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# A LIMNOLOGICAL SURVEY OF SINKHOLE PONDS IN THE VICINITY OF DOE RUN, MEADE COUNTY, KENTUCKY<sup>1</sup> <sup>2</sup>

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#### Introduction

This study was initiated on November 1, 1960, in conjunction with a radioecological study of the biota of Doe Run, Meade County, Kentucky, and had a twofold purpose: 1) to make a limnological reconnaissance of a previously unstudied area, and 2) to obtain data for a comparison of the lentic waters in sinkholes with the lotic waters of Doe Run. Since the springs that feed Doe Run arise from a subterranean system, it was deemed advisable to determine whether or not there might be some biological and chemical continuity between the ponds and the stream. A comprehensive study on the ecology of Doe Run has been presented by Minckley (1963).

The study area, a rectangle about 8.4 by 5.5 miles in northern Meade County, is delimited on the east and west by the drainage basin of Doe Run as determined by surface contours, on the north by the Ohio River, and on the south by U.S. Highway 60.

Doe Run deeply incises the area. From its source at 575 feet above mean sea level (msl), Doe Run flows 9.7 miles north-northeast to enter the Ohio River at 374 msl. The upland area through which the creek has cut is at an elevation of 680 feet msl near the source of the stream and slopes gently to an elevation of 600 feet msl at the bluffs bordering the Ohio River flood plain. To the south in the study area, scattered knobs (Buck Knobs, Bee Knob) attain an altitude above 900 msl.

The entire area is karstic, and all of the principal tributaries to Doe Run arise from caves or springs. A preliminary perusal of the study area indicated that there was a wide range in age of the various sinkholes, some of them being quite active whereas others obviously had contained water for many years.

#### Acknowledgments

Many persons graciously offered assistance and critical advice throughout the course of this study. We are especially grateful to Dr. W. L. Minckley, Arizona State University, for his many helpful

institution MAR 2 4 1964

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criticisms and suggestions, and to him and to Richard Goodyear, James Craddock, Judy Minshall, and Donald Tindall for their assistance in the field. We gratefully acknowledge the willing assistance of Jack Seilheimer and Rudolph Prins, University of Louisville; Dr. Gerald A. Cole, Arizona State University; Dr. Harry Yeatman, University of the South; and Dr. Clyde Goulden, Yale University; in the verification and identification of various plankton organisms.

This study could not have been completed without the cooperation of the many landowners who permitted access to their ponds. Mr. B. S. Jordan deserves special thanks for permitting the bimonthly sampling of his two ponds.

# Description of the Area Geology and Geography

The study area is in the Western Pennyroyal-Limestone Predominant Division of Ligon and Karraker (1949), and in the Elizabethtown Area of the Central Pennyroyal by Sauer (1927). This area, along with many others to the south and west, was named "the barrens" by early settlers because of the lack of trees (McFarlan, 1943). At present, about 75 per cent of the land in the study area is cleared. The remainder is in stands of western mixed mesophytic forest (Braun, 1950).

Cummings (1905) reported that the soil over the St. Louis formation in Indiana, although derived from limestone, was deficient in lime. His analyses showed the soil to contain from 67 to 80 per cent silica, 8 to 14 per cent aluminum, 6 to 7 per cent iron oxide, and very small percentages of lime, potash, magnesium, and other minerals. It is the iron oxide that gives the soil its characteristic orange-red color. Solution of the calcium carbonate has been so effective that it is necessary to add agricultural lime to cultivated lands.

In Meade County, soil samples from the barren hill district contained primarily aluminum, iron oxide, silica, phosphate, and calcium carbonate (Owen, 1857). The present soil of the area undoubtedly has been altered by land use and the addition of fertilizers, but probably fits best into the Bewleyville soil type of Ligon and Karraker (1949).

McFarlan (1943) described the area as a rolling plain with mature karst features developed in upper St. Louis limestone, which outcrops from central Indiana through Kentucky to central Tennessee (Swinnerton, 1942). Of Mississippian origin, the limestone is nearly 600 feet thick in the Mammoth Cave region of central Kentucky (Jillson, 1927), but becomes progessively thinner to the north so that in the study area it is about 400 feet thick (Greene, 1908). Analyses has shown it to be a very pure limestone of more than 90 per cent calcium carbonate (Cummings, 1905).

Since the formation is compact, there are many vertical joint planes, which, along with the bedding planes, probably are more numerous in this than in any other of the Mississippian limestones (Greene, 1908). The fractures are oriented in two directions, one running  $10^{\circ}$  east of north, and the other  $80^{\circ}$  west of north.

The area was peneplained during the Teritary Period. Since then the country has been mildly elevated to its present state (Greene, 1908). Weller (1927) pointed out that pure, dense limestone with joint planes offers an ideal situation for sinkhole formation. The continuing uplift has caused the surface water to cut deeper and deeper into the limestone, a process favored by the warm, humid climate (mean annual rainfall, 41.5 inches; mean annual temperature,  $56.5^{\circ}$  F; mean annual relative humidity, 79 per cent). Jillson (1927) attributed the lack of extensive cavern development in the study area, as compared with that in the Mammoth Cave region, to the thinning out and increased impurity of the limestone. However, on the basis of flow data from Doe Run (Minckley, 1962), there is an extensive cavern system in connection with the source of that stream.

Sinkholes of the area can be classified either as simple or compound sinks according to Scott (1909), who defined a simple sink as having the shape of an inverted cone with no secondary depressions within its slopes, and a compound sink as a large depression with secondary sinks within its slopes. In the compound sinks, the secondary depressions may become incorporated into the primary depression when their common divides are reduced by erosion. Scott pointed out the variability within its classification that the sinks may be opened or plugged. Frequently a secondary depression in a compound sink may become plugged and contain water whereas the primary depression remains open at its bottom.

The majority of plugged sinks (ponds) in the study area are in simple depressions on cleared farmland. There, the surface soil is openly exposed to erosion and surface run-off, particularly in cultivated areas, and carries the loose silt into the bottom of the depression to plug the hole. Conversely, there are not many sinkhole ponds in wooded areas because of the protection from erosion. Farmers in the study area frequently used sinks as places to dump brush and rubbish, thus assisting in plugging the outlet. Whatever the method of closure, the sinkhole pond, once established, is cut off from the ground water system and the water level rises and falls with precipitation. In a solution pond near Bloomington, Indiana, White (1930) reported that evaporation was the principal cause of water loss, although a small amount was lost through seepage.

Sinkhole ponds on the study area are neither large nor deep; the maximum size is less than three acres and the maximum depth no more than eight feet. The very shallow ponds remain dry in all but the wettest parts of the year. Since most ponds are in simple sinks, they are circular, subcircular, or ovoid in shape, and have saucer-like contours because of siltation in the deeper parts. About 80 per cent of the ponds are less than an acre in extent and less than five feet deep; more than a third of all the ponds are less than two feet deep.

Because of their shallow nature and the generally agrarian environment, the ponds usually fill with silt quite rapidly. Scott (1910) listed the deposition of plant remains and silt as the principal factors in the filling and eventual extinction of sinkhole ponds. He reported that one pond diminished in depth from eight to four feet within 24 years. As such siltation proceeds, ponds become shallower until water remains only during the wettest periods. On the other hand, residents of Meade County reported that some ponds which have held water for many years have suddenly gone dry. Such instances usually occurred during heavy rains when pressures either from above or below removed the plug and allowed the water to escape.

#### Stations A and B

Two ponds, A and B in Figure 1, on the B. S. Jordan farm on the eastern edge of the study area were chosen for intensive study. Station A is a simple sinkhole pond approximately 370 by 260 feet with a surface area of 2.0 acres. It was mapped and sounded when the pond was covered with ice in late December 1960 (Fig. 2). At that time the maximum depth was 7 feet 11 inches and the bottom was a relatively firm clay-mud mixture interspersed with chert and limestone fragments. The bottom was softer at the four corners of the pond where small undeveloped gullies entered. The pond is in a pasture used primarily by hogs and sheep. There were hog wallows present, but because of the firmness of the pond bottom they had little effect on the turbidity of the water. Except for some Eleocharis acicularis and Juncus effusus along the margin of the pond, there was no vascular flora. Because of its relatively deep basin, firm bottom, and general lack of vascular flora, Station A probably is relatively very young in geological time.

Station B (Fig. 3) is much shallower and much more eutrophic than Station A. Its kidney-shaped area, covering the basins of two

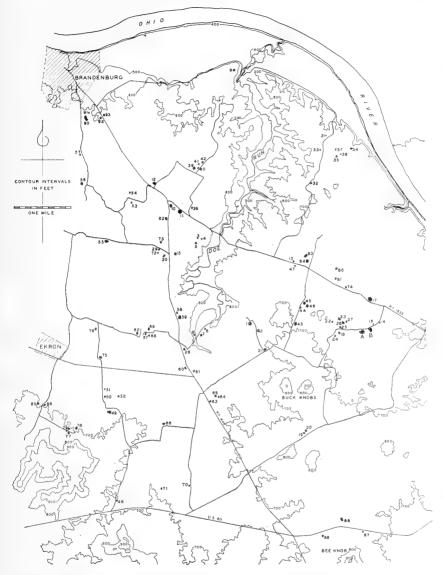


Figure 1.—The study area in northern Meade County, Kentucky, showing the locations of Stations A and B and the 93 survey ponds in relation to Doe Run.

adjacent sinks indicates that it is a compound sinkhole pond. There is deep water in the basin at either end with a shallower area in between. The pond was mapped during a period of ice cover in February 1961, and the approximate dimensions are 430 by 185 feet with

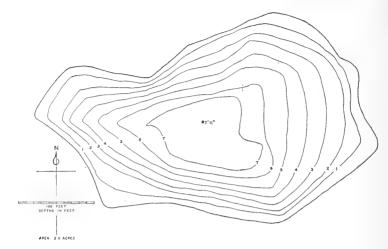


Figure 2.—Station A on the B. S. Jordan farm, Meade County, Kentucky, showing the one-foot depth contours.

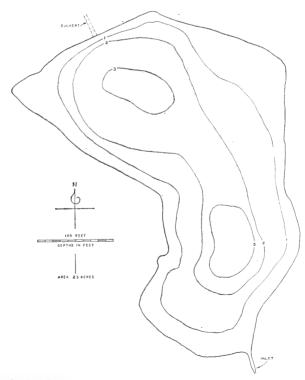


Figure 3.— Station B on the B. S. Jordan farm, Meade County, Kentucky, showing the one-foot depth contours.

a surface area of 2.3 acres. Soundings on October 15, 1961, indicated a maximum depth of 3 feet 6 inches near the center of the north basin. A rich vascular flora is dominated by Ceratophyllum demersum, and other common submerged forms are Chara sejuncta and Potamogeton diversifolius, each of which are not uncommon in other ponds of the area. P. diversifolius is scattered sparsely along the east and west shorelines, whereas C. sejuncta is confined to a dense stand in the southwest corner. Eleocharis acicularis found in most ponds visited, grew in the wet mud along the shore as well as in the water, but is largely confined to a narrow band around the pond perimeter, with a more extensive growth in the shallow water of the south end. Button bush, Cephalanthus occidentalis, formed a thicket and false shoreline along most of the eastern shore, and is less abundant midway along the western shore with water primrose, Jussiaea diffusa. Also, Eleocharis obtusa, E. palustris, Iris Shrevi, Juncus effusus, J. acuminatus, Polygonum sp., and Salix sp. were scattered along the shore or in shallow water. It was readily apparent that decaying plant material has been contributing to the bottom sediments for a long time because the bottom of hard clay was covered with a thick layer of black ooze.

At the beginning of the study, the pond was surrounded by pasture, but early in March 1961, the field on the east side was plowed and planted to corn. The effect of this change in land use was reflected in limnological conditions on various occasions for the remainder of the study.

#### Methods

All together, 95 ponds were sampled between October 1960 and mid-December 1961. Two ponds, Stations A and B, were studied in detail from November 1, 1960, through November 1, 1961, each being sampled 22 times in order to note any seasonal fluctuations in physical, chemical, and biological characteristics. In addition, preliminary data on water chemistry were collected on October 1, 1960. The remaining 93 ponds were sampled only once each, the majority being visited during the summer, autumn, and early winter of 1961. Of those 93 ponds, 4 were man-made, 3 were modified natural basins enlarged by a bulldozer, and the remaining 86 were natural sinkhole ponds.

General morphology and physical characteristics of each pond were noted and the area estimated. Measurements were made of maximum depth, water temperature at a depth of six inches, and turbidity. Turbidity was measured with a Bausch and Lomb Spectronic 20 colorimeter following the procedure outlined in Hach Chemical Company's "Water and Sewage Analysis Methods Manual" (Anonymous, n.d.) using distilled water as a standard. Results are expressed as "turbidity units" roughly equivalent to milligrams per liter (mg/1). Determinations of pH were made with an Analytical Measurements Corporation pocket pH meter or with a LaMotte color standard kit; alkalinity, using methyl purple as an indicator, and titrating with N/50 sulfuric acid; free carbon dioxide, by calculation using the formula of Ruttner (1953) and the dissociation constant K'<sub>1</sub> in Hutchinson (1957); and total iron, nitrate and nitrite nitrogen, and phosphate phosphorus with a Bausch and Lomb Spectronic 20 colorimeter with the procedures of "Water and Sewage Analysis Methods Manual." Alkalinity was expressed as milligrams per liter of calcium carbonate, whereas other nutrients were expressed as milligrams per liter.

Carbonate alkalinity was not distinguished from bicarbonate alkalinity although pH values were high enough in a few ponds so that the bound form could have been present in significant concentrations. This may have been true at Stations A and B, especially in late summer when the pH rose as high as 8.7.

In order not to disturb the bottom and roil the water, samples were taken from a 9-foot aluminum boat whenever feasible.

In addition to the above measurements, fluctuations in water level were recorded at Stations A and B, and fluctuations in water temperatures were recorded from maximum-minimum thermometers. Also, determinations for dissolved oxygen were made biweekly at Stations A and B using the unmodified Winkler method (Ellis, *et al.*, 1948). Samples were fixed in the field and taken to the laboratory for titration. Concentrations were corrected to sea level and percentages of saturation were obtained by using the nomogram of Hutchinson (1957). All samples used for oxygen determinations were collected six inches below the pond surface.

Plankton samples were taken every two weeks from Stations A and B, and once only from each of 81 of the 93 survey ponds. The survey ponds not sampled were Nos. 1, 2, 4-8, and 10-14. All plankton collections were made with a No. 25 silk bolting cloth net using a single vertical tow in the deepest water of each pond. In very shallow ponds where a vertical tow was impractical, plankton samples were obtained by pouring several bucketfuls of water through the net.

Tressler (1939) stated that in lakes with even bottom contours, a single central station gives a fairly good representation of the whole lake. Yeatman (1956), who used horizontal tows in his study of Woods Reservoir, Tennessee, and Ricker (1938) pointed out that yertical tows have an inherent error because the horizontal distribution of plankters is not always uniform. Since plankton nets do not yield catches that furnish accurate quantitative data for phytoplankton and small zooplankters (Strickland, 1960), such a net was relied on to provide a convenient, easy means for obtaining comparable qualitative data from the different ponds.

Plankton samples were preserved in four per cent formalin in the field and taken to the laboratory for identification and counting. Each sample was concentrated in a Foerst centrifuge and counted in a Sedgwick-Rafter chamber, using a field count, a strip count, and a chamber survey as outlined in Standard Methods for the Examination of Water and Wastewater (Anonymous, 1960). Whenever possible, l-ml aliquots from 15-ml concentrates were counted, but occasionally it was necessary to dilute the concentrate to facilitate counting. In such instances, an appropriate dilution factor was used in the calculations.

#### **Physical and Chemical Characteristics**

Since the single collections from each of the 93 survey ponds were made at all seasons of the year, no comparisons between ponds can be made, but these data may be used to indicate general trends in physical and chemical characteristics. Any information on seasonal changes must come from the data collected at regular intervals at Stations A and B.

Data from Stations A and B indicate that the water temperatures were very near the air temperatures (Fig. 4). Temporary thermal stratification occurred on hot, sunny days, but because of the shallowness of the ponds no thermoclines developed. On August 15, 1961, in Station A, there was a  $4^{\circ}$  C difference between the surface and bottom at a depth of seven feet.

On December 27, 1960, and January 29, 1961, an ice cover was present at Stations A and B. Other observations indicated that in each instance the cover lasted about a week. A very thin ice cover was present on some small survey ponds on December 3, 1960, and December 12, 1961.

The relations between precipitation and water levels at Stations A and B are illustrated in Figure 4B. The spring of 1961 was marked with heavy rains in March and May causing many ponds to overflow and spread over neighboring fields; water levels at Stations A and B rose more than two feet. Scott (1910) pointed out that such changes in water levels in such ponds are relatively great because the ponds are not very deep to begin with. The maximum depth of Station B increased almost 66 per cent during the periods of highest water level.

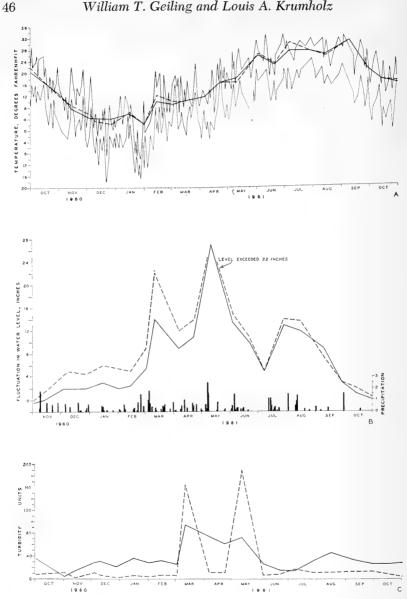


Figure 4.

A.—Daily maximum-minimum air temperatures (in degrees Centigrade) at Godman Field, Fort Knox, Kentucky, and water temperatures (in degrees Centigrade) at Station A (solid line) and Station B (broken line). Daily fluctuations of less than 2° C in air temperature usually were disregarded.

B.—Relationships between fluctuations in water levels at Station A (solid line) and Station B (broken line) and precipitation at Godman Field.

C.—Flucuations in turbidity at Stations A (solid line) and B (broken line). Values are expressed as "turbidity units," roughly equivalent to milligrams per liter.

Turbidity was greatest during periods of heavy precipitation (Fig. 4), but also was affected by disturbances by livestock. Agricultural use of the drainage basin caused higher turbidities at Station B than at Station A where the land laid fallow. At Station A, the turbidity values ranged from 95 turbidity units on March 11 to 4 on November 11, 1960, while at Station B they ranged from 188 on May 11 to 2 on November 13, 1960. In the 93 survey ponds, turbidity values ranged from 594 to 6 turbidity units.

