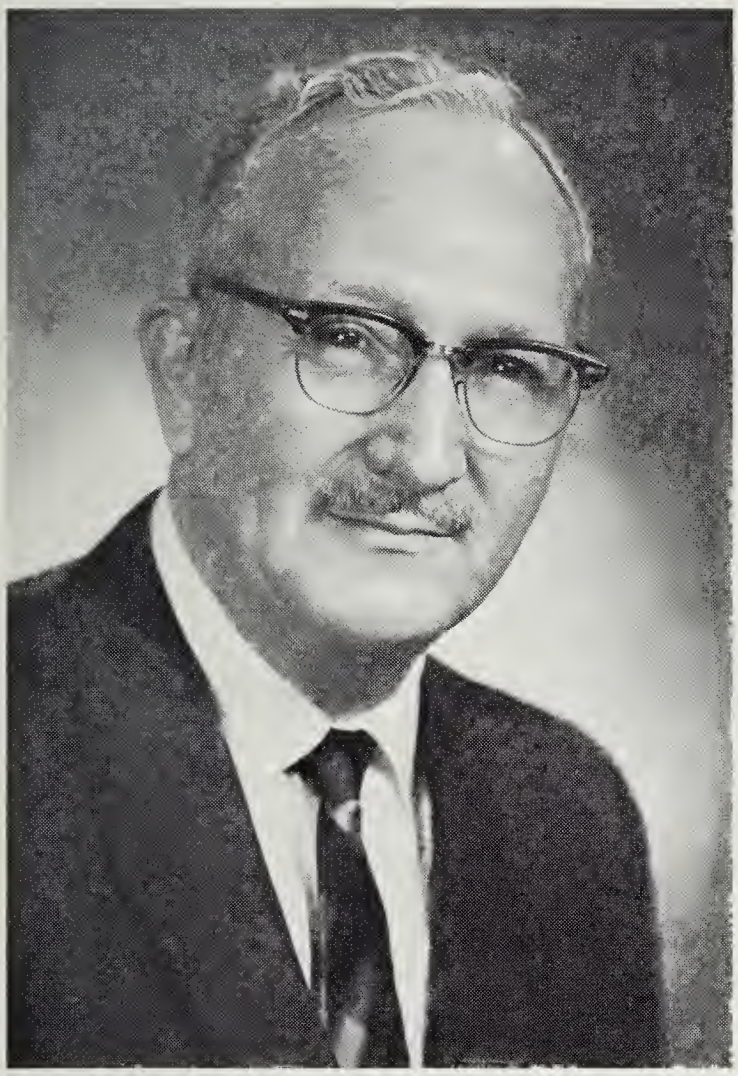

FIGURE 1. H. B. Mills, March, 1963.

Harlow Burgess Mills (Figure 1), retired Chief of the Illinois Natural History Survey, retired visiting professor of entomology at the University of Wisconsin, Racine, and a long time member of the Illinois State Academy of Science, died in Breckenridge Hospital, Austin, Texas, on April 5, 1971, at the age of 64 years, 7 months, and 16 days. Dr. Mills had suffered a stroke five days before his death.

Harlow B. Mills was born August 20, 1906, in Le Grand, Marshall County, Iowa, son of E. M. and Anna Burgess Mills. His boyhood was spent in this small, central Iowa community. After graduation from high school, he entered Iowa State College of Agriculture and Mechanic Arts (now Iowa State University) and received his B.S. degree in 1929 and his M.S. degree from the same institution in 1930. During the year 1929-1930, he was also an assistant in the Iowa Experiment Station.

On August 27, 1930, Harlow B. Mills and Esther Winifred Brewer of Central City, Iowa, were married. They moved to Texas where he served as assistant professor of entomology at Texas Agricultural and Mechanical College (now Texas A. & M. University) for the year 1930-1931. He was also associated with the Texas Experiment Station. The couple returned to Iowa in 1931 and Harlow continued his studies at Iowa State. In 1932 he was field assistant in the experiment station. He completed his Ph.D. degree in 1934. His doctoral dissertation was "A monograph of the Collembola of Iowa."

Dr. Mills spent the next 13 years in the west. From 1934-1935 he was Ranger Naturalist and Wildlife Technician at Yellowstone National Park. In 1935, he became Assistant State Entomologist for Montana and, in 1937, he joined the faculty of Montana State College (now Montana State University), Bozeman, as professor of zoology and entomology and head of the department. The same year he received a grant from the Bache Fund for a study of Collembola. During his professorship at Montana State he published numerous articles on economic entomology.

On March 1, 1947, Dr. Mills took over the duties of Chief of the Illinois Natural History Survey. His broad biological background plus his ability to get people to cooperate were instrumental in the development of the Survey during his administration. He retired from the Survey on September 30, 1966.