Fluctuation in oxygen concentration and percentage of saturation reflect the net metabolism for Stations A and B (Fig. 5). The winter maximum and summer minimum illustrate that solubility,

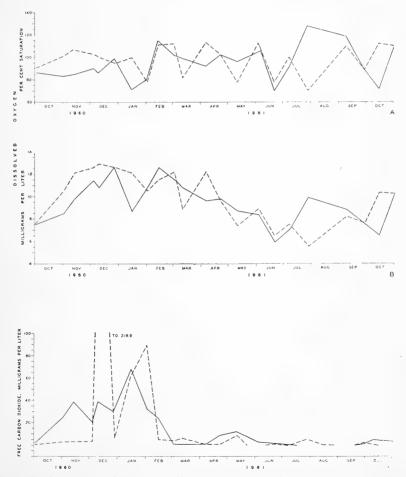


Figure 5.—Percentages of saturation (A) and concentration (B) of dissolved oxygen, and concentrations of free carbon dioxide (C) at Stations A (solid lines) and B (broken lines).

greater at lower temperatures, is of great importance in determining the dissolved oxygen.

It was difficult to correlate net phytoplankton productivity and oxygen saturation for various dates at Station A since density of net phytoplankton did not always coincide with high oxygen saturations. However, supersaturation of oxygen did occur only during periods of rather high numbers of net phytoplankton, and did not occur during the winter months when those numbers were reduced. Similar fluctuations in oxygen saturation occurred at Station B. The oxygen concentration was never low enough on any sampling day to limit the change of ferrous to ferric iron or the conversion of ammonia and nitrites to nitrates.

Diel variations in oxygen concentrations are illustrated by the data from 24-hour studies (Table 1). Maximum values usually were recorded late in the afternoon and minimum values just before dawn. Schmitz (1962) pointed out that although the maximum photosynthetic rate occurs during the morning hours, oxygen values continue to rise throughout the afternoon until respiration exceeds photosynthesis, sometimes as late as 8:00 PM.

Under optimal conditions (low temperature, low pH, and high alkalinity), very high concentrations of carbon dioxide are possible. Such conditions existed at Station B on December 10, 1960, when there were 219.9 mg/1 of free carbon dioxide. Photosynthetic activity, which takes carbon dioxide from the water, was minimal at that time. Reid (1961) described lakes with a pH near 6.0 and nearly 200 ppm free carbon dioxide. Optimal conditions exist during the winter months, as reflected in the concentration values at Stations A and B (Fig. 5). In February, with increasing water temperature and photosynthetic activity, carbon dioxide concentrations became depressed, and in lated summer there was a deficiency of carbon dioxide.

Of the 93 survey ponds, only 2 had a pH higher than 9.5 (Fig. 6), which indicates that half-bound bicarbonate was the prime form of alkalinity. Pond 29, sampled during a heavy bloom of *Anabaena spiroides*, had a pH of 10.4. The water had the appearance of green paint.

The range of pH values during the study period at Stations A and B (Fig. 7) indicates that Station B, the more productive pond, had higher values than Station A on all but 4 sampling dates. The lowest measurements in midwinter and the maximum values in late summer can be explained by the fact that photosynthesizing plants take up carbon dioxide which reduces the concentration of carbonic acid.

#### Table 1.—Chemical data at Stations A and B for 24-hour periods during winter, spring, and summer 1961. All values except pH are in milligrams per liter.

Station A

Station B

3.	l J	anuar	y-1	Fe	$\mathbf{br}$	uary
----	-----	-------	-----	----	---------------	------

	6 PM	12 MID	8 AM	12 N	6 PM	12 MID	8 AM	12 N
Fe	0.41	0.41	0.38	0,38	0.20	0.14	0.14	0,20
$PO_4$	0.23	0.09	0,06	0.06	0.07	0.06	0.02	0.06
$NO_3$	0.18	0,20	0.26	0.22	<0.01	<0.01	0.10	<0,01
NO2	0.02	<0.01	0,02	<0.01	0.02	0.02	0.00	0,01
<u>p</u> H	6.0	5,8	6.2	5.9	6.6	6.7	6.5	6.4
02	12.7	12.7	10.4	10.8	10.4	9.0	10.7	10.5
Alk.	8	8	11	8	78	67	55	70
			22-23	3 March				
	6 PM	12 MID	6 AM	1 PM	6 PM	12 MID	6 AM	l PM
Fe	0.61	0.71	0,65	0.69	3	0,10	0.20	0.16
$PO_4$	0.36	0.09	0.01	0.11	-	0.05	0.01	0.03
$NO_3$	0.25	0.30	0.20	0.20	-	0,01	<0.01	<0.01
$NO_2$	0.06	0.05	0.02	0.06	-	0.00	0.02	0.03
$\underline{p}^{H}$	7.4	7.1	7.0	6.4	8.1	7.9	8.1	8.1
· 0 <sub>2</sub>	9.6	9.2	9.3	8.9	10.7	9.8	9.4	10.1
Alk.	14	10	9	10		62	55	60
			27	-28 July				
	6 PM	12 MID	6 AM	12 N	6 PM	12 MID	6 AM	12 N
Fe	0.22	0.14	0.18	0.15	0.04	0.02	0.05	0,08
PO4	0.01	0.61	0,20	1.40	0.98	0.82	0.07	0.02
NO3	0.06	0.06	0.03	0.06	0,03	0.02	0.05	0.05
NO2	<0.01	<0.01	<0.01	<0.01	0.03	0,02	0.04	0.02
₽Ħ	9.4	8.8	8.6	8.9	8.0	7,2	7.2	7.3
02	10.8	9.6	8,7	9.8	8.4	7.6	5.1	5.5
Alk.	17	18	16	19.	55	56	57	57

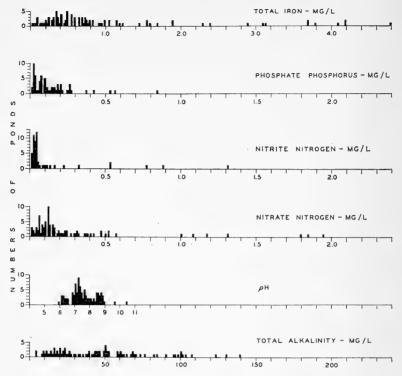


Figure 6.—Ranges and modal concentrations of total iron, phosphate phosphorus, nitrate nitrogen, nitrite nitrogen, hydrogen ions (as pH), and total alkalinity for the survey ponds. No phosphate was detected in 24, no nitrate in 10, and no nitrite in 39 of these ponds.

Also, as Ruttner (1953) pointed out, true aquatic plants are able to take up bicarbonate ions from the water and replace them with hydroxyl ions. Mann (1958) reported comparable annual fluctuations in some English ponds. Similar fluctuations on a much smaller scale are illustrated by the diel rhythms from Station A on July 27 and 28 (Table 1). The pH at 6:00 PM on July 27 was 9.4. With respiratory carbon dioxide being given off during the night, the pH was lowered to 8.6 at 6:00 AM on July 28. With the return of photosynthetic activity, the pH rose to 8.9 at noon. During this period the fluctuations in alkalinity were too small to be significant.

Alkalinity values at Station B were consistently higher than at Station A, and the concentrations at Station B were higher than the modal value for the 93 survey ponds and those at Station A, comparable to the softer ponds in the area.

Dissolved iron as the ferrous or the ferric form in the surface waters of the ponds studied is not indicated on the basis of the reviews by Ruttner (1953) and Hem (1959). Ruttner (1953) pointed out that iron in the ferrous form is unstable in the presence of oxygen and is immediately oxidized and precipitated as ferric hydroxide.

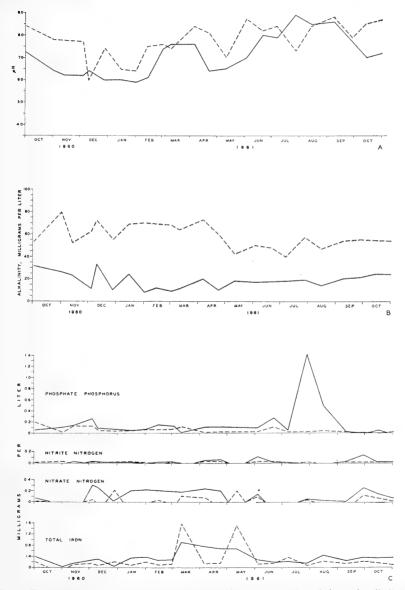


Figure 7.—Hydrogen ion concentrations (as pH) (A), amounts of methyl purple alkalinity (B), and phosphate phosphorus, nitrate nitrogen, nitrite nitrogen, and total iron (C) for Stations A (solid lines) and B (broken lines).

In the pH range of natural waters, the amount of ferric iron present is theoretically limited by the virtual insolubility of ferric hydroxide. It is only at levels below pH 3.0 that the solubility of ferric iron is increased to a point where it is measurable (Hem, 1959). However, iron can be present as ferric hydroxide adsorbed to suspended materials and in complex organic compounds (Hutchinson, 1957).

The range of concentrations of total iron (dissolved and suspended) at Stations A and B (Fig. 7) indicates the twofold increase at Station B over Station A during two periods of spring rains when large amounts of soil were washed into Station B from a freshly plowed field.

There was a positive correlation between turbidity values (Fig. 4) and total iron at Stations A and B, and there was a strong positive correlation between turbidity and total iron among the 93 survey ponds (Fig. 8). In late fall 1960, when the turbidity at Station B was low and there was a sparse plankton population, no iron was detected in the water.

The range of nitrate and nitrite nitrogen for the 93 survey ponds and for Stations A and B, respectively, are shown in Figures 6 and 7. There is little known about the workings of nitrite nitrogen in natural waters. The presence of nitrite nitrogen in concentrations in excess of those of nitrate nitrogen, or as Hutchinson (1957) stated, "any appreciable nitrite content in surface waters," has long been regarded as an indication of sewage contamination. In the 93 survey ponds, there were nine with greater concentrations of nitrite nitrogen than nitrate nitrogen. However, the nitrite concentrations generally were so low (less than 0.1 mg/1) that no conclusions could be drawn.

Nitrate concentrations in Stations A and B appear correlated with the biological activity of those ponds. Station B was consistently low in nitrates, presumably because of the abundant vascular flora. Nitrate concentrations at Station A, although usually higher, became depleted during periods of high phytoplankton production. Concentrations were at a stable maximum from midwinter through midspring, and the annual minimum occurred during the summer. Apparent algal succession in response to changes in nitrate concentration will be discussed later.

Diel variations in concentrations of nitrates were small and quite irregular (Table 1).

Fluctuations in concentrations of phosphate at Stations A and B, except for the extreme high concentrations in late July and mid-August, were quite stable (Fig. 7). In four of the six 24-hour studies, however, surface-water concentrations of phosphates fluctuated widely

#### A Limnological Survey of Sinkhole Ponds

(Table 1). On July 27, at 6:00 PM at Station A, there was 0.01 mg/l of phosphate in the surface-water sample, and 18 hours later a value of 1.40 mg/l was recorded (Fig. 7). Although such variation makes analysis of the data difficult, a possible explanation might lie in the vertical movements of plankton organisms and a consequent concentration of organic phosphorus within the stratum of densest plankton.

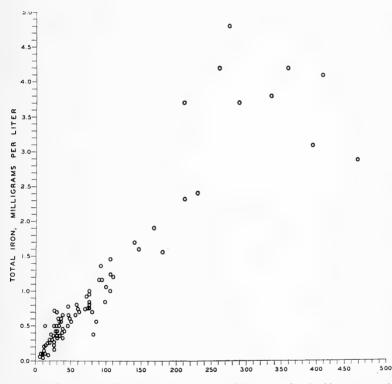


Figure 8.-Relationship between total iron and turbidity units for the 93 survey ponds.

Hutchinson (1957) stated that at the height of summer there may be a great increase in total phosphorus in the surface waters at times of algal blooms. On August 15, the value for phosphates in the surface-water sample was eight times greater than that recorded at the bottom (0.48 mg/1 and 0.06 mg/1). However, no comparison could be made between the plankton and the phosphate because an integrated sample of plankton was collected at the time.

There was no detectable phosphorus present in 24 of the 93 survey ponds when sampled. Otherwise, the ranges and modal concentrations were quite like those at Stations A and B (Fig. 6). One pond, No. 79, had an abnormally high concentration of 0.84 mg/1, which may be explained by its location in a barnyard. Still, several other ponds in similar locations did not have such high concentrations.

The mean value for phosphates for ponds in the study area was higher than means listed for several lake regions in the world (Reid, 1961). The concentrations in the study ponds were exceeded only by lakes in the phosphatic rock regions in Florida. The favorable ratios between water volume and bottom surface, and the agrarian environment undoubtedly contributed to the high values for phosphates.

In Doe Run (Minckley, 1962), concentrations of total iron were highest in periods of high flow, but when exceedingly high turbidities were recorded, concentrations of iron were not as great as those in the sinkhole ponds. It is possible that the soil washed into the cave aquifer may have come from a different horizon, lower in iron content, than that surrounding the sinkhole ponds, or it may indicate that the source of water feeding Doe Run is not related to the sinkhole area studied.

The mean phosphate and nitrate concentrations in Doe Run were much higher than those in the sinkhole ponds (Minckley, 1962).

The greatest difference in the water chemistry of Doe Run and that of the sinkhole ponds was in the data on alkalinity. The average total alkalinity at the source of Doe Run (Minckley, 1962) in periods of low flow was about 230 mg/l whereas the modal alkalinity for the sinkhole ponds was about 50 mg/l. In periods of high flow, when much surface run-off entered Doe Run, values for alkalinity dropped. These differences between the sinkhole ponds and Doe Run probably are due to the effects of the subterranean passage of the water where it may have accumulated much nutrient material before it entered the stream, and to the effects of the biota, including man, on the water after it entered the sinkhole ponds. Run-off water collected by Minckley (1962) from a tributary to Doe Run after a heavy rain, had an alkalinity intermediate to the modal alkalinities of the stream and the sinkhole ponds.

Ponds 39, 40, 41, and 42 were man-made, having been dug with a bulldozer. Ponds 51, 52, and 80 had been modified by being made deeper or larger. The water chemistry of these ponds was about the same as that of the natural sinkhole ponds. However, since the manmade and modified ponds are used primarily for sport fishing, they are occasionally treated with inorganic fertilizers to increase productivity.

#### Net Plankton Flora and Fauna

All together, more than 140 kinds of organisms were identified (see Appendix). Identifications were made to species whenever possible, and in all instances the most feasible definitive classification was used. The localities and relative abundances of all organisms taken during the study were listed by Geiling (1962). This discussion includes the spatial and temporal distributions of the various groups of organisms. The seasonal distributions of the more abundant plankters at Stations A and B are given in Table 2 and are illustrated graphically in the different figures.

## Chlorophyta

#### Phytoplankton

More than 40 species of green algae, the highest number for any algal group, were collected and identified. Of those, 20 were desmids, which rarely were abundant, but usually were represented by one or two species as a minor planktonic element. These findings are similar to those of White (1930) for a solution pond in southern Indiana. Many desmids were grouped as Cosmarium spp. and Closterium spp. primarily because of the lack of a modern comprehensive key. Cosmarium sp. exhibited minor pulses at Station A in May and June and in July and August (Fig. 9). At Station B, however, Cosmarium reinforme (Ralfs) Archer and Cosmarium sp. were the dominant green algal forms most of the year. The large (600 to  $800\mu$ ) Closterium turgidum Ehrenberg, Staurastrum gracile Ralfs, and Cosmarium spp. were the most common desmids among the survey ponds. Prescott (1962) stated that desmids are found most abundantly in standing waters, rich in organic acids and low in calcium. The alkaline pH and the moderate hardness of most of the Meade County ponds probably limited the development of large desmid populations.

Certain small ( $\langle 25\mu \rangle$ ), unicellular- coccoid forms, numerically abundant at Ponds 21, 24, 27, and Station A, were recorded merely as green coccoids. The spring bloom at Station A was caused first by an even smaller form ( $\langle 10\mu \rangle$ ), possibly *Chlorella* sp., and later by a larger species (*Chlamydomonas* sp.?). A bloom in June also was dominated by the larger species.

Among the survey ponds, several species occurred in bloom proportions, but usually only in a relatively few ponds. Among those species were Actinastrum hantzschii var. fluviatile Schroeder, Closterium longissima Lemmerman, Scenedesmus sp., and Volvox globator L. The only other chlorophytes having a more-than-limited distribution were Pediastrum duplex Meyen and the colonial Eudorina elegans Ehrenberg, which also was a major plankter at Station B during mid and late summer. Cole (1957) considered *E. elegans* a warm-water form.

#### Chrysophyta

Fourteen different kinds of chrysophytes, of which nine were diatoms, were identified. Like the desmids, the diatoms rarely were of numerical importance. Cole (1957) found that diatoms rarely were abundant at Tom Wallace Lake, near Louisville, Kentucky, and Eddy (1931) made similar observations in sinkhole ponds in southern

Table 2.—Seasonal occurrence (semimonthly intervals) of major plankton organisms found in sinkhole ponds in Meade County, Kentucky. Data from Stations A and B and the 93 survey ponds are combined.

	196		1	e	AL	A	IN a			961		۸.		500	0.	-+	Not		20
Organism	Nov	Dec	Jan	FeD	mar	Apr	ma	y	JU		uı	Â	g	sep	1	- 1			Je
Chlorophyta																			
<u>Closterium turgida</u>						×	×			×		×	×		×	×	× >		
Cosmarium spp.	×	× ×			×	× ×			×	××	×	×	×	×	×	×	,		
Eudorina elegans		ļ				×			×	×	×	×	×					þ	×
Pediastrum duplex										××	×	×	×	×		×	×	<	
Staurastrum gracile			1				×		×	××		×	×	×	×	×		1	×
Chrysophyta																			
Botryococcus protuberans										×	×	×	×	×	×				
Botryococcus sudeticus															×	×	×		
Dinobryon cylindricum						×	×			××	×	×				×			×
Pyrrophyta															-				
Ceratium hirundinella					× ×	× >	×	×	×	×	×	×	×	× ×	×	×	×	×	×
Peridinium cinctum					× ×	×		×	×	×	×	×	×	× ×	×	×	×		
Euglenophyta																			
Phacus longicauda	×	×			×	,	< ×	×	×	×>	×	×	×	× ×	×	×	×	×	×
Trachelomonas hispida												×				×		×	×
Trachelomonas volvocina																×	×		
Cyanophyta																			
<u>Anabaena spiroides</u>										×	<			>					
Microcystis aeruginosa												×	×	,	< x	×	×		
Oscillatoria sp.												×	×	,		×			
Rhizopoda																			
Difflugia lobostoma										× :	<	×	×	_× ,		×	×	×	>

#### A Limnological Survey of Sinkhole Ponds

Organism	No	v	De	с	Ja	n	Fe	eb	Ma	r	Ap	r	Ma	У,	Jι	in	Jt	11	Au	10	I <sup>S e</sup>	ep	100	st	No	v,	Dec
Ciliophora																											
Codonella cratera	×		×	×	×	×	×		×		×	×	×	×	×	×			×	×	×	×	×	×	×	×	×
Rotifera																											
Asplanchna sp.													×				×			×		×		×	×	×	×
Brachionus angularis	×		×	×			×		×				×			×	×	×	×	×	×	×	×	×	×		
Brachionus havanaensis	×										×		×	×	×	×	×	×	×	×	×	×	×	×			
<u>Filinia longiseta</u>	×	×	×	×	×	×	×		×						×	×	×	×	×	×	×	×	×	×	×	×	×
<u>Kellicottia</u> <u>bostoniensis</u>	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×
Keratella cochlearis	×	x	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	× :
<u>Polyarthra</u> sp	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×		×	×	×	×	×	×
Cladocera																											
<u>Bosmina</u> longirostris	×	×	×	×	×				×	×	×		×		×	×	×	×	×	×	×	×	×	×	×	×	× :
<u>Ceriodaphnia</u> pulchella																×	×	×	×	×	×	×	×	×			
Daphnia ambigua			×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Diaphanosoma brachyurum															×	×	×	×	×	×	×	×	×		×		
<u>Diaphanosoma</u> leuchtenbergiamum																×			×	×			×				
Moina affinis																×		×	×				×				
Copepoda																											
Diaptomus pallidus			×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		
Cyclops vernalis									×		×		×			×	×	×				×		×		×	×
Mesocyclops edax													×	×	×	×	×		×	×			×	×			
Tropocyclops prasirus																×	×		×	×		×	×	×	×	×	×

Table 2. (continued)

Illinois. The only exceptional occurrence of diatoms in this study was a spring bloom of *Fragilaria crotonensis* Grunow at Station A which yielded 3,200 organisms per foot of tow. That diatom also was the most common one among the survey ponds; it was collected from 11 ponds.

Dinobryon cylindricum Imhof was a spring and midsummer form in the Meade County ponds. It was present in bloom proportions at Station B early in April, at Station A late in June, and at four survey ponds, one of which was sampled on December 2, 1961. That was the only winter record of that species for this study although Cole (1957) found D. sertularia Ehrenberg throughout the year at Tom Wallace Lake.

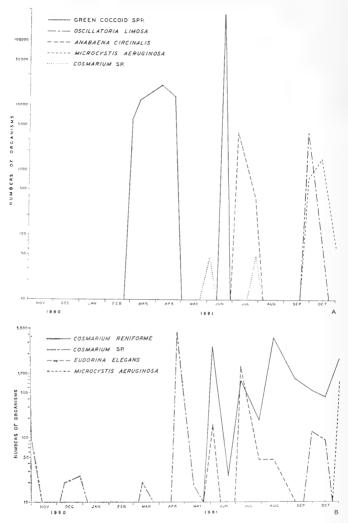


Figure 9.—Seasonal occurrence of Chlorophyta and Cyanophyta at Stations A (A) and B (B). Numbers of organisms are given per foot of tow.

Botryococcus protuberans G. M. Smith and B. sudeticus Lemmerman were the only abundant chrysophytes collected in late summer and early autumn, and they often were associated with the blue-green alga *Microcystis aeruginosa* Keutz. Prescott (1939) stated that an increase in temperature often accompanies excessive growth of many species of Myxophyceae and certain Heterokontae (= Xanthophyceae) such as *Botryococcus braunii* Keutz. The taxonomic status

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of the genus *Botryococcus* is uncertain, and authorities do not agree whether it should be placed in the Chlorophyceae or the Xanthophyceae. The inclusion of the genus in the Xanthophyceae allows the Chrysophyta a summer and autumnal representative among the plankton. *B sudeticus* was present in large numbers in November 1960 and October 1961 at Station A.

#### Pyrrophyta

The dinoflagellates were represented by only two species, Ceratium hirundinella (Mueller) Schrank and Peridinium cinctum (Mueller) Ehrenberg, but they combined to dominate the net plankton of most of the ponds from midspring through late autumn (Fig. 10). Eddy (1931) considered *Ceratium* a common pond form, and *P*. cinctum was one of the species that characterized the plankton of the sinkhole ponds he studied. Yeatman (1956) found Ceratium present during the summer, with its first appearance usually as a strong surge. Tressler, et. al. (1940) reported large numbers of Peridinium in the summer plankton of the large but shallow Buckeye Lake, Ohio. At Stations A and B, Ceratium appeared earlier than Peridinium, and at Station A the Ceratium was still present after the Peridinium. Both species were equally abundant in the spring collections from the survey ponds, but *Peridinium* disappeared before *Ceratium* in the autumn. These findings agree with those of Cole (1957) for Tom Wallace Lake, where both species bloomed during the summer. P. cinctum was not collected when the water temperature was below  $15^{\circ}$  C, whereas C, hirundinella was collected on eight occasions when the water was below that temperature. C. hirundinella was present in 52 of the 81 ponds sampled for plankton, and P. cinctum was present in 48.

#### Euglenophyta

Three genera of euglenophytes were found in the ponds studied; they were Euglena, Phacus, and Trachelomonas. Euglena spp. and Phacus longicauda were common in collections of summer and autumn, whereas Trachelomonas hispida (Perty) Stein, T. superba (Swir.) Deflandre, and T. volvocina Ehrenberg were taken only in the autumn. P. longicauda was present at Station A from mid-April through mid-September but never in large numbers (Fig. 11). Euglenophytes at Station B occurred only occasionally. Phacus and Euglena occasionally were present in abundance in the survey ponds, but for the most part they occurred in moderate numbers. Smith (1950) found that Euglena is a common organism in waters rich in organic matter, such as pools in barnyards.

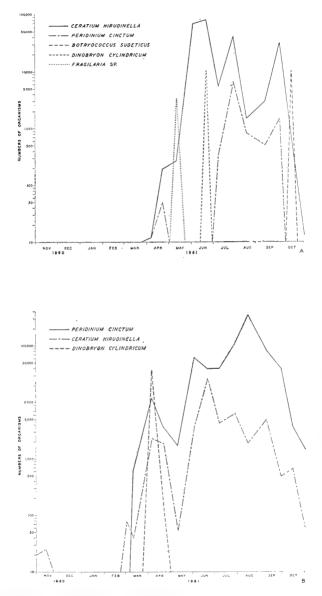


Figure 10.—Seasonal occurrence of Chrysophyta and Pyrrophyta at Stations A (A) and B (B). Numbers of organisms are given per foot of tow.

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Prescott (1962) pointed out that the lorica of *Trachelomonas* may be clear to brownish depending on the presence of iron compounds. Most *T. volvocina* collected from the survey ponds were bright orange, which may indicate that iron was readily available to the pond plankton.

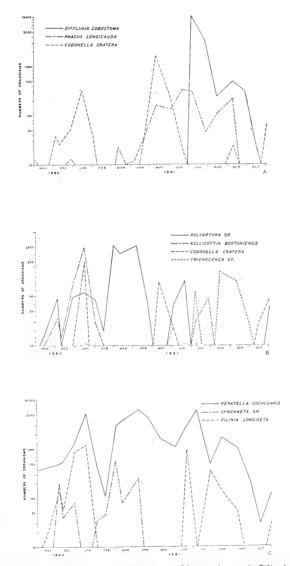


Figure 11.—Seasonal occurrence of Euglenophyta, Rhizopoda, and Ciliophora at Station A (A), and of Ciliophora and Rotifera at Station B (B and C). Numbers of organisms are given per foot of tow.

#### Cyanophyta

Thirteen different kinds of blue-green algae were identified. Some species were present in bloom proportions, the cyanophytes were not as abundant as might be expected for such warm, highly eutrophic waters, Prescott (1960) stated that eutrophic lakes, by their chemical nature, may be veritable garden spots for blue-green algae when phosphates and nitrates are not limiting, and (Prescott, 1939) listed blue-green algae as a dominant form, both in total numbers and variety of species, for eutrophic lakes. At Stations A and B and most of the survey ponds, the cyanophytes were overshadowed by the large numbers of pyrrophytes.

White (1930) found that shallow ponds do not support large populations of Myxophyceae. Although he did not elaborate that statement, the shallowness of the sinkhole ponds may have been unfavorable for the development of blue-green algae.

The first blue-green alga to appear at Station A was Anabaena circinalis Rabenhorst (Fig. 9). It was collected in two of the July samples but was more common early in the month. No other bluegreen algae were taken until late September when two species, Oscillatoria limosa Agardh and Microcystis aeruginosa Keutz., were present in bloom proportions. Microcustis was still present but Oscillatoria had disappeared when the last collections was made November 1, 1961. It is probable that O. limosa was not planktonic in origin. Blum (1960) reported that at times of rapid photosynthesis, individual masses of Oscillatoria filaments were detached from the substrate and buoyed upwards by trapped oxygen bubbles. Although that report was for stream conditions, the phenomenon was observed at Station A when floating algal mats still carried soil particles. Blum also stated that floating algal mats usually could be observed between noon and midafternoon on sunny summer days. Samples at Station A usually were taken at that time.

The only blue-green alga taken other than occasionally at Station B was *Microcystis aeruginosa* which was in pulse proportions on November 1, 1961 (Fig. 9). It was abundant in 4 of the 16 survey ponds from which it was collected between early August and mid-November. From these data, it appears that *M. aeruginosa* populations develop only in the late summer and autumn. Other species of blue-green algae, *Anabaena spiroides* Klebahn and *Aphanizomenon flos-aquae* (L.) Ralfs, were collected in large numbers in late June and July. Only two other species, *Anabaena felisii* (Menegh) Bornet and Flahault and *Coelosphaerium neagelianum* Unger, were observed in large numbers.

#### Rhizopoda

#### Zooplankton

Only two rhizopods were observed, Difflugia lobostoma Leidy and one recorded only as "unidentified rhizopod." At Station A, D. lobostoma formed a strong pulse early in July and continued in gradually reduced numbers until late September. By mid-October it had virtually disappeared (Fig. 11). D. lobostoma was taken in 28 survey ponds, in many instances abundantly. It was not collected from any of the ponds in the spring, but was taken in large numbers in summer and autumn. Three ponds sampled in mid-October and early November 1961, contained large populations. Cole (1957) found D. lobostoma limited almost entirely to warm-water periods at Tom Wallace Lake, but it was present throughout the year in sinkhole ponds studied by Eddy (1931) in southern Illinois, being most abundant during summer. Yeatman (1956) reported D. lobostoma as very abundant in late summer at Woods Reservoir, Tennessee.

The unidentified rhizopod was taken only at Survey Ponds 91 and 92 in mid-December 1961, but in each instance it was abundant.

#### Ciliophora

The sole ciliophoran observed was *Codonella cratera* Leidy. It was taken at Station A four times during the study as follows (Fig. 11:1) a winter pulse that lasted from early December to early February; 2) a large late-spring to early-summer pulse that lasted from mid-April to the end of June; 3) a minor occurrence in September; and 4) a late autumn occurrence of moderate numbers on November 1, 1961. There were three pulses in Station B; one in January, one in May, and one in October. That last-mentioned pulse was still in evidence when the study was terminated November 1, 1961.

C. cratera was present in 35 survey ponds and was abundant in 14, 6 of which were sampled during the first half of November 1961. From this information it is apparent that this ciliophoran is a late-summer and autumn form. It was not taken in large numbers in any pond before late August.

Eddy (1931) stated that *C. cratera* is a common pond form and was abundant at all seasons. Cole (1957) also reported year-round distribution of the species at Tom Wallace Lake, but with greatest abundance in spring.

#### Nemata

Pennak (1953) stated that the rare free-swimming nematodes move through the water just above the substrate. Probably because of that habit, nematodes (unidentified) were collected in small numbers from only two ponds, Survey Ponds 30 and 56.

#### Rotifera

Rotifers were represented by more species and usually were present in greater numbers than any other group of zooplankton. This appears true for most small lakes and ponds (Cole, 1957; Tressler, *et al.*, 1940; Chandler, 1940; Pennak, 1957; and others). A total of 21 different kinds of rotifers were identified during this study. It is possible that other forms were collected but not identified since the illoricate rotifers become distorted and shriveled when preserved, and are difficult to distinguish. Unidentified rotifers occurred, sometimes in great abundance, in 22 survey ponds. Eight identified species were collected regularly in significant numbers.

Three species of *Brachionus* occurred abundantly, *B. angularis* Gosse, *B. calyciflorus* Pallas, and *B. havanaensis* Rousselet. Two other species, *B. bidentata* Anderson and *B. quadridentatus* Hermann, each were taken on one occasion. Eddy (1931) recorded the genus *Brachionus* as scarce in sinkhole ponds in southern Illinois, and Edmondson (1959) stated that it characteristically is found in hard water.

B. angularis was collected in 25 survey ponds from mid-May to early November. Usually it was abundant during the summer but on two occasions large numbers were present in autumn collections. At Station A, this species was taken throughout the year (Fig. 12). It was present in moderate pulse proportions in December 1960 and February 1961, and also in moderate to small numbers from July to mid-September. It was most abundant as a major pulse in mid-October. B. angularis was not recorded from Station B. B. calcyciflorus was collected from seven survey ponds and on scattered occasions at Stations A and B. It occurred at all times of the year but was abundant only during the summer. B. havanaensis was present in samples from 27 survey ponds and Station B, and it was a major summer plankter at Station A. It was essentially a summer form, frequently occurring in large numbers from June to September; it was present at Station B in small numbers in spring and autumn. Ahlstrom (1940) reported B. havanaensis from ponds at Cincinnati, Ohio, and Lexington, Kentucky, but was not reported from the region of Louisville by Cole (1957) or Bunting (1959).

Asplanchna sp., a large  $(500 \ \mu)$  illoricate rotifer was taken in 15 survey ponds and was thought to be a non-seasonal form although it was not taken in the spring samples. It was abundant only at Pond 44.

*Kellicottia bostoniensis* Rousselet was taken in every sample from Station A except the one of November 1, 1960 (Fig. 12). In November of each year its numbers were minimal, but for the rest of the year

it was a major constituent of the plankton and from early May until late August it was present in very large numbers. At its maximum on June 21, there were more than 9,000 individuals per foot of tow. After the decline in late August, there was a moderate resurgence in October. *K. bostoniensis* was abundant in midwinter at Station B and occurred as a minor element of the plankton for a short time in midsummer. The early November decline at Station A is coincident with its absence in the collections from 19 survey ponds sampled between October 23 and November 18, 1961; it was taken only from 18 other ponds sampled before or after that period.

Keratella cochlearis (Gosse) was by far the most abundant zooplankter. It occurred in 76 of the 81 ponds sampled for plankton and was abundant or very abundant in 39 of those. Although usually abundant at Station A (Fig. 12), it disappeared late in March but reappeared in April and remained abundant for the remainder of the study period. It was generally abundant throughout the year at Station B (Fig. 11).

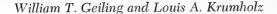
*Filinia longiseta* (Ehrenberg) was present throughout the year and was abundant in 15 of the 34 ponds where it was collected. At Station B (Fig. 11) it was present as a midwinter pulse and as an interrupted midsummer pulse. At Station A (Fig. 12) it was present in moderate numbers from November 1960 to early February 1961, for a short time in early June, and from mid-July to mid-October.

The last major rotifer, *Polyarthra* sp., was collected from 32 survey ponds at all seasons of the year and was abundant in 8 ponds.

Synchaeta sp., listed by Edmondson (1959) as a common limnetic rotifer, was collected only during the winter at Station B (Fig. 11). Cole (1957) stated that Synchaeta was a cold-water species and that he never found it during the summer at Tom Wallace Lake. The genus was entirely wanting in Eddy's (1931) collections. The warm summer and autumn temperatures of the ponds in the Doe Run area probably inhibit the development of Synchaeta.

#### Anostraca

The spiny-tailed fairy shrimp, *Streptocephalus seali* Ryder, was the only anostracan collected. The only previous record of this species from Kentucky was from a temporary pond in Warren County (Weise, 1957). A single specimen was collected in the net plankton at Pond 31, July 23, 1961, and great numbers were collected with a minnow seine on August 8, 1961, from Pond 34. Fairy shrimp are found most often in temporary ponds although periods of drought are not a requisite part of the life cycle (Moore, 1951). Because of its size and depth, Pond 34 probably contained water all year, but



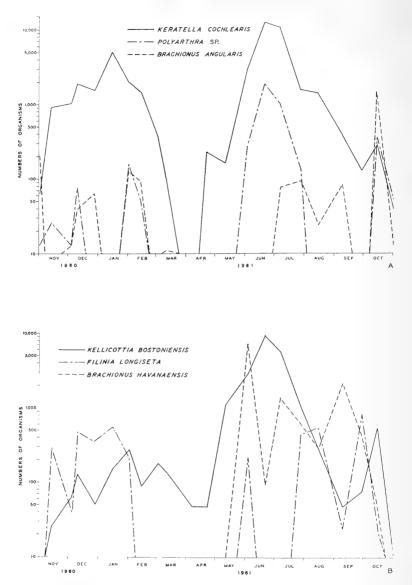


Figure 12.—Seasonal occurrence of Rotifera at Station A. Numbers of organisms are given per foot of tow.

the lack of fish probably allowed the fairy shrimp to become established. It may be that the presence of fishes in the other ponds restricts the distribution of *Streptocephalus seali*.

### Cladocera

Nineteen cladocerans were identified from the study area. With the exception of *Bosmina longirostris* (O. F. Mueller) and, to a lesser extent, *Daphnia ambigua* Scourfield, none were extensively distributed.

Four species of cladocerans were collected at Station A, and three of them were present in relatively large numbers. *Daphnia ambigua* 

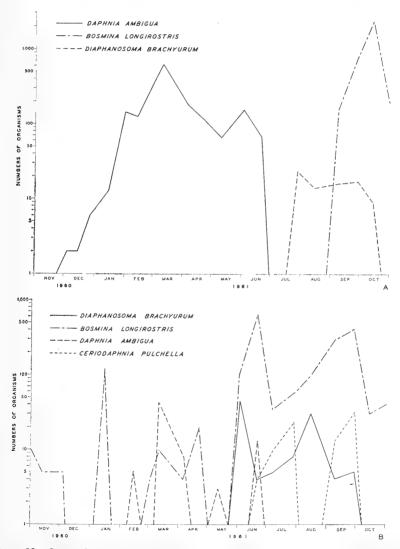


Figure 13.—Seasonal occurrence of Cladocera at Stations A (A) and B (B). Numbers of organisms are given per foot of tow.

was a vernal species; the numbers increased through late winter to a peak in mid-March (Fig. 13). After that maximum, the numbers remained high until late June when the species disappeared. Armitage (1961) recorded *D. ambigua* as a spring species in ponds in north-eastern Kansas.

In late July there was a moderate pulse of *Diaphanosoma brachyurum* (Liéven) (Fig. 13). It remained moderately abundant until mid-October when it disappeared abruptly. Eddy (1931) recorded this species only in July and September in southern Illinois, and Armitage (1961) called it an autumnal species.

Bosmina longirostris appeared in large numbers in early September and reached a maximum of more than 2,000 individuals per foot of tow by mid-October (Fig. 13). It was still present in large numbers on November 1, 1961, when sampling at Station A was terminated.

The fourth cladoceran from Station A was a littoral form, *Alona* guttata Sars which was taken in small numbers in two autumn samples.