After retiring from the Survey, Dr. Mills became visiting professor of entomology at the University of Wisconsin, Racine, and also served as acting dean for a time. He retired from university duties in 1970, and moved to San Marcos, Texas.

Dr. Mills was active in the Illinois State Academy of Science. He had been in Illinois but two months when he spoke at the Peoria meeting, May 2, 1947, in the general session on "The Newer Concept of Predation." He was a member of the Conservation Committee from 1947-1963, its chairman from 1947-1949. He was also a member of the Public Relations Committee from 1948-1957, the Research Development Committee, 1948-1949, and the Nominating Committee,

1961-1962. He became First Vice-President for 1957-1958 and President, 1958-1959. He was a member of the Council for the year 1959-1960.

Other professional societies in which Dr. Mills was active included the American Association for the Advancement of Science, the American Fisheries Society, the American Ornithologists Union, the Ecological Society of America, the Entomological Society of America, and the Wildlife Society (editor of its journal, 1947-1949). He served as President of the Montana Academy of Science in 1943. He was a member of honor and professional fraternities: Gamma Sigma Alpha, Phi Mu Alpha, Phi Sigma, and Sigma Xi. His civic activities included membership on the Council of the University Y.M.C.A. and he was for many years active in Rotary.

In 1950, Dr. Mills was a Gugenheim Fellow and spent six weeks in Jamaica, Haiti and eastern Cuba in the study of the Collembola. In 1958, he was a consultant for both the U.S. Department of Agriculture and the Department of Interior on the fire ant control programs in the southern states. His duties were to find the strong and weak points of the program and to effect a reconciliation between divergent viewpoints. From August 1962 to August 1963, he was Chief Scientist in the Latin American Office of the National Science Foundation, Rio de Janerio, Brazil. In this position he represented American science to the Latin American scientist, and *vice versa*, and sought to obtain the best possible rapport between these two groups. From January 14, 1964 to September 30, 1966, he was an advisor to the Illinois Nature Preserves Commission.

Dr. Mills had a broad knowledge of plants and animals. It was a pleasure to be in the field with him and he enjoyed being in the field with Survey staff members. He had a great interest in birds and, as a result of that interest, one never traveled with Harlow Mills without listing the birds observed during the trip. He knew the birds of the midwest by sight and many by their song. A visit in the woods with him was a rewarding experience. His biological knowledge and his love for children resulted in approximately 25 articles on scientific matters, published in children's magazines. He enjoyed poetry and was, himself, a poet by avocation. His love of birds was reflected in his poems: The Nighthawk, The Wood Thrush, The Horned Lark.

Usually Dr. Mills was a happy man but tragedies strike all families. Tragedy struck Dr. Mills in June 1969, when his beloved and devoted wife passed away. That year he published a booklet of poems, "Eleven Personal Poems of Harlow B. Mills,", dedicated to the memory of Esther Brewer Mills. The love and devotion of this couple are reflected in these poems. Following his wife's death, Harlow went back to Wisconsin and then to Texas where his life came to a close.

Dr. Mills is survived by three children: Dr. David Mills of Silver Spring, Maryland, Gary Mills of Edina, Minnesota, Mrs. Timothy (Judith Anne) Lewis of Champaign, Illinois, and 5 grandchildren. He is also survived by three brothers—Max of Marshalltown, Iowa, Ralph of Marion, Iowa, and Glenn of King Hill, Idaho—and three sisters—Mrs. Helen Grimes of Marshalltown, Iowa, Mrs. Ursula Johnson of LeGrand, Iowa, and Mrs. Marjorie Vendervelde of Emmetsburg, Iowa.

The world has lost a gentle man and a gentleman.

Technical Publications of Harlow Burgess Mills

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The life history and thoracic development of Oligotoma texana (Mel.) (Embiidina). Ann. Entomol. Soc. Am. 25 (3):648-652.

1933

Collembola from the State of Washington (with A. R. Rolfs). Pan-Pacific Entomol. 9(2):77-83.

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1935

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1936

The Virginia creeper leafhopper (with J. H. Pepper). Mont. Agr. Sta. Bull. 314, 4 p.
Observations on Yellowstone elk. J. Mammal. 17(3):250-253.
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1939

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Errata:

In the article, "The First Record in Illinois of a population of *Stethaulax marmoratus* (Say) (Hemiptera: Scutelleridae) with information on Life History." 64:198-200, the photograph for Fig. 1 is reversed in terms of caption, i.e. read left for right.

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Doe, J. H. 1951. The life cycle of a land snail. Conchol., 26: 21-32, 2 tables, 3 figs.

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