Eight species of Cladocera were taken at Station B. The greater number of species here than at Station A probably is traceable to the "littoral" nature of the pond. Four species, *Chydorus sphaericus* O. F. Mueller, *Alona guttata, Alona karua* King, and *Pleuroxus denticulatus* Birge, were taken only occasionally. Of the other four species, *Bosmina longirostris* was most abundant, but in contrast with Station A was present throughout the year and was most abundant from June 1961 throughout the remainder of the study. *Daphnia ambigua* occurred in four pulses from February to June, *Diaphanosoma brachyurum* was taken in moderate numbers from June until October, and *Ceriodaphnia pulchella* Sars was present in moderate pulses in July and September.

Bosmina longirostris was the most common cladoceran in the survey ponds, being taken in 31 of the 81 ponds sampled. Other species, besides those taken from Stations A and B include Daphnia laevis Birge, D. parvula Fordyce, Diaphanosoma leuchtenbergianum Fischer, and Moina affinis Birge.

Using Cole (1957) as a point of reference, there are recorded here new state records for five cladocerans. Cole recognized that daphnids reported as *D. longispina* and *D. pulex* actually included several species. In his monograph on North American daphnia, Brooks (1957) solved the problem by elevating several varieties to species status with the necessary nomenclatural changes. On the basis of Brooks' revision, *Daphnia ambigua*, *D. laevis*, and *D. parvula* are reported as new state records, and *Alona karua* and *Diaphanosoma leuchtenbergianum* also are reported from Kentucky for the first time.

# A Limnological Survey of Sinkhole Ponds

#### Copopoda

Eleven species of copepods, five calanoid and six cyclopoid, were identified. *Diaptomus pallidus* Herrick was by far the most common, being abundant at Stations A and B and being taken in 57 survey ponds. At Station A, *D. pallidus* was present throughout the year and, although there was much fluctuation in abundance, the numbers generally increased to a maximum in October. Although not as abundant as at Station A, it occurred throughout the year also at Station B, where the largest numbers were present in summer and autumn. Ward (1940) pointed out that *D. pallidus* was common throughout the year in the Cincinnati region, and Armitage (1961) stated that it was taken at all seasons in Kansas. Cole (1953) stated that *D. pallidus* was the common calanoid copepod in the Louisville area.

Diaptomus sanguineus S. A. Forbes, described by Yeatman (1956) as a cold-water species and by Ward (1940) as a spring plankton form, was collected in only one pond and that sample was taken in mid-May.

*Diaptomus stagnalis* S. A. Forbes, the large, bright-red copepod, was collected only once in a temporary pond in a woodlot. It was, however, also collected from a small marsh at the junction of Buck Grove Road and Kentucky state highway 1638 by W. L. Minckley and J. E. Craddock.

The following two species of *Diaptomus* are reported from Kentucky for the first time. *D. leptopus* S. A. Forbes was collected from six different survey ponds. Although usually not occurring in large numbers, this species is widespread in North America. Cole (1959) did not include it in his list of crustaceans from Kentucky. *D. birgei* Marsh was found in only one pond. It was not listed by Cole (1959), and Wilson (1959) listed it as occurring along the eastern coast of the United States and in Louisiana. *D. birgei* was recorded from Sullivan County, Indiana, about 150 miles northwest of Meade County, Kentucky, by Plew and Pennak (1949).

The most common cyclopoid copepod was *Tropocyclops prasinus* (Fischer), which was taken in 31 survey ponds and in late autumn at Station B. It was never abundant in the collections. Cole (1957) reported the species as common in Tom Wallace Lake.

Cyclops vernalis Fischer, a species of variable form, was abundant in several survey ponds. Yeatman (1944) discussed some of the recognized forms and cautioned against using these groups as subspecies. Most Meade County specimens were of the "brevispinosus" form.

Mesocyclops edax S. A. Forbes, although never abundant, was

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found in 12 survey ponds. However, it formed a pulse at Station A (Fig. 14) indicating a seasonal occurrence. There was an occurrence of copepodids in March followed by an abundance of adults in May. Then there was another pulse of copepodids that began in June and lasted through September but which was not followed by a pulse of adult M. edax. This conforms with the findings of Comita (1961) that M. edax has two generations during the ice-free period from March to November in temperate regions. Coker (1933), in Cole,

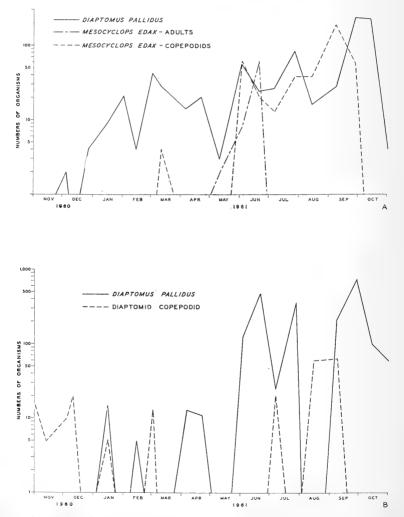


Figure 14.—Seasonal occurrence of Copepoda at Stations A (A) and (B). Numbers of organisms are given per foot of tow.

1953) and Elgmork (1955) reported instances where cyclopoid copepods ceased development for long periods after reaching the fourth copepodid stage. Elgmork reported that this resting stage normally lasted over the summer for *Cyclops strenuus* Fisher. Both Coker and Elgmork, however, reported that after the resting periods the animals passed through the fifth copepodid stage and reached adulthood. The fact that no adult M. edax were taken at Station A between the end of the copepodid pulse in October and the end of sampling at Station A on November 1, may be explained by the findings of Smyly (1961) who reported three generations of M. leuckarti Claus in Esthwaite Water, England. Members of the third generation, occurring in July and August, did not develop past the fifth copeodid stage, but sank to the bottom after the fall overturn and lay dormant in the mud of the substrate.

The most unlikely species taken in this study was *Mesocyclops* tenuis (Marsh), collected from the same two ponds that contained the fairy shrimps. Yeatman (1959) described M. tenuis as "rare in Arizona and probably other states bordering Mexico." This, of course, is a new record for Kentucky, and extends the known range more than 1,000 miles.

Two other cyclopoid copepods, *Eucyclops agilis* (Koch) and *Macrocyclops alibidus* (Jurine), both littoral forms, were taken occasionally.

Although *Cyclops bicuspidatus thomasi* (Forbes) is widely distributed in Kentucky and nearby states, it was not taken in any of our collections. It has been reported from the area by Cole (1953), Eddy (1931), Ward (1940), and Yeatman (1956).

# Seasonal Distribution of Organisms

Frequently in studies where physical and chemical data have been collected along with plankton samples, there is an attempt to correlate environmental conditions with the abundance of a species or a group of species. It is evident that such correlation can be done only within the confines of the data and with a knowledge of the complex ecological interactions in the body of water concerned. Ruttner (1953) concluded that "No direct relationship exists between the quantity of total plankton or of individual species present at any one moment and the content of nutrients measurable at the same time, for the reason that any quantities of these productionlimiting materials are completely utilized as they are introduced." He further stated that "Since the nutritional physiology of plankton organisms is still understood only to a very small extent, we shall have to be content to perceive only the gross outlines of the results of these dynamics." Hutchinson (1944) stated that the physiological condition of a plankton population and its relation to populations of other species are likely to explain many inconsistencies between water chemistry and the occurrence of certain species. Thus, there is little basis for attempting any correlation between the physicochemical data and the occurrence of plankton in the survey ponds which were sampled only once.

The validity of these statements by Ruttner and Hutchinson becomes even more evident when an attempt is made to correlate the chemical data from Stations A and B with the occurrences of plankters there. Phosphate values remained essentially unchanged during a succession of various algal blooms in the spring and early summer. Hutchinson (1944) and Prowse and Talling (1958) pointed out that blue-green algae appear when inorganic nutrients (nitrates and phosphates) are low. Anabaena circinalis appeared at Station A in July (Fig. 9) after a period when nitrate concentrations were low (Fig. 7), but the September blooms of Microcystis aeruginosa and Oscillatoria limosa (Fig. 9) took place when nitrate concentrations were high. Conversely, concentrations of phosphates were high in July and low in September.

A pulse of diatoms occurred at Station A after heavy rains early in May, as would be expected according to Pearsall's (1923, *in* White, 1930) "theory of diatom periodicity," but there was no such pulse at Station B nor at either station after the March rains. Concentration of phosphates at Station B was never depleted during the spring and summer in spite of heavy production of vascular plants and an extensive bloom of *Peridinium cinctum*. Thus it is obvious that most fluctuations in abundance of plankton organisms in this study are unexplained at present, and that they can be solved only by an increased understanding of the complex ecological relationships between the organisms and their environment.

The seasonal distributions of the more frequently occurring organisms provides a general picture of the dynamics of the net plankton populations of the sinkhole ponds of Meade County, Kentucky. The more abundant plankters in the survey ponds as well as at Stations A and B indicate that there are marked seasonal differences (Table 2). The species referred to here can be arranged in four groups:

1) Species present throughout the year: Codonella cratera, Kellicottia bostoniensis, Keratella cochlearis, Polyarthra sp., Bosmina longirostris, Daphnia ambigua, and Diaptomus pallidus. Of particular interest here are Bosmina longirostris and Daphnia ambigua, which although present at all times, seldom occurred together. At Stations A and B, *D. ambigua* was a vernal species and *B. longirostris* was autumnal. *B. longirostris* was taken in 31 survey ponds and *D. ambigua* in 13; they occurred together in only three ponds.

2) Species absent during only one season: *Ceratium hirundinella*, *Phacus longicauda*, *Peridinium cinctum*, *Brachionus havanaensis*, and *Filinia longiseta* were most frequently absent during the winter.

3) Species present during only one or two seasons: Eudorina elegans, Pediastrum duplex, Staurastrum gracile, Botryococcus protuberans, B. sudeticus, Trachelomonas hispida, T. volvocina, Anabaena spiroides, Microcystis aeruginosa, Oscillatoria sp., Difflugia lobostoma, Asplanchna sp., Brachionus angularis, Ceriodaphnia pulchella, Diaphanosoma brachyurum, D. leuchtenbergianum, Moina affinis, Mesocyclops edax, and Tropocyclops prasinus were present most frequently in the summer and autumn.

4) Species scattered throughout the year: *Closterium turgidum*, *Cosmarium* spp., *Dinobryon cylindricum*, and *Cyclops vernalis*.

In nearly all instances the planktonic species were most abundant during the summer, but numbers remained high through late October. Also, more different species were present during the summer. It is noteworthy that the numbers of individuals and species were low during winter. Bunting (1959) found that the winter ratio of net zooplankton to net phytoplankton was two to one in Cherokee Lake, Louisville, Kentucky. There, the winter zooplankters, primarily rotifers, probably relied on nannoplankton for food.

The net plankton of the sinkhole ponds of Meade County can be characterized by the absence of such cold-water forms as *Synchaeta* and the rare occurence of diatoms. Similarly, the relative abundance of the above-mentioned species is characteristic.

# A Comparison of the Biota of Doe Run with that of the Sinkhole Ponds

Three typical pond plankters, *Difflugia lobostoma, Mesocyclops* edax, and *Keratella cochlearis*, were collected in very small numbers from Doe Run by Minckley (1962), who suggested that they had been washed into the stream from neighboring sinkhole ponds during periods of heavy rainfall. Eddy (1931) offered incomplete evidence of the connections between established sinkhole ponds and nearby springs. All planktonic species found by Scott (1909) in Shawnee Cave were epigean, and since they occurred only in small numbers, he concluded that they were transient. Scott pointed out that the epigean plankters probably were washed into the cave system during periods of high water when sinkhole ponds overflowed into open sinks.

On these bases it is assumed that there is no valid comparison between the lentic biota of the sinkhole ponds with the lotic biota of Doe Run, at least so far as the planktonic organisms are concerned.

# A Comparison of the Biota of the Natural Ponds with that of the Artificial Ponds

The species composition of the biota of the man-made ponds was very similar to that of the natural sinkhole ponds. Also, there were no appreciable differences in the physical and chemical characteristics of the waters of the two kinds of ponds. Although most of the artificial ponds contained no vascular flora, there were several natural ponds that also contained no such plants. *Nelumbo nucifera* Gaertn., the oriental water lily that is exotic to this region, had been introduced to Pond 39 and was well established. Many of the ponds, both artificial and natural, were heavily used for recreational purposes.

## **Summary and Conclusions**

The study area, bordered on the north by the Ohio River, is rectangular (approximately 8.4 by 5.5 miles in extent) and lies immediately southeast of Brandenburg, Meade County, Kentucky. Underlain by Mississippion limestone, the region is characterized by a rolling plain with mature karst topography, the only interruption being Doe Run, a spring stream that bisects the study area. The character of the bedrock and the gradual elevation of the area are optimal for the development of sinkholes. These sinks, upon becoming plugged, form small ponds, and are most often found in cleared farmland where erosion and run-off are maximal.

From November 1, 1960, through mid-December 1961, 95 ponds were sampled to obtain data on the physico-chemical and biological limnology which could be compared with similar data from Doe Run. Two of the ponds, Stations A and B, were each sampled 22 times from November 1960 through November 1, 1961, to detect seasonal fluctuations as they occurred.

The survey ponds ranged up to slightly less than three acres in extent and in depths up to about eight feet; most were less than two-and-a-half feet deep. Station A had a maximum depth of seven feet, 11 inches and was 2.0 acres in extent, whereas Station B was only three-and-one-half feet deep and was 2.3 acres in extent.

Water temperatures in the ponds usually were very near that of the air. Temporary thermal stratification occurred on hot sunny days, but the shallowness of the ponds precluded the development of thermoclines. There were complete ice covers at Stations A and B for two one-week periods during the winter of 1960-1961.

Water levels were dependent primarily on rainfall and evaporation. During the study, the levels fluctuated more than two feet from the spring maximum to the autumn minimum.

Turbidity was dependent on rainfall, the character of the pond bottom, and any disturbance by livestock.

Concentrations of oxygen and carbon dioxide were influenced primarily by their solubilities as determined by water temperature, being highest in the winter and lowest in the summer. Percentage saturation of dissolved oxygen could not always be correlated with phytoplankton abundance even though supersaturation occurred only during phytoplankton blooms. Oxygen concentrations never were observed to be low enough to limit chemical and biological oxidation processes.

There was little correlation between alkalinity, pH, and free carbon dioxide. Most ponds ranged in alkalinity from soft to medium hard and were slightly basic. Measurements of total iron, phosphates, nitrites and nitrates indicate that concentrations of iron were positively correlated with turbidity; phosphates exhibited large fluctuations and were present in amounts excessive of most major lake districts of the world (this was attributed to shallowness and agrarian environment); and nitrates and nitrites showed apparent depletion during periods of high plant production. Man's use of the ponds was reflected in the high concentrations of phosphate and nitrate as a result of the application of fertilizers to the drainage basins, or because of direct application of such fertilizers to increase fish production, or to excreta of livestock using the ponds. Total iron increased when livestock roiled the bottom muds and resuspended iron-rich sediments. The attendant increase in turbidity reduced the transparency, which in turn reduced photosynthesis, and affected the uptake of nutrients and the exchange of gases.

Plankton was collected with a 25-mesh silk bolting cloth net using a vertical tow in the deepest part of each pond. More than 140 kinds of plankton organisms were collected and the most abundant groups are discussed individually. Net phytoplankton was scarce during the winter, and in spring, summer, and autumn, the phytoplankton was dominated in most ponds by the pyrrophytes, *Peridinium cinctum* and *Ceratium hirundinella*. Desmids were rarely abundant but were present in many ponds throughout the year. Diatoms usually were rare or absent, whereas the cyanophytes, usually abundant in eutrophic waters, were second in abundance to the pyrrophytes, especially in the late summer and autumn.

Rotifers were represented by more species and were present in greater numbers than any other group of zooplankton. *Keratella cochlearis* was taken in all but six of the 125 plankton collections. *Bosmina longirostris* was the most common cladoceran, and the calanoid, *Diaptomus pallidus*, was the most abundant copepod. *Tropocyclops prasinus* was the most abundant cyclopoid copepod.

There was little correlation between the presence or abundance of plankters and the chemistry of the water. The best correlation was in the seasonal occurrence of organisms as indicated by four groups: 1) species present throughout the year, 2) species absent during only one season, 3) species present during only one or two seasons, and 4) species present occasionally throughout the year. The number and variety of species was highest in the summer and remained high until mid-October. Organisms absent during only one season usually were absent during the winter; those absent during two seasons usually were absent during the winter and spring.

There was little correlation between the chemistry and biology of the sinkhole ponds with similar data from Doe Run. Conversely, there was little difference between the limnological findings for the manmade ponds and those of the natural ponds of the study area.

The net plankton of the Meade County sinkhole ponds may be characterized by the presence of the following organisms: Cosmarium spp., Ceratium hirundinella, Peridinium cinctum, Codenella cratera, Brachionus angularis, B. havanaensis, Filinia longiseta, Kellicottia bostoniensis, Keratella cochlearis, Polyarthra sp., Bosmina longirostris, Daphnia ambigua, and Diaptomus pallidus.

The following species are listed as new records for the state of Kentucky: Daphnia ambigua, D. laevis, D. parvula, Alona karua, Diaphanosoma leuchtenbergianum, Diaptomus leptopus, D. birgei, and Mesocyclops tenuis.

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## Appendix

Plankton organisms collected from the 95 ponds sampled:

### Chlorophyta

Actinastrum hantzschii var.fluviatile Schroeder Closteriopsis longissima Lemmerman Closterium acerosum var. alongatum Brebisson Closterium acutum (Lyngbye) Brebisson Closterium braunii Reinsch Closterium dianae Ehrenberg Closterium didymotocum Ralfs Closterium gracile Brebisson Closterium intermedium Ralfs Closterium liebleinii Kuetzing Closterium lunula (Mueller) Nitzsch Closterium spp. Closterium turgidum Ehrenberg Coelastrum microporum Naegeli Coleochaete irregularis Pringsheim Cosmarium moniliforme (Turpin) Ralfs Cosmarium reniforme (Ralfs) Archer Cosmarium turpinii Brebisson Dictyosphaerium ehrenbergianum Naegeli Euastrum johnsonii West and West Euastrum oblongum (Greville) Ralfs Eudorina elegans Ehrenberg Unidentified green coccoid Micrasterias radiata Hass Micrasterias sp. Micrasterias truncata (Corta) Brebisson Mougeotia sp. Oedogonium sp. Pediastrum duplex Meyen Pediastrum simplex Meyen Pleuroteanium trabecula (Ehrenberg) Naegeli Scenedesmus arcuatus Lemmerman Scenedesmus dimorphus (Turpin) Scenedesmus opoliensis Richter Scenedesmus sp. Sphaerocystis schroeteri Chodat Spirogyra sp. Staurastrum gracile Ralfs

Staurastrum sp. Tetraedron gracile (Reinsch) Hansgirg Tetraedron limneticum Borge Tetraedron sp. Volvox globator Linnaeus Xanthidium antilopaeum Nordst

#### Chrysophyta

Botryoccocus protuberans G. M. Smith Botryococcus sudeticus Lemmerman Dinobryon cylindricum Imhof Dinobryon sertularia Ehrenberg Dinobryon sp. Fragilaria crotonensis Grunow Fragilaria spp. Gomphonema sp. Gyrosigma sp. Navicula sp. Pinnularia nobilis Ehrenberg Pinnularia sp. Stauroneis phonenicentron (Nitzsch) Ehrenberg Stauroneis sp. Tribonema affine G. S. West

### Pyrrophyta

Ceratium hirundinella (Mueller) Schrank Peridinium cinctum (Mueller) Ehrenberg

#### Euglenophyta

Euglena spp. Phacus longicauda (Ehrenberg) Dujardin Trachelomonas hispida (Perty) Stein Trachelomonas superba (Swir.) Deflandre Trachelomonas volvocina Ehrenberg Trachelomonas sp.

#### Cyanophyta

Anabaena circinalis Rabenhorst Anabaena felisii (Menegh) Bornet and Flahault Anabaena sp.

### William T. Geiling and Louis A. Krumholz

Anabaena spiroides Klebahn
Aphanizomenon flos-aquae (Linnaeus) Ralfs
Chroococcus limneticus Lemmerman
Coelosphaerium naegelianum Unger
Lyngbya latissima Prescott
Merismopedia glauca (Ehrenberg)
Kuetzing
Microcystis aeruginosa Kuetzing
Oscillatoria limnosa Agardh
Oscillatoria sp.
Spirulina sp.

#### Rhizopoda

Difflugia lobostoma Leidy Unidentified rhizopod

#### Ciliophora

Codonella cratera (Leidy)

### Nemata

Unidentified nematode

#### Rotifera

Asplanchna sp. Brachionus angularis Gosse Brachionus bidentata Anderson Brachionus caluciflorus Pallas Brachionus havanaensis Rousselet Filinia longiseta (Ehrenberg) Gastropis sp. Hexarthra sp. Kellicottia bostoniensis Rousselet *Keratella cochlearis* (Gosse) Lecans sp. Lepadella sp. Monostyla quadridentata Ehrenberg Monostyla sp. Platyias patulus Muller Poluarthra sp. Synchaeta sp. Trichocerca multicrinis (Kellicott) Trichocerca similis Wierzejski Trichocerca sp. Unidentified rotifer

#### Anostraca

Streptocephalus seali Ryder

#### Cladocera

Alona guttata Sars Alona karua King Alona rectangula Sars Bosmina coregoni Baird Bosmina longirostris (O. F. Muller) Ceriodaphnia pulchella Sars Ceriodaphnia quadrangula (O. F. Muller) Chydorus sphaericus (O. F. Muller) Daphnia ambigua Scourfield Daphnia laevis Birge Daphnia parvula Fordyce Daphnia sp. Diaphanosoma brachuurum (Lieven) Diaphanosoma leuchtenbergianum Fischer Leydigia acenthocercoides Fischer Moina affinis Birge Pleuroxis denticulatus Birge Scapholebris kingi Sars Simocephalus serrulatus (Koch)

### Ostracoda

Unidentified ostracod

#### Copepoda

Cyclops vernalis Fischer Diaptomus birgei Marsh Diaptomus leptopus Forbes Diaptomus pallidus Herrick Diaptomus sanguineus Forbes Diaptomus stagnalis Forbes Eucyclops agilis (Koch) Macrocyclops albidus (Jurine) Mesocyclops deax Forbes Mesocyclops tenuis (March) Tropocyclops prasinus (Fischer)

#### Diptera

Chaoborus sp.

# DISPERSION OF MACROFAUNA IN A SECTION OF MUDDY FORK, JEFFERSON COUNTY, KENTUCKY

ELIZABETH DIETRICH LOSE and W. L. MINCKLEY Medical School, University of Louisville, Kentucky, and Department of Zoology, Arizona State University, Tempe

The spatial distribution of the abundant benthic invertebrates was studied in a section of Muddy Fork, Jefferson County, Kentucky, to determine the relations of the animals to each other an dto certain characteristics of the stream. Muddy Fork flows through heavily populated, residential areas, and receives domestic sewage from septic tanks as well as various pollutants from automotive shops and other businesses. A riffle was selected as the study area and a section 16 feet long was mapped in detail (Fig. 1). The riffle had bedrock bottom, covered in places with algae, gravel, sand, rubble, and mud. Most rubble was 2 to 3 inches in diameter and was mixed with smaller materials. A large stone at the upper end of the section caused a quiet eddy, and other eddies were present along the banks.

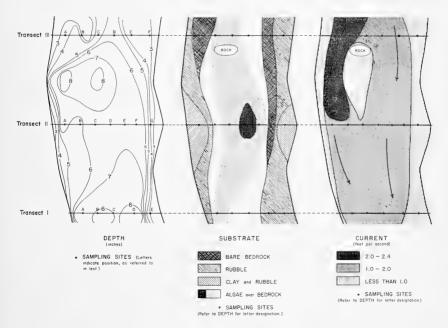


Figure 1.—Maps of a section of Muddy Fork, Jefferson County, Kentucky. Dots marking the sampling sites are placed in the center of the area covered by the square-foot quadrats. The distance from Transect I to Transect III is 16 feet; width is drawn to scale. See text for further explanation.

Currents generally were less than 1.5 feet per second in the main channel. Preliminary observations were begun in January, 1962, and quantitative sampling was done on March 3, 1962, between 1:00 and 3:00 P.M. Water temperature was  $48^{\circ}$  F. in the period of sampling, and air temperatures ranged from  $42^{\circ}$  to  $44^{\circ}$  F.

An unmodified Surber stream sampler (Surber, 1937), covering 1 square foot of bottom and with 40-mesh netting, was used for collections. Three transects were placed at intervals of 8 feet along the section (Fig. 1), and 18 quadrats were sampled: 5 from the downstream transect, 7 from the middle, and 6 from the upstream transect. The sampler was placed to obtain a visibly uniform substrate within each quadrat. Each sample was preserved *in toto* in 10 per cent formalin; the organisms were later sorted to taxonomic categories and preserved in ethanol.

# Results

The major benthic alga was *Cladophora glomerata* (L.) Kuetz.; *Batrachospermum* sp. was rare. Diatoms, all epiphytic on *C. glomerata*, were *Rhoicospheria curvata* (Kuetz.) Grun. and *Diatoma vulgare* Boyr. *Gomphonema* sp. and *Navicula* spp. were rare, but consistently present. *Cladophora glomerata* and associated diatoms occur as a part of a characteristic stream community that requires swift water, relatively high light intensities, and solid substrates (Blum, 1956).

Amphipoda, Isopoda, and Diptera were the most abundant groups of invertebrates, comprising collectively 86.5 per cent, 86.7 per cent, and 81.8 per cent of the total numbers of animals taken at each transect, respectively. Because of the relative scarcity of other animals, and because of taxonomic difficulties, only amphipods and isopods were determined to species. Three tendipedid dipterans, *Metriocnemius* sp. and 2 species of the subfamily Hydrobaeinae, were present, with 1 of the last 2 species making up an estimated 90 per cent of the total number of flies obtained.

Malacostracans were present in all but 4 samples (Table 1). Totals of 69 *Crangonyx gracilis* Smith, 78 *Synurella dentata* Hubricht, 75 *Asellus intermedius* Forbes, and 330 *Lirceus fontinalis* Rafinesque were obtained. Of the *C. gracilis*, 49 were females (none gravid), 29 were males, and the sex of 1, less than 4 mm long, could not be determined; there was no observed segregation on the basis of size or sex. The average length of females of *C. gracilis* was 7.3 mm; males averaged 7.5 mm long. Only 5 mature females and 1 mature male of *S. dentata* were obtained. Sex of 72 specimens, all less than 4 mm long, was not determined; their over-all average length was 3.0 mm. Of the specimens of *A. intermedius*, 7 were immature, 38 were

### Dispersion of Macrofauna

Organisms         A         B         C         D         E         F         G           Amphipoda:         S. dentata         10         15          11         C. gracilis         2         2          21         Isopoda:         2         2          21         Isopoda:         10         15          11         C. gracilis          21         Isopoda:         2         2          21         Isopoda:          21         Isopoda:         43         A. intermedius         6         9          14         Diptera         9         11         1          40         TRANSECT III         40         TRANSECT III         40         1         40         1         40         15          13         1         4         4         13         2          13         1         4         4         13         2          1         4         13         2          1         4         1         4         13         2          1         4         13         2          1         1							
Amphipoda:       10       10       15        11         C. gracilis        2       2        21         Isopoda:        2       2        21         Isopoda:        16       50        43         A. intermedius        6       9        14         Diptera       2       57       39       16       1       5         Other <sup>1</sup> 9       11       1        40         TRANSECT III         Amphipoda:        6       9       7        3         S. dentata         6       9       7        3         Isopoda:          6       9       7        3         L. fontinalis         1       4       4       13       2           Diptera        2       7       117       63       21        2         Other        2       7			Quadrats				
S. dentata       10       15       11         C. gracilis       2       2       21         Isopoda:       16       50       21         Isopoda:       16       50       43         A. intermedius       6       9       14         Diptera       2       57       39       16       1       5         Other <sup>1</sup> 9       11       1        40         TRANSECT III       7       3       6       9       7        3         Amphipoda:       5       12       29       47       22        7         A. intermedius       1       4       4       13       2           Diptera       2       47       117       63       21        2         Other       2       7       30       10       2        30         TRANSECT II       11       4       1       9       13       10       2        30         Other       2       7       30       10       2        30         Isopoda:       2	Organisms A	В	С	D	E	F	G
C. gracilis       2       2       2       21         Isopoda:       16       50       43         A. intermedius       6       9       14         Diptera       2       57       39       16       1       5         Other <sup>1</sup> 2       57       39       16       1       5         Other <sup>1</sup> 9       11       1       40       40         TRANSECT III       7       3       6       9       7       3         A. intermedius       4       5       4       16       4       3         Isopoda:       1       4       4       13       2           L. fontinalis       5       12       29       47       22           Diptera       2       47       117       63       21        2         Other       2       7       30       10       2        30         TRANSECT III       1       4       1       9         10       2        30         TRANSECT III       2       7       6	Amphipoda:						
Isopoda:       16       50        43         A. intermedius       6       9        14         Diptera       2       57       39       16       1       5         Other <sup>1</sup> 9       11       1        40         TRANSECT III         Amphipoda:       6       9       7        8         S. dentata       6       9       7        8         Isopoda:       4       5       4       16       4          L. fontinalis       5       12       29       47       22        7         A. intermedius       1       4       4       13       2            30         Diptera       2       7       63       21        2	S. dentata	10	15	••••		11	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C. gracilis	2	2			21	
A. intermedius       6       9       14         Diptera       2       57       39       16       1       5         Other <sup>1</sup> 9       11       1        40         TRANSECT III         Amphipoda:        6       9       7        3         S. dentata         6       9       7        3         Isopoda:        4       5       4       16       4        3         Isopoda:         6       9       7        3         L. fontinalis        5       12       29       47       22           Diptera        2       47       117       63       21        2         Other        2       7       30       10       2        30         TRANSECT III        4       1       9        30         S. dentata        2       7       6        2       C. gracilis        4	Isopoda:						
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Depend 1 $2$ $2$ $1$ $1$ $1$ $4$ Other 1 $3$ $1$ $1$ $1$ $4$ $4$ Amphipoda: $3$ $5$ $6$ $9$ $7$ $3$ S. dentata $4$ $5$ $4$ $16$ $4$ $3$ Isopoda: $4$ $5$ $16$ $4$ $3$ $L$ fontinalis $5$ $12$ $29$ $47$ $22$ $$ $A$ intermedius $1$ $4$ $4$ $13$ $2$ $$ $2$ $Other$ $2$ $7$ $30$ $10$ $2$ $$ $30$ TRANSECT II $A$ $11$ $4$ $4$ $13$ $2$ $$ $30$ TRANSECT II $A$ $A$ $10$ $2$ $$ $30$ Maphipoda: $2$ $7$ $6$ $$ $2$ $C$ $gracilis$ $a$ $a$ $L$ fontinalis $4$ $13$ $22$ $10$ $50$ <td>A. intermedius</td> <td>6</td> <td>9</td> <td>••••</td> <td>••••</td> <td>14</td> <td></td>	A. intermedius	6	9	••••	••••	14	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Diptera 2	57	39	16	1	~	
Amphipoda:       S. dentata       6       9       7       3         C. gracilis       4       5       4       16       4       3         Isopoda:       1       4       4       13       2       7         A. intermedius       1       4       4       13       2          Diptera       2       47       117       63       21        2         Other       2       7       30       10       2        30         TRANSECT II         Amphipoda:       2       7       6        2        30         L fontinalis       2       7       6        2        30         Isopoda:         L. fontinalis       4       13       22       10       50         A. intermedius       2       1       3       3       13         Diptera       23       21       11       2       3         Other        3       6       14		9	11	1	••••	40	
S. dentata	TRANSI	ECT II	I				
S. dentata	Amphipoda:						
Isopoda:       5       12       29       47       22        7         A. intermedius       1       4       4       13       2        2         Diptera       2       47       117       63       21        2         Other       2       7       30       10       2        30         TRANSECT II       Amphipoda:       2       7       6        2       7         S. dentata       2       7       6        2       7       6        30         Isopoda:       2       7       6        2       7       6        2         L. fontinalis       4       13       22       10       50       6        4       13       13       13       13       13       13       13       13       13       14       14       14       14       14       14       14       14       14       16       16       16       16       16       16       16       16       16       16       16       16       16       16       16			6	9	7		3
Isopoda:       5       12       29       47       22        7         A. intermedius       1       4       4       13       2        7         Diptera       2       47       117       63       21        2         Other       2       7       30       10       2        30         TRANSECT II           30         30         Amphipoda:        2       7       6        2        30         Isopoda:        2       7       6        2        3       13         L. fontinalis       4       13       22       10       50          A. intermedius       2       1       3       3       13 </td <td>C. gracilis</td> <td>5</td> <td>4</td> <td>16</td> <td>4</td> <td></td> <td>3</td>	C. gracilis	5	4	16	4		3
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$		12	29	47	22		7
Other       2       7       30       10       2        30         TRANSECT II       TRANSECT II       TRANSECT II       2       7       6        2       2       7       6        2       7       6        2       7       6        2       7       6        2       7       6        2       2       7       6        2       1       3       13       10       10       2        30       10       2        30       10       2        30       10       2	A. intermedius 1	4	4	13	2	••••	
Other       2       7       30       10       2       30         TRANSECT II         Amphipoda:       2       7       6       2       2       6       2       2       10       2       10       2       10       10       2       10       10       2       10       50       10       2       10       50       10       2       10       50       10       2       13       3       14       14       14       14       14       14       14       16       1	Diptera 2	47	117	63	21		2
Amphipoda:       2       7       6        2         S. dentata       2       7       6        2         C. gracilis        4       4       1       9         Isopoda:        4       13       22       10       50         A. intermedius        2       1       3       3       13         Diptera        23       21       11       2       3         Other        3       6       14	Other	7	30	10	2		30
Amphipoda:       2       7       6        2         S. dentata       2       7       6        2         C. gracilis        4       4       1       9         Isopoda:        4       13       22       10       50         A. intermedius        2       1       3       3       13         Diptera        23       21       11       2       3         Other        3       6       14	TRANS	ECT II	•				
S. dentata       2       7       6        2         C. gracilis        4       4       1       9         Isopoda:        4       13       22       10       50         A. intermedius        2       1       3       3       13         Diptera        23       21       11       2       3         Other        3       6       14							
C. gracilis		7	6		2		
Isopoda: $L$ . fontinalis $4$ $13$ $22$ $10$ $50$ $A$ . intermedius $2$ $1$ $3$ $3$ $13$ Diptera $23$ $21$ $11$ $2$ $3$ Other $3$ $6$ $14$		4	4	1			
A. intermedius       2       1       3       3       13         Diptera       23       21       11       2       3         Other		13	22	10	50		
Diptera         23         21         11         2         3           Other          3         6         14		1	3	3	13		
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### Table 1.—Numbers of benthic invertebrates in square-foot quadrats along 3 transects crossing Muddy Fork, Jefferson County, Kentucky. See Figure 1 and text for further explanation.

<sup>1</sup> Includes Oligochaeta, Tubellaria, Decapoda, Trichoptera, Coleopetera, Pelecypoda, and Gastropoda.

females, and 30 were males; adults ranged to 13 mm in length. The collection of L. fontinalis contained 120 immature individuals, 123 females, and 87 males. Eleven (8.9 per cent) of the female L. fontinalis, and 12 (31.6 per cent) of the female A. intermedius were carrying young. The average length of gravid L. fontinalis was 9.8 mm, whereas the over-all average length of females in the collection was 8.0 mm. Gravid A. intermedius were no larger than the over-all average length of the females (8.8 and 8.9 mm, respectively). As with C. gracilis, no size or sexual segregation was detected in either of the isopods.

# Discussion

The results were surprising in the apparent preference of all the abundant invertebrates for the same habitats (*Cladophora* beds in the channel and coarse rubble), and in the occurrence of 2 species each of Amphipoda and Isopoda in the same habitats. The greatest densities occurred in the thickest beds of vegetation (Table 1, Fig. 1). Synurella dentata tended to be slightly more common in the midchannel, and to be absent or rare in eddies; C. gracilis followed the same pattern, but occurred along the banks in larger numbers than did S. dentata. The 2 isopods were likewise found in the same areas, but L. fontinalis was more common in all samples than was A. intermedius. Tendipedid larvae were perhaps more closely associated with the beds of algae than were the malacostracans.

Little specific data are available on the habitats and behavior of the malacostracans found in Muddy Fork, especially in their interspecific relations. Synurella dentata differs greatly from C. gracilis in behavior, however, scuttling about on the bottom in an upright position, whereas C. gracilis swims freely or "scuds" on its side on the bottom. Thus, one may speculate that the 2 species occupy different niches, at least in their mode of locomotion and probably in feeding. Both L. fontinalis and A. intermedius are large, robust isopods that superficially resemble one another, and the greater abundance of the former in all samples (Table 1) may indicate some competition between them. Lirceus fontinalis is the most abundant species of aquatic isopod in the area, and is almost ubiquitous in habitat, whereas A. intermedius is more restricted in its occurrence (Cole, 1957).

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# OPTICAL ROTATIONS OF SOME SUGARS IN DIMETHYL SULFOXIDE SOLUTIONS

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Dimethyl sulfoxide (DMSO) exhibits greater dissolution power toward sucrose and other sugars than any other known non-protogenic solvent. Kononenko and Herstein (1956) reported this for sucrose; the observation for several other sugars have been made in this laboratory. Solutions containing as much as 38-40 weight per cent of sucrose, D-glucose, and D-fructose in DMSO are obtainable at 25° C. although complete dissolution of sucrose is especially slow for the more concentrated solutions.

Polarimetric methods for determinning the concentration of an optically active substance such as a sugar are convenient but it is necessary to obtain calibration data for each system under study and also to make sure that equilibrium values are being observed. The influence of the solvent on the optical rotation behavior of the solute is complex and presently not fully understood.

The objectives of this investigation were to obtain basic information concerning the optical rotation behavior of sucrose, D-glucose, and D-fructose in DMSO solutions and to make comparisons with known results for the same solutes in aqueous solutions.

## Experimental

Commercial grade DMSO was purified by the fractional freezing method. After several cycles, a final product having a freezing point of  $18.50^{\circ}$  C. was obtained. Sucrose and D-glucose (both Fisher Certified Reagent Grade) were pulverized and dried overnight at  $105^{\circ}$  C. prior to use. D-fructose (Fisher Reagent Grade) and sulfamic acid (99.99% assay) were used without additional treatment. Distilled water was further treated by passing it through an Illco-Way, Research Model, ion-exchange column.

Solutions were prepared on a weight basis using calibrated weights. Densities were determined using 25-ml. Reischauer pycnometers. Appropriate buoyancy corrections were applied prior to the calculation of concentrations and densities.

A Schmidt and Haensch double-field polarimeter was used to measure angles of rotation. Its scale was graduated directly into quarters of degrees and, with the aid of a vernier and magnifying lens, the angles could be read to  $0.01^{\circ}$ . A sodium-vapor lamp was used as a monochromatic light source. Water from a  $25.0 \pm 0.1^{\circ}$  C. thermostat was pumped rapidly through water-jackets of the 2-decimeter polarimeter tubes. Prior to a measurement, each tube was rinsed several times with distilled water and then several times with acetone before drying with filtered, dry air. The tube then was rinsed once or twice with the solution or solvent, filled, closed from the atmosphere with a polyethylene cap, and connected into the pumping circuit associated with the thermostat. A period of 10 minutes was allowed for the attainment of temperature equilibrium. The polarimeter was zeroed with a tube filled with the solvent. Measurements of the angle of rotation for each solution were made using two polarimeter tubes and the mean value of the rotation angle has been reported and used in calculations.

### **Results and Discussion**

The specific rotation,  $\left[ \propto \right]_{\lambda}^{^{O}C}$ , of an optically active substance in solution may be calculated by the following equation:

(1)

$$\left[ \, \swarrow \, \right]_{\lambda}^{^{\mathrm{O}}\mathrm{C}_{=}} \quad \frac{100 \, \, \swarrow}{1 \, \mathrm{p} \, \mathrm{d}}$$

in which	X	=	observed angle of rotation in degrees
	1.	=	length of light path in decimeters
	p	11	concentration of solute, g. solute/100 g
			solution
	đ	n	density of solution, g./ml.
	°C	= '	temperature in degrees Centigrade
	$\lambda$	<del>,</del>	wavelength of light

For measurements at  $25^{\circ}$  C. involving the D-line of sodium light and a 2-decimeter polarimeter tube, equation (1) becomes

(2) 
$$\left[ \propto \right]_{D}^{25} = \frac{50 \propto}{p d}$$

At the outset of this investigation, measurements of the specific rotation of sucrose in aqueous solutions were made to check on procedural aspects. The results, which are summarized in Table I, show good precision and agree within 0.3% with the calculated comparison data.

Weight Per Cent	Specific F Experimental	Rotation Calculated*	Per Cent Difference
	( Rur	1)	
4.97	66.66	66.42	0.36
9.72	66.67	66.45	0.33
14.25	66.63	66.47	0.24
18.84	66.76	66.47	0.44
	( Run	2)	
5.31	66.59	66.42	0.26
10.20	66.64	66.45	0.29
14.54	66.77	66.47	0.45
20.50	66.63	66.46	0.26

Table 1.-Specific Rotation of Sucrose in Water.

<sup>•</sup> "Handbook of Chemistry and Physics," 40th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1958-1959, p. 3017.

Data concerning the specific rotations of sucrose, D-glucose, and D-fructose in DMSO solutions are summarized in Table II. Specification of the time after mixing has been included inasmuch as the specific rotations of D-glucose and D-frutose change with time presumably owing to mutarotation. The specific rotation values for the sucrose solutions did not vary with time and consequently may be taken as equilibrium values which are about two units less than corresponding values for aqueous solutions.

р	d	α	$\begin{bmatrix} & \\ & \end{bmatrix}_{D}^{25}$
	Suc	rose	
4.58	1.1125	6.59	64.61
9.36	1.1305	13.63	64.44
13.68	1.1472	20.26	64.54
17.76	1.1633	26.64	64.48
	D-Gh	ucose	
4.85	1.1136	12.18	112.8
9.11	1.1298	23.12	112.4
13.53	1.1469	34,65	111.6
18.14	1.1650	46.99	111.2
	D-Fru	ictose	
5.20	1.1142	-16.30	-140.7
10.12	1.1322	-31.99	-139.6
14.66	1.1491	-46.82	-139.0
19.79	1.1685	01.00	-138.5

Table 2.--Specific Rotations of Some Sugars in DMSO About One Hour After Mixing.

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Table III contains a limited amount of data concerning the rates of catalyzed and uncatalyzed mutarotations of D-glucose in DMSO. An inspection of these results reveals that the uncatalyzed mutarotation proceeds extremely slowly. However, the rate of mutarotation is increased considerably by the presence of sulfamic acid even though sulfamic acid normally exists in dipolar ionic form in DMSO solutions as reported by Hovermale and Sears (1956). Assuming that the rate of catalyzed mutarotation follows first-order kinetics as supported by several studies such as those of de Grandcham-Chaudun (1950) and of Johnson and Moelwyn-Hughes (1941), it is possible by successive approximations to deduce the value of the equilibrium specific rotation,  $(S.R.)_{eq}$ , which gives a linear plot based on the following equation:

(3) 
$$\log \left[ (S.R.)_t - (S.R.)_{eq} \right] = \left( \frac{-k}{2.303} \right) t + \text{ constant}$$

Such calculations indicate that the equilibrium value for the specific rotation is 54.3 and that the specific rate constant for the catalyzed mutarotation is  $4.1 \times 10^{-2}$  hr.<sup>-1</sup>

Weight Per Cent D-Glucose	Weight Per Cent Sulfamic Acid	Time 2	After Mixing in 72	Hours 138
10.21	0.000	112.3	109.9	109.6
10.16	0.432	107.3	57.1	54.5

Table 3.-Specific Rotation of D-Glucose in DMSO as a Function of Time and Catalyst.

The values for the specific rotation of D-glucose listed in Table II probably are 0.1-0.2 unit lower than those characteristic of D-glucose immediately after dissolution in DMSO. The initial and equilibrium specific rotations of about 112.5 and 54.3, respectively, for solutions of D-glucose in DMSO are very similar to the corresponding values of 111.2 and 52.5 reported by Nelson and Beegle (1919) for aqueous solutions of D-glucose at  $25^{\circ}$  C.

The rate of mutarotation of D-fructose is much more rapid than that of D-glucose in DMSO solutions. Nevertheless, an equilibrium rotation for D-fructose is obtained within 30 minutes after dissolution in water at  $25^{\circ}$  C. whereas it is not obtained even after 8 days for uncatalyzed DMSO solutions. Experimental data for the uncatalyzed mutarotation of D-fructose in DMSO are given in Table IV. It may be observed that the four solutions behaved very similarly showing that the rate of change in optical rotation with time is essentially independent of concentration. Similar behavior has been reported by Browne (1912) for mutarotation rates in other systems. Again, assuming the applicability of first-order kinetics, the value of (S.R.)<sub>eq</sub> for each solution needed for a linear plot based on equation (3) can be calculated. For each solution the value of (S.R.)<sub>eq</sub> was found to be in the -10 to -9 range and the specific rate constant to be (1.2-1.3)  $\times 10^{-2}$  hr. <sup>-1</sup>

Weight Per Cent		Time After M	ixing in Hours	
D-Fructose	1	22	96	192
5.20	-140.7	-115.3	-65.0	-43.1
10.12	-139.6	-115.6	-66.6	-43.8
14.66	-139.0	-114.0	-65.9	-44.1
19.79	-138.5	-113.9	-65.5	-44.5

Table 4.—Specific Rotation of D-Fructose in DMSO as a Function of Time and Concentration.

A DMSO solution of D-frutose containing some sulfamic acid also was prepared but interaction of the components yielded a yellowish-brown solution after a few hours and before an equilibrium value for the specific rotation could be ascertained. Although this was not observed for solutions of D-glucose in DMSO containing sulfamic acid, it has been observed in other experiments in this laboratory that aqueous sucrose solutions containing sulfamic acid also tend to become similarly colored after a few days.

The specific rotation values for D-fructose in Table II probably are about 1.2 units lower than those for a solution of D-fructose in DMSO immediately after dissolution. For comparison purposes, the initial and equilibrium specific rotation values for aqueous D-fructose solutions at 25° C. according to Nelson and Beegle (1919) are -131and-88, respectively, and differ appreciably from the corresponding values of -141 and -10 for DMSO solutions in this study. These data in conjunction with those for several other media summarized in the International Critical Tables (1928) and by Browne (1912) clearly illustrate the pronounced solvent effect on the optical rotation behavior of D-fructose.

For concentrations up to 20 weight per cent, no significant change in specific rotation with sucrose concentration occurs in either aqueous or DMSO solutions. The small variations in specific rotations with concentration for D-glucose and D-fructose are reversed for aqueous and DMSO solutions.

### Summary

The optical rotation behavior of sucrose, D-glucose, and D-fructose in DMSO solutions has been investigated at  $25^{\circ}$  C. The behavior of sucrose and D-glucose has been found to be similar in aqueous and DMSO solutions whereas that of D-fructose was found to be quite different. Specific rotations have been determined as a function of both concentration and time. D-glucose and D-fructose were observed to undergo slow mutarotation in DMSO. Assuming the application of first-order kinetics to these two systems, equilibrium specific rotations and specific rate constants have been calculated.

## Acknowledgment

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# ECONOMIC SIGNIFICANCE OF KENTUCKY'S AREAL GEOLOGIC MAPPING PROGRAM—A PROGRESS REPORT

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Kentucky's statewide areal geologic mapping program, conducted cooperatively by the State and Federal governments through the Kentucky Geological Survey and the United States Geological Survey, is aiding soil scientits and agriculturists in soils mapping and planning land utilization and soil conservation programs as well as providing essential knowledge for locating and developing our mineral wealth.

Since soil classifications are in large part based on the character of rock formations at or near the surface of the ground, geologic maps are used to explain soil formations, thus effecting a substantial time savings in field mapping of soils and in report writing. The new maps are of greatest benefit in areas where the geologic picture is complicated. Some of the soil-mapping teams in Kentucky have consulted freely with the field geologists on special problems. An explanation of the nature and occurrence of unusual limestone bodies in rock usually devoid of such formations aided soil scientists in unravelling a particularly knotty problem in the Columbia area.

As a result of conferences between representatives of the U.S. Department of Agriculture and the Kentucky areal geologic mapping team, a Kentucky farmer was advised to change his allotted tobacco acreage from an area on his farm of silty and clayey soil to a place where the parent material of the soil was limestone. The result was an increase of 500 pounds of tobacco without additional fertilizer or extra treatment. In these instances the farmers were the first to feel the economic benefit of the mapping program.

Numrous companies and individuals have standing orders for new maps as they are issued because they are convinced that the structtural and allied geologic data shown thereon will materially assist them in their exploration and development programs.

Published maps have pointed out such mineral commodities as clay, coal, limestone, and shale. The fault pattern on the Salem quadrangle in Crittenden and Livingston Counties will guide prospecting for fluorspar and other vein minerals such as barite, galena and sphalerite. Structure contours on the published maps will encourage exploration for petroleum and natural gas resources. At least one large company has used the Big Clifty quadrangle as a guide in their attempt to evaluate natural rock asphalt deposits in Grayson County.

### Preston McGrain

Several companies and individuals have studied the published maps in south-central Kentucky with great interest, attempting to relate the nature and trends of limestone reefs to occurrences of petroleum in the areas to the west and northwest. The term "reef" is a magic word in many oil circles. The discovery of reef-type limestone bodies in the Lake Cumberland and nearby areas by field geologists of the Columbia. Kentucky office has attracted numerous petroleum geolists to the region. It is most likely that additional knowledge of the size, trend, and physical properties of the reefs will assist and encourage future oil and gas exploration in southern Kentucky (Thaden and others, 1961). The development of Kentucky's coal resources will be similarly benefitted-for example, the Varney quadrangle in parts of Martin and Pike Counties records the presence of 13 coal beds ranging in thickness from 14 to 120 inches. The Haldeman and Wrigley quadrangles in Carter, Rowan, and Morgan Counties show the presence of flint clay and high-calcium limestone. Maps in Adair and Russell Counties have disclosed limestone deposits which were heretofore unknown. Prospecting for a new quarry site is currently under way in that area.

The Kentucky Department of Commerce used published maps to answer a specific inquiry about coal resources in an area near Corbin. Also, Department of Commerce personnel with assistance from the members of the mapping team from the Princeton office were instrumental in locating a plant site in Caldwell County which assured the construction of a new factory.

The Materials Division of the Kentucky Department of Highways is currently using the new maps for soil profile studies along rights-ofway of new and proposed roads and in their investigations of aggregate materials.

As a by-product of the mapping program the Kentucky Geological Survey has published oil and gas maps of Adair County (Helton, 1963) and the Hanson area (Rose and Smith, 1963) in Hopkins County. Data gathered by personnel of both the Kentucky Geoligical Survey and the United States Geological Survey were used in the preparation of these maps.

Each field geologist is on the alert for geologic occurrences of economic significance; pertinent data are passed on to the Kentucky Geological Survey for further investigation (Hagan, 1961). Probably the most important discoveries to date are the gypsum deposits in Meade and Hardin Counties.

Stratigraphic tests drilled near Guston in Meade County and near Summit in Hardin County for the purpose of learning more about the rock sequence occurring at or near the surface of the ground in that area encountered gypsum-bearing rock in the lower St. Louis Limestone at 336 feet and 460 feet respectively. The deposit near Guston consisted of 54 feet of interbedded limestone and evaporities, with gypsum and anhydrite constituting an estimated 35 percent of the interval. The deposit near Summit was 49 feet of interbedded carbonates and evaporites, with 28 percent being gypsum and anhydrite. These cores are on file at the Kentucky Geological Survey Well Sample Library at the University of Kentucky in Lexington; they have been inspected by numerous company and private geologists.

Gypsum is an important nonmetallic mineral which is used widely in the building industry in wallboard, building plasters, cements, and the like. While the deposits encountered in the Kentucky test holes just described are not large enough for economical recovery they do represent a significant clue which warrants further exploration by private interests. The fact that two major gypsum-producing companies are presently mining gypsum and manufacturing wall board at sites in Indiana less than 75 miles northwest of the Kentucky area from the same sequence of rocks gives the discovery added significance. The Indiana mines are 450 to 515 feet deep and are recovering a bed of gypsum 13 to 14 feet thick. The presence of gypsum in commercial quantities could give Kentucky an important new multimillion-dollar industry and produce a significant impact on the economy of the region.

The geologic mapping program is providing an important tool for systematic exploration and progressive development of Kentucky's natural resources. Although the industries related to agriculture, forestry, and minerals will appear to be the first affected, every segment of our economy will be benefited.

Observations by staff members of the Kentucky Geological Survey for more than a century have pointed out many potential mineral resources. The new geologic maps are giving the earlier work a new dimension in that it provides a pattern of occurrence and a more accurate and precise framework for natural resource evaluation. This new dimension in Kentucky geology is causing geologists, engineers, and others to take a closer look at our State. The economic impact of the geologic mapping program is already significant; it will increase as the project progresses.

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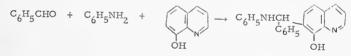
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### THE BETTI REACTION

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The reaction of a primary aromatic amine, an aromatic aldehyde and a phenol (naphthols (1-3), kojic acid (4), 8-quinolinol (5-9) and 8-hydroxy-quinaldine (10) are the only known examples of phenols) is sometimes called the Betti reaction (11), although it is undoubtedly a special case of the Mannich reaction. Indeed, an occasional successful use of a secondary amine or of an aliphatic aldehyde or amine in the reaction demonstrates the relationship clearly. The Betti reaction should be an especially valuable form of the Mannich for studies of theoretical and mechanistic factors in that a variety of substituents in the aromatic aldehydes and amines may be used and their effects interpreted in terms of Hammett constants, ionization constants, electronegativities and the whole apparatus that has been developed to explain the reactions of aromatic compounds.

An example of the Betti reaction is the production of 7-(*a*-anilinobenzyl)-8-quinolinol from either benzylideneaniline or, as shown in the equation below, a mixture of benzaldehyde and aniline. (The reaction with benzylideneaniline is much slower than with the mixture of aldehyde and amine.)



More than fifty Betti reactions of 8-quinolinol or 8-hydroxyquinaldine have been reported in the last ten years, and it is now possible for the first time to see some regularities in the reactions that have succeeded and some reason for those that failed. The experimental procedures have not been altogether uniform but the following conclusions appear fairly definite:

1. There is a correlation of yields with Hammett sigma constants for substituents in either the aromatic amine or aldehyde (Table I), positive values being associated with high yields, negative with low or no yields.

2. The time required to yield a precipitate of product at room temperature has been generally recorded in these reactions and is roughly proportional to the yield. Obviously the existence of supersaturation and other factors make the time value a defective measure of reactivity and certainly an inadequate indicator of kinetic data, but

А.	Substituted a	nilines, benza	ldehyde and 8-	quinolinol	
	Substitute	0	Yield %	Reference	
	p-NO2	0.778	96	5	
	m-Cl	0.373	74	5	
	p-COOH	0.265	43	6	
	p-Me	-0.170	25	5	
	p-OEt	-0,250	0	8	
B.	Substituted b	enzaldehydes,	aniline and 8-	quinolinol	
	p-NO <sub>2</sub>	0,778	70	4	
	m-NO <sub>2</sub>	0,710	85	8	
	p-C1	0.227	30	8	
	m-Me	-0.069	15	8	
	p-iso-Pr	-0.151	22	4	
	p-Ome	-0.268	12	5	
	p-OH	-0.357	0	8	

Table 1.-Correlation of Betti Reaction Yields with Hammett Sigma Constants.

the fastest reactions generally furnish the highest yields. Reaction times range from two hours to over a hundred days.

3. Heterocyclic amines or aldehydes with the functional group adjacent to the ring hetero atom appear to react with unusual speed.

4. 8-Hydroxyquinaldine reacts more slowly and with generally poorer yields than 8-quinolinol, perhaps because of its slightly greater basicity. It is curious that the 2-methyl group does not enter into the reaction with the one exception of anthranilic acid as the amine and benzaldehyde; in this case benzaldehyde attacks the active methyl at the same time that the Betti reaction occurs at the 7-position to yield 2-styryl-7-[a-(o-carboxyanilino)benzyl]-8-quinolinol. Since a similar involvement of the 2-methyl is not observed when anthranilic acid is replaced by p-aminobenzoic acid, this is a rather specific anomaly.

5. It is possible that a steric hindrance factor operates to diminish yields and slow the reactions of amines or aldehydes with o-substituents. For example, p-nitroaniline gives the fastest reactions and highest yields of any amine (5), but o-nitroaniline requires more than a month to precipitate a negligible yield in a parallel reaction. Other available

examples of a possible ortho effect are not so striking, and in some instances no such effect is detected at all.

The Mannich reaction mechanism in basic solutions is considered to involve the reaction of the carbanion of the active methyl compound with HOCH<sub>2</sub>NR<sub>2</sub>, and in acid solution to be a slower attack of the carbonium ion +CH<sub>2</sub>NR<sub>2</sub> on the active hydrogen (12). Since the only catalysts in Betti reactions have been the acid or base nature of the reactants, it is difficult to say which class of reaction is involved. The extraordinarily high reactivity of p-nitroaniline suggests acid catalysis.

It seems safe to assume that the Schiff's base formed between the amine and aldehyde components of the reaction is not an active intermediate, because benzylideneaniline reacts more slowly than the aldehyde-amine pair. Duplicate runs with 8-hydroxyquinaldine were not very reproducible (see figure), but do show a great difference from corresponding reactions of ethyl or methyl anthranilate and benzaldehyde in place of the Schiff's base. A comparison with aniline and benzaldehyde would carry more weight, but this mixture gave crystalline products with 8-hydroxyquinaldine at first but oils later, and it was thus impossible to get useful data.

The only reactions of Betti products explored in any detail are chelation with metals (6,7,13) and hydrolysis (14). Some use as anti-oxidants in internal combustion engines has been claimed (15).

In Table II a few new examples of the Betti reaction with 8hydroxyquinaldine are summarized.

## Experimental

All reactions were run by dissolving the appropriate aldehyde and amine in ethanol and then adding an equimolar amount of 8hydroxyquinaldine. Time of initial precipitation was noted but the products were not filtered and recrystallized until 21 days had passed.

In the investigations of the rate of formation of product the precipitate was removed at intervals, and the solution (with a few seed crystals present) allowed to stand again until enough to weigh had precipitated.

The reaction of equimolar amounts of benzaldehyde, anthranilic acid and 8-hydroxyquinaldine precipitated a red product, m.  $60-2^{\circ}$ , that analyzed for the structure 2-styryl-7-[*a*-(o-carboxyanilino)benzyl]-8-quinolinol. Calcd. for  $C_{31}H_{24}N_2O_3 \cdot H_2O$ : C, 75.90; H, 5.34; N, 5.71. Found: C, 75.28; H, 5.16; N, 6.07.

A comparison of the infrared spectrum of this compound with a mixture of 7-[a-(o-carboxyanilino)benzyl]-8-quinolinol and 2-styryl-8-quinolinol showed similar bands below 11  $\mu$ .

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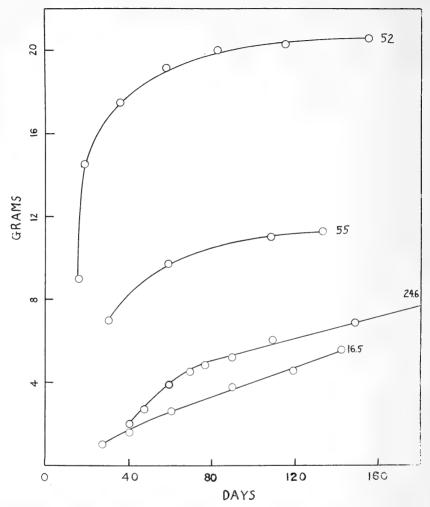


Figure 1.—Weight of product as a function of time for Betti reactions of 8-hydroxyquinaldine, benzaldehyde and methyl anthranilate (top curve) or ethyl anthranilate (next to top); graphs for benzylideneaniline and 8-hydroxyquinaldine duplicate runs are at bottom. Nos. at right are percent yields.

### Summary

A review of more than fifty published examples of the reaction of 8-quinolinol or 8-hydroxyquinaldine with aromatic amines and aldehydes has shown that the reaction is favored by positive sigma constants of substituents in either amine or aldehyde component, that heterocyclic aldehydes or amines are very active in the reaction, and that a Schiff's base or anil is probably not an intermediate. In

### The Betti Reaction

some instances a steric hindrance by ortho substituents in the aldehyde or amine may exist. A few new examples of the reaction are also given.

# Acknowledgment

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Betti Reaction Products from 8-Hydroxyquinaldine <sup>*</sup> : ArNHCH							
Ar	Ar	Formula	N % Calcd. F	ound.	<u>М.р., °С.</u>	Yield, %	
Ph	2-Pyridyl	$C_{22}H_{19}N_{3}O$	12.33	12,44	129-30	60	
Ph	4-Pyridyl	$C_{22}H_{19}N_{3}O_{1}$	12.33	12.40	154-5	55	
4-C1-2-Me- C <sub>6</sub> H <sub>3</sub>	Ph	$C_{24}H_{21}C1N_{2}O$	7,21	7.30	142-4	23	
4-C1-2-Me- C6H3 #	Ph	C <sub>23</sub> H <sub>19</sub> C1N <sub>2</sub> O	7.47	7.41	136-8	35	
5-Me-2-pyridyl	Ph	$C_{23}H_{21}N_{3}O$	11.82	11.78	167-8	28	
11 11	2-Pyridyl	$\mathrm{C}_{22}\mathrm{H}_{20}\mathrm{N}_{4}\mathrm{O}$	15.72	15.69	185-6	29	
11 11	2-Thienyl	$\mathrm{C_{21}H_{19}N_3OS}$	11.63	11.52	183-5	.40	
$p-HOOCC_6H_4$	Ph	$C_{24}H_{20}N_2O_3$	7.29	7.58	228-30	31	

1	a	bl	е	2.

\*Times required for initial precipitation were 3, 1, 102, 30, 7, 1, 1 and 21 days respectively from top to bottom of table. Except for the third, fourth and eighth compounds (which were not determined) ultraviolet spectra were similar in  $\lambda_{max}$  at 250 ± 1 mu (log  $\epsilon$  4.7) and at 290-312 mu (log  $\epsilon$  3.8).

#8-Quinolinol used in place of 8-hydroxyquinaldine.

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# A STUDY OF HYDROGEN BONDING IN ALCOHOL-DIOXANE SYSTEMS BY INFRARED SPECTROSCOPY

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In recent years, there has been much infrared spectroscopic work on hydrogen-bonding in alcohol solutions (Pimentel and McClellan, 1960). Alcohols present an interesting study for hydrogen bonding not only because of the possibility of bonding between the alcohol molecules to form dimers and polymers but also because of the ability of alcohols to hydrogen bond with Lewis bases.

One absorption frequency of the alcohol which shows a marked effect when hydrogen bonding occurs is the fundamental O-H stretching frequency around 3610 to 3640 cm.<sup>-1</sup> Upon formation of a hydrogen bond, the frequency\_for the bond O...H...:X, where: X represents an electron-donor, is shifted to the 3500 cm<sup>-1</sup> region. The extent of the shift ( $\Delta \gamma_1$ ) is dependent upon several factors; and there have been several attempts to correlate the frequency shift, the strength of the hydrogen bond and the thermodynamic properties of its formation. These attempts have met with only moderate success due to the many factors which can influence the hydrogen-bond formation (Pimentel and McClellan, 1960).

In a recent study, Becker (1961) investigated the hydrogen-bond eneagies of methanol, ethanol and t-butyl alcohol in six Lewis bases. Becker determined the frequency shift  $(\Delta \gamma_1)$  for each system and showed relationships between the strengths of the bases and the effects of temperature as well as thermodynamic properties and equilibrium constants for the bond formation.

In this paper, the results of a study of the hydrogen bonding between several aliphatic alcohols and only one Lewis base—p-dioxane are reported. It is hoped that this work can show a rough relationship between the structure of the alcohols and the frequency shift due to hydrogen bonding of these alcohols to p-dioxane.

The structure of p-dioxane reveals that it is a suitable Lewis base, having two oxygen atoms with unshared electron pairs to which bonding can occur. Since Becker (1961) and others have found that the equilibrium relation between the existence of the alcohol in its monomer form and the hydrogen-bonded complex form is shifted

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toward the existence of the monomer on dilution, the concentration of the alcohol is kept much smaller than that of the dioxane. In all probability under these circumstances, only one of the available sites on p-dioxane for hydrogen bonding is used. The small concentration of the alcohol also insures that the alcohol which is not bonded to the p-dioxane exists in these soultions as a monomer rather than as dimers and polymers.

The alcohols which were investigated in dilute solutions of pdioxane were: methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, t-butyl, n-amyl, and 3-pentyl. These alcohols were grouped into four classes in order to determine the effect of structure on the frequency shift of the spectra. The five normal alcohols were arranged together to observe the effect of lengthening the carbon chain. Secondly, the effect of methyl groups substituted for hydrogen atoms on the carbinol carbon was studied with methyl, ethyl, iso-propyl, and t-butyl alcohol. The two "iso" alcohols were also compared to observe the spectral shift when the branched methyl groups are on the carbinol carbon and when they are on the alpha carbon. A final comparison was made between iso-propyl, sec-butyl, and 3-pentyl alcohol to show the spectral relationships when the OH group is moved toward the center of a carbon chain.

## Experimental

Spectra were obtained on a Perkin-Elmer model 12C infrared spectrometer which was converted from single beam single pass to single beam double pass with a kit available from the Perkin-Elmer Corporation. A NaC1 prism was used, and the frequency accuracy was estimated to  $\pm 10 \text{ cm}^{-1}$  in the three-micron region over which the data was taken.

The room was air-conditioned, and the temperature held fairly constant at  $25^{\circ} \pm 2^{\circ}$ C. The slit width was kept constant while all the spectra were taken at 0.035-0.040 mm depending upon the alcohol being used. The cell width was 1.0 mm in all cases.

The alcohols and p-dioxane were of reagent grade, meeting A.C.S. purity specifications. The reagents were also checked for purity on an Abbe refractometer. All the solutions of alcohol and p-dioxane were run in reagent-grade carbon tetrachloride which also met A.C.S. purity specifications. The concentration of the alcohol in each case was approximately 0.025 to 0.045 M, and the concentration of the p-dioxane ranged from 0.45 to 0.55 M. Several spectra for each alcohol-dioxane system were taken, and an average of the absorption frequencies was obtained.

The obsorption peaks in the 3880 to 3430 cm<sup>-1</sup> range were read from a calibration curve relating wavenumber to arbitrary drum-turn units on the spectrometer. The peaks used for calibration were the atmospheric water peaks at 3881 cm<sup>-1</sup>, 3740 cm<sup>-1</sup> and 3619 cm<sup>-1</sup> (Downie, 1953) and the N-H stretching frequency of diphenylamine at 3433 cm<sup>-1</sup> (Richards, 1949). A method outlined by Davis (1943) gave a linear relationship between wavenumber and drum-turn units and was used in determining the absorption frequencies.

# **Discussion of Results**

The absorption frequencies for the OH stretch of the monomer and the hydrogen-bonded complex and the shift  $\Delta_{\gamma_1}$  between the two are listed in Table 1. The values found in the literature (Barrow, 1955; Kuhn, 1952) for the free OH of several alcohols are also given.

		Tuble IV	10. 1		
	EXPERIMEN	LITERATURE VALU			
Alcohol with p-dioxane	Free OH in cm <sup>-1</sup>	H-bond Complex in cm <sup>-1</sup>	$\begin{array}{c} {\rm Shift} \\ \Delta \gamma_1 \end{array}$	Free OH in cm <sup>1</sup>	References
Methyl	3640	3517	123	3642	Kuhn
Ethyl	3632	3511	121	3634	Barrow
n-Propyl	3626	3511	115		
iso-Propyl	3617	3507	110	3620	Barrow
n-Butyl	3627	3514	113	3632	Kuhn
iso-Butyl	3626	3513	113		
sec-Butyl	3620	3509	111	3623	Barrow
t-Butyl	3614	3507	107	3614	Barrow
n-Amyl	3623	3506	117		
3-Pentyl	3622	3512	110	•••••	

Table No. 1

As shown by Table 2, the shift in frequency due to hydrogen bonding of the alcohol to p-dioxane is seen to decrease generally as the length of the carbon chain in the aliphatic alcohol is increased. This decrease for the low-numbered alcohols is substantiated by Becker (1961) for methanol and ethanol. The deviation of n-amyl

n-Alhocol with	Shift $\Delta \gamma_1$ in cm <sup>-1</sup>
p-dioxane	in cm <sup>-1</sup>
Methyl	
Ethyl	
n-Propyl	115
n-Butyl	
n-Amyl	117

Table No. 2

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alcohol from this pattern cannot be fully explained from the limited data available, and a further examination of the OH stretching frequencies and of hydrogen bonding for alcohols larger than the amyl alcohols is needed.

The effect of substitution of methyl groups in place of hydrogen atoms on the carbinol carbon is determined by a comparison of frequency shifts for methyl, ethyl, iso-propyl, and t-butyl alcohol; and Table 3 is given showing these shifts. The reduction in shift which accompanied an increase in substitution may be explained by the

Alcohol with p-dioxane	Shift $\Delta \gamma_1$ in cm <sup>-1</sup>
Methyl	123
Ethyl	
iso-Propyl	
t-Butyl	

10010 110. 3	Tab	le	No.	3
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electron-pushing effect of the additional methyl groups which makes the hydroxyl hydrogen less available for hydrogen bonding than the hydroxyl hydrogen of alcohols with no substitution. Steric hindrance of iso-propyl and t-butyl alcohol may also cause this decrease in shift.

A comparison of the shifts caused by iso-propyl and iso-butyl alcohol reveals that the frequency shift is increased when the branched methyl groups are on the alpha carbon instead of on the carbinol carbon. The electron-donating effect of the methyl groups is more reduced in iso-butyl alcohol than in iso-propyl alcohol.

No general relationship can be ascertained from this data concerning the shift in solutions of iso-propyl, sec-butyl and 3-pentyl alcohol. The OH group in the middle of a carbon chain seems to affect the shift to approximately the same degree without regard to the carbon chain's length.

The absorption frequency of the hydrogen-bonded complex is affected greatly by temperature changes (Bellamy, 1960), and a temperature-controlled cell is needed to obtain extremely accurate results. The low spectral dispersion of the NaCl prism also hinders the accurate measurement of absorption frequencies in the threemicron range, and a LiF prism should be used for exact measurements. In most works concerning hydrogen boning in alcohol-base systems, a double-beam spectrometer is used instead of a single-beam.

In general, substitution of alkyl groups on the carbinol carbon shifts the electron density of the alcohol molecule toward the oxygen atom, making it more negative and attracting the proton to a greater extent than teh oxygen of alcohols with little substitution. The protonattractive power of a base such as p-dioxane is thus lessened with substituted alcohols, and the hydrogen bonds formed between the base and such alcohols will cause a smaller shift in frequency than with alcohols having little or no substitution on the carbinol carbon.

## Summary

The absorption frequencies for the free or monomer OH stretch and the hydrogen-bonded complex along with the shift  $\Delta_{\gamma_1}$  are given for ten alcohols in p-dioxane, which acts as a Lewis base. An attempt is made to relate the amount of these shifts to the structure of the alcohols.

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# THE PREPARATION OF PYRIDINE ANALOGS OF p-DIMETHYLAMINOAZOBENZENE

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In the course of our studies (Brown 1954a, 1954b) on the carcinogenic activity of pyridine analogs of p-dimethylaminoazobenzene (Butter yellow), all the p-dimethylaminophenylazopyridines, their 1-oxides and a number of methyl homologs of these were prepared. These compounds were fed to male Sprague-Dawley rats at the level of .06% in "semi-synthetic" diet (Brown 1954a). The following did not produce liver tumors in any of the animals after periods of 10-12 months: 2'-(4-dimethylamino)azopyridine, its 4'-methyl homolog, its 6'-methyl homolog and the 1'-oxides of these three compounds. 3'-(4-Dimethylamino)azopyridine was somewhat less active than Butter yellow while 4'-(4-dimethylamino)azopyridine, its 1'-oxide and the 2',6'-dimethyl homolog of the 1'-oxide were all more active. The details of the testing for carcinogenic activity have been reported (Brown, 1954a, 1954b).

We have observed that 2-, 3-, and 4-aminopyridine-1-oxides, under the usual conditions of diazotization and coupling, behave like aniline in forming azo compounds. This is true of 3-aminopyridine (Binz, 1931) but 2- and 4-aminopyridine behave somewhat differently (Faessinger, 1951).

Although it has not been possible to successfully diazotize and couple 2-aminopyridine and its homologs, we have, by a modification of the diazotization procedure, been able to obtain good yields of the 4-(p-dimethylaminophenylazo) pyridines. Data on these compounds are found in Table 1.

2-Aminopyridine-1-oxide was originally prepared by (Newbold,

Substituent	Formula	MP		Analyses, %						
		°C	Calcd.				Found			
			С	H	<u>N</u>	C	H	N		
	с <sub>13</sub> н <sub>14</sub> N4	207-9	69.02	6.20	24.77	69.20	6.25	24.64		
2-Methyl	$C_{14}H_{16}N_4$	142-3	70.00	6.67	23.33	69.92	6.70	23.36		
3-Methyl	$C_{14}H_{16}N_{4}$	186-8	70,00	6.67	23.33	70.15	6.65	23.12		

Table 1

4-(p-Dimethylaminophenylazo)pyridines

1949) a series of reactions starting with the 1-oxide of picolinic acid. Preparation by this method is involved and gives a low overall yield.

Pyridine reacts with 40% commercial peracetic acid to yield pyridine 1-oxide almost quantitatively, but 2-aminopyridine, so treated, yielded principally an oil of unknown composition and a small amount of 2-aminopyridine 1-oxide (10-15%). When 2-aminopyridine was treated with a 10-15% solution of commercial hydrogen peroxide in acetic acid, the results were essentially the same, an only slightly higher yield of 2-aminopyridien 1-oxide being obtained. However, when 2-(N,N-dibenzoyl)aminopyridie or 2-acetylaminopyridine (Brown, 1957) was treated with hydrogen peroxide in acetic acid or commercial peracetic acid good yields of the corresponding acylaminopyridine 1-oxides were obtained.

Alkaline hydrolysis of these intermediates yielded 2-aminopyridine-1-oxide. The dibenzoyl and acetyl derivatives of the various 2-aminopicolines treated in the same manner yielded the corresponding 1-oxides. Similarly, the monobenzoyl and acetyl derivative of 3-aminopyridine yielded 3-(acylamino)-pyridine-1-oxide which, on alkaline hydrolysis, was converted to 3-aminopyridine-1-oxide. The various acylaminopyridines are listed in Table II.

The aminopyridine-1-oxides diazotize in the presence of nitrous acid and couple readily with dimethylaniline to provide the expected pyridine-1-oxide azo compounds. The data for these azo compounds are given in Table III.

Pyridine 1-oxides							
Acylamino derivative	Formula	MP °C	Ni Calcd.	trogen Found			
2-(N,N-dibenzoyl)amino-	C <sub>19</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	98-9°	8,80	8.77			
2-Acetylamino-	C7H8N202	(Huntress, 1948)					
2-(N,N-dibenzoyl)amino-3-methyl	C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	124-5°	8.43	8.38			
2-Acetylamino-3-methyl-	C8H10N202	20 <u>4</u> ~5°	16.87	17.00			
2-(N,N-dibenzoyl)amino-4-methyl-	C20H16N203	114-5°	8.43	8.35			
2-Acetylamino-4-methyl-	C8H10N202	144-5°	16.87	16.91			
2-(N,N-dibenzoyl)amino-5-methyl-	C20H16N203	95-6°	8.43	8.39			
2-Acethyamino-5-methyl-	C8H10N202	179-80°	16.87	16.73			
2-(N,N-dibenzoyl)amino-6-methyl-	C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	65-6°	8.43	8.50			
2-Acetylamino-6-methyl-	C8H10N202	(Huntress, 1948)					
3-Benzoylamino-	C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	209-10°	13.08	12.99			

Table 2

## Experimental

Pyridine-4-azo-p-dimethylaniline.--4-Aminopyridine, (Tomcufcik, 1962) 1.8 g. (.02 mole), was dissolved in a cold mixture of 10 ml. of 85% phosphoric acid and 5 ml. of concentrated nitric acid. At 0° C. a cold concentrated solution of 1.4 g. of sodium nitrite was added beneath the surface of the amine solution with stirring. After the addition of the sodium nitrite, the solution was diluted by addition of 25 g. of ice-water. To the diazonium solution was added 2.4 g. of dimethylaniline dissolved in 20 ml. of 30% phosphoric acid. After standing in the ice-bath for  $\frac{1}{2}$  hour, the solution was made basic with sodium carbonate. The product was filtered and after recrystallization from ligroin, the yield of pyridine-4-azo-p-dimethylaniline was 3.5 g.

The picoline-4-azo-p-dimethylanilines were prepared in the same way. (Table I).

2-(N,N-dibenzoyl)aminopyridine 1-oxide.—To a solution of 3 g. (.01 mole) of 2-(N,N-dibenzoyl)aminopyridine (Huntress, 1948) in 25 ml. of glacial acetic acid was added 15 ml. of 27.5% hydrogen peroxide. The mixture was heated at 70-80° C. for five hours. The resulting solution was evaporated to dryness *in vacuo* and the residue was recrytallized from ethanol-water. The yield of 2-(N,N-dibenzoyl)aminopyridine 1-oxide was 3 g.

2-(N,N-dibenzoyl)aminopicoline 1-oxides were prepared in the same way. Table II.

Pyridine-1-oxide-2-azo-p-dimethylaniline.-The hydrolyses of the 2-acylaminopyridine 1-oxides were conducted as previously reported (Brown, 1957). 2-Aminopyridine 1-oxide hydrochloride, 1.4 g. (.01 mole) was dissolved in 15 ml. of 17% hydrochloric acid and the solution was cooled to 0° C. An ice-cold solution of 0.7 g. of sodium nitrite was added slowly beneath the surface of the amine solution with vigorous stirring. To this cold solution was then added, with stirring, a cold solution of 1.2 g. of dimethylaniline in 10 ml. of concentrated hydrochloric acid. The reaction mixture was allowed to stand in the ice-bath for 1/2 hour. The deeply colored solution was then made basic with sodium carbonate and extracted with chloroform. Concentration of the chloroform extracts and addition of petroleum ether precipitated the product. Upon recrystallization from chloroformpetroleum ether, the yield of pyridine-1-oxide-2-azo-p-dimethylaniline was 1.8 g. Analytical samples were usually chromatographed on alumina from benzene solution.

The corresponding azo compounds from 2-aminopicoline-1-oxides

(Brown, 1957) were prepared in the same way. The products are listed in Table III.

*Pyridine-1-oxide-3-azo-p-dimethylaniline.* - 3-Aminopyridine 1-oxide (Murray, 1954) was prepared in the above manner. After diazotization and coupling, when the solution was made alkaline with sodium carbonate, the product precipitated and was recrystallized from ethanol. (Table III)

Pyridine Moiety	Formula	MP		Analyses, % Calcd. Found				
	ronmara	<u> </u>	C	H	N	C	Found H	N
4-pyridine 1-oxide	с <sub>13</sub> н <sub>14</sub> N40	210-11	64.46	5.83	23.14	64.52	6.00	22.97
2-methyl-4-pyridine l-oxide	$c_{14}H_{16}N_{4}O$	191-2	65,62	6,30	22.04	65.71	6,21	21.69
3-methyl-4-pyridine 1-oxide	$C_{14H_{16}N_{4}O}$	193-4	65,62	6.30	22.04	65.75	6.35	21.71
2,6-dimethyl-4-pyridine 1-oxide	C <sub>15</sub> H <sub>18</sub> N <sub>4</sub> O	197-8	66.67	6.72	20.70	66.58	6.91	20,66
2-pyridine 1-oxide	с13н14и40	181-2	64.46	5,83	23.14	64.25	5.92	23.06
3-methyl-2-pyridine l-oxide	$c_{14}H_{16}N_{4}O$	193-4	65.62	6.30	21,87	65.57	6.42	21.69
4-methyl-2-pyridine 1-oxide	$C_{14}H_{16}N_{4}O$	184-5	65.62	6,30	21.87	65.43	6.45	21.69
5-methyl-2-pyridine l-oxide	$c_{14H_{16}N_{40}}$	201-2	65.62	6.30	21,87	65.54	6,28	21.80
6-methyl-2-pyridine l-oxide	$c_{14H_{16}N40}$	170-1	65,62	6.30	21,87	65.67	6.47	21.78
3-pyridine 1-oxide	C <sub>13</sub> H <sub>14</sub> N <sub>4</sub> O	i89-91	64.46	5.83	23.14	64.52	6.01	23.08

Table 3 (p-Dimethylaminophenylazo)-Pyridines

Pyridine-1-oxide-4-azo-p-dimethylaniline.—The hydrochlorides of 4aminopyridine 1-oxide, the two 4-aminopicoline 1-oxides and the 4-amino-2,6-lutidine 1-oxide were prepared by nitration and reduction of the corresponding pyridine 1-oxides according to Ochiai (Ochiai, 1953). A .01 mole solution of the hydrochloride was cooled to 0° C. and an ice-cold solution of 0.7 g. of sodium nitrite was added beneath the surface of the amine solution with stirring. To the diazonium solution was added a solution of 1.2 g. of dimethylaniline in 10 ml. of concentrated hydrochloric acid. After standing in the ice-bath for  $\frac{1}{2}$  hour, the solution was made basic with sodium carbonate and the product was filtered. Upon recrystallization from ethanol-water the yield of pyridine 1-oxide-4-azo-p-dimethylaniline was 1.6 g.

The picoline-1-oxide- and 2,6-lutidine-1-oxide-4-azo-*p*-dimethylanilines were prepared in the same way. The compounds are listed in Table III.

#### Robert W. Faessinger and Ellis V. Brown

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## THE STATUS OF THE JUNIOR ACADEMY OF SCIENCE IN KENTUCKY

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## Introduction

The Kentucky Junior Academy of Science is an organization for high school students in the state of Kentucky. The goals of this organization can best be stated by quoting from Article II of the Junior Academy Constitution: "The object of this organization shall be to stimulate and to create interest, promote understanding and appreciation in science among the teachers and students in the high schools of Kentucky, and to promote friendships and the exchange of ideas among those who have similar interests."

The Kentucky Junior Academy of Science is an auxillary of the Kentucky Academy of Science and its existence and operation is authorized in Article XII of the Constitution of the Kentucky Academy of Science as revised November 26, 1951.

Since the activities and goals of the Junior Academy are the responsibility of the parent organization, this report is presented to inform all the members of the Kentucky Academy of Science of the present status and potential of the Kentucky Junior Academy of Science.

#### **Historical Background**

The idea of a Junior Academy of Science in Kentucky was first conceived by Dr. Anna A. Schneib of Eastern Kentucky State College in 1932 during her tenure as President of the Kentucky Academy of Science. Dr. Schneib appointed a committee of four senior members of the Academy to investigate the possibility and desirability of such an organization under the sponsorship of the Academy. She was given much moral support and helpful information in the endeavor by Professor Louis A. Astell, then Director of the Illinois Junior Academy of Science. Following a favorable report by the committee, forty six high school students attended a meeting of the Kentucky Academy of Science at Lexington in 1933 for the purpose of organizing the Junior Academy. An Executive Committee for the purpose of guiding the affairs of the Junior Academy was appointed. This committee consisted of Dr. Schneib, who served as chairman and director of the Junior Academy, Dr. V. F. Pavne of Transylvania College, Miss Alice Gossett of Shawnee High School, Mr. W. R. Sebastian of Bellevue High School and Mr. W. Gayle Starnes of Maysville High School.

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On May 19, 1934, the first meeting of the Junior Academy was held in Berea with over five hundred students and teachers in attendance. Mr. Frank Edwards, a student from Waco was elected as the first President and the Junior Academy began its life with a membership consisting of three hundred ten students from nine science clubs. Mr. James Speed of the Louisville Post spoke to the first meeting of the Junior Academy with foresight when he told the members of the importance such an organization could have in encouraging science education. The dues of the organization were set at fifteen cents, ten cents of which was to go to the parent organization. The first issue of the official organ, *The Junior Science Bulletin*, was published in November, 1934 with Dr. Schneib serving as editor.

Some of the advantages of membership in the Junior Academy as set forth by the founders of the organization included the following:

- 1. Speakers for local club meeting—Members of the Kentucky Academy volunteered their time to visit science clubs of schools affiliated with the Junior Academy. Member clubs contacted the President of the Kentucky Academy who made the necessary arrangements.
- 2. Each club affiliated received a copy of the *Proceedings of the Kentucky Academy of Science* as it was published.
- 3. The Junior Science Bulletin which was published a minimum of three times a year was distributed to all members of affiliated clubs. This publication provided an opportunity for members of the Junior Academy to report on their observations and science projects. Papers contributed by guest scientists were also a valuable feature.
- 4. The opportunity to compete for awards and recognition at the annual meeting for the best exhibit and the best discussion.
- 5. Members of the Junior Academy were entitled to wear a pin in the form of the official insignia of the Junior Academy. This insignia was designed by Miss Eleanor Mebane of the Art Department of Eastern Kentucky State College.

The early interest in the activities of the Junior Academy can be seen in the record of its first ten years of growth:

1932–Committee of four senior members appointed by Dr. Anna A. Schneib.

1933-Forty six students meet to organize the Junior Academy.

1934-310 members-9 clubs. First official meeting.

1935—327 members—15 clubs.
1936—510 members—20 clubs.
1937—621 members—24 clubs.
1938—764 members—32 clubs.
1939—842 members—36 clubs.
1940—783 members—33 clubs.
1941—938 members—41 clubs.
1942—Over 1000 members in 45 clubs.

Within ten years of its inception in 1932 the Kentucky Junior Academy of Science grew to become the largest scientific organization in the state. The growth of the organization was reflected not only in the quanity of its membership but in the quality of its activities as well. Throughout these years, the guidance and leardership of Dr. Anna Schneib kept the members constantly striving to improve their efforts in science. This can especially be seen in the improvement of the quality of the student papers published in The Junior Science Bulletin over a period of years. It was Dr. Schneib's great drive and energy which made possible the publication of several lengthy issues of the Bulletin each year in addition to the other activities of the Junior Academy. The annual meeting of the Junior Academy was a highlight of each year. This meeting was held in the schools of various member clubs throughout the state. An important guest speaker was always featured and awards were presented to winners of the various categories of competition.

Some of the highlights of Dr. Schneib's tenure follow. In 1938, the Junior Academy sent five members and an exhibit to the meeting of the American Association for the Advancement of Science in Indianapolis, Indiana. In 1941, an arrangement was made whereby all clubs affiliated with the Junior Academy would receive the *Kentucky Warbler*, the official organ of the Kentucky Ornithological Society. In 1942, through the efforts of Dr. Harvey B. Lovell, then President of the Kentucky Ornithological Society, an invitation was extended to the Junior Academy to affiliate with the society.

The year 1942 can only be described as a banner year for the Junior Academy. With a membership of over 1000 it was now the largest scientific organization in the state. The organization published four eight page issues of the *Bulletin* and one six page issue and would have published another issue if funds had been available. The Kentucky Academy appointed district chairmen to aid in bringing the organization to all the schools in the state. Dr. Schneib was ap-

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pointed as a member of the AAAS Standing Committee on Junior Academies. Two members of the Junior Academy were given honorary membership in the AAAS. Several articles representing the work of Junior Academy members were published in *The Science Teacher*, the journal of the National Science Teachers Association. The annual meeting of the Junior Academy had been expanded to the point that awards were made in twelve categories of competition.

In 1944, the Kentucky Junior Academy of Science was recognized by the AAAS and the New York City Library as having a noteworthy publication. In the fall of 1952, Dr. Anna Schneib, having been associated with the Kentucky Junior Academy of Science for twenty years, retired. Dr. Schneib was succeeded by Dr. Harvey H. LaFuze, Professor of Biology at Eastern Kentucky State College, who served as Counselor of the organization until the fall of 1954 when the pressure of other duties forced his resignation.

In the Fall of 1954, Mr. Robert M. Boyer of the Chemistry Department of the University of Kentucky became Counselor of the Junior Academy. He served in this capacity with distinction, until the fall of 1960 when he was succeeded by Mr. Maurice Christopher of the Chemistry Department of Murray State College. In the first issue of the *Bulletin* which he edited, Mr. Boyer published a letter to the membership in which he emphasized that he was interested in excellence primarily, and numbers secondarily. His leadership of the organization reflected the accomplishment of the goal of excellence. There developed an increased interest in science projects and by the time of the Spring Convention in 1958, there were three hundred students in attendance with one hundred nine science projects and nineteen discussion entries.

In the Fall of 1961, the writer became the successor to Mr. Christopher and has served until the present. During this period the Junior Academy has extended its affiliation to include, in 1962, the Science Clubs of America, and in 1963, the National Science Fair-International. Because he is leaving the state, the writer is resigning effective September, 1963 and will be succeeded by Mr. Ray Jordan of Eastern Kentucky State College. Mr. Jordan is currently serving as the Regional Director of the Middle Eastern Science Fair Region.

# **Present Status**

The leadership of the Junior Academy is appointed by the President of the Kentucky Academy in accordance with Article XII, Section 2 of the Constitution of the Kentucky Academy which states: "The President of the Academy shall appoint three active members of the Kentucky Academy of Science to the governing committee of the Junior Academy. He shall designate one of these as Chairman." The leadership is further delimited by Section 3 which reads: "The Chairman of the governing committee shall be director of the affairs of the Junior Academy." In actual practice, a single member of the Kentucky Academy is usually appointed as Counselor to the Junior Academy and the remaining members are not appointed. In October, 1962, the Constitution of the Junior Academy was revised to comply with the Constitution of the Kentucky Academy and further specified that one of the counselors is to serve as Director, a second is to serve as Membership Chairman, and a third is to serve as Editor of *The Junior Science Bulletin*.

The remainder of the governing committee is composed of four Governors appointed by the Director from among the sponsors of the affiliated clubs. The governing committee then establishes the policies of the Junior Academy and looks to the Director for the implementation of these policies. Four members of the governing committee constitute a quorum and all business is decided by a majority vote. In order to give the necessary continuity to the committee, the Governors serve for four years. A system of rotation, yet to be established, allows for the appointment of one of new Governor each year. The present Governors are Mrs. Harriet Williams, Lexington Junior High School, Mrs. Ethel Lovell, Durrett High School, Miss Hazel Nollau, Breckenridge Training School, and Mrs. Thelma Whitlock, Model Laboratory School, who also serves as treasurer of the Junior Academy. These teachers have served the Junior Academy in various capacities for a number of years and are responsible for the continued stability of the organization.

The membership of the organization presently consists of approximately 880 students from 30 affiliated clubs. This is comparable to the membership of the organization in 1940. When a comparison is made of the growth of the organization with the growth of the school population during the last 23 years it becomes apparent that the Junior Academy does not serve as large a percentage of the students in the state as it formerly did. Since the organization has become affiliated with Science Clubs of America, the membership list of the SCA has been made available to the Junior Academy. This list shows over three hundred science clubs from Kentucky affiliated with SCA during this period. In effect, the Junior Academy serves about ten percent of these clubs. While size of membership is not only the concern of the organization, the leaders of the Junior Academy feel that the organization does provide worthwhile opportunities which should be available to every student in the state. The majority of the clubs presently affiliated are from Jefferson, Fayette, and Madison Counties.

The major activity of the Junior Academy is the Kentucky State Science Fair, held annually in the Spring. Through the interest of Dr. Frank Dickey, former President of the University of Kentucky, the University has assumed a joint sponsorship of the event with the Junior Academy. This cooperation has made possible the use of the facilities at the University and has greatly reduced the costs of presenting the fair. Whereas the fair has traditionally been held in various localities, depending upon invitation, the governing committee, in the Spring of 1963, voted to make the University of Kentucky the permanent site of the state fair. This should prove to be an important decision, since availability of competent judges as well as physical facilities is one of the major considerations in presenting a science fair.

Scientists from the University of University of Kentucky, Transylvania College, Eastern Kentucky State College, and many industrial and research concerns responded with great enthusiasm to the call for judges at the 1963 fair. As a result of affiliation with National Science Fair-International, the Junior Academy was entitled to send two top winners to the National Science Fair in Albuquerque, New Mexico during May, 1963. Due to inadequate finances to pay the expenses of two students, a single winner was selected for the honor. This winner was Mr. Fred Gittes of Fort Knox High School, whose project was entitled "The Effect of Vitamin K on the Coagulation Time of Cobalt Irradiated Mice."

The annual fee for affiliation with the NSF-I is one hundred dollars. Awards made available at no additional expense to the Junior Academy as a result of this affiliation include a two week cruise with the U.S. Navy for a male participant, a set of of the World Book Encylopedia, the Handbook of Physics and Chemistry, fifty paperback science books, and certificates in various categories from the U.S. Air Force and the National Aeronautics and Space Adminstration. At the 1963 fair, the Air Force and NASA provided their own judges for the certificate awards, NASA flying its judge to Lexington from Huntsville, Alabama. First, second, third, and fourth place ribbons were awarded to participants in various categories and each entrant received a certificate of merit signifying his participation. More than 200 students prepared projects for the 1963 science fair and many hundreds of students and parents viewed the proceedings during the two day exhibition. Recent editorials and letters have expressed justifiable concern over the emphasis present in many fairs. However, this does not negate the important work which is being accomplished through this growing event.

Of particular interest is the recognition being given by colleges to the scientific achievement of the young people participating in science fairs. This recognition is being given in a very material and valuable way, through college scholarships. At the 1962 State Science Fair, Eastern Kentucky State College gave a scholarship to the top winner of the fair exhibition. In 1963, the University of Kentucky selected an outstanding participant to receive a scholarship. Scholarships are also given at the various regional fairs. This promises to be a significant method of identifying students with interest and ability in science and should serve to encourage many of these young people to continue a career in science.

The awards for the 1963 State Science Fair were presented at an Awards Banquet at the Georgian Hotel, where Dr. Beardsley Graham, President of Spindletop Research, Inc., addressed the students. This was the first such banquet held and the leadership of the Junior Academy is hopeful that it will be possible to make this an annual event at which the members of the Junior Academy will be exposed to contact with prominent scientists from various fields.

As a result of action by the governing committee, the 1963 fair was opened to all students in the state, regardless of affiliation with the Junior Academy. This was a departure from past policy. However, another very important event held in conjunction with the fair is restricted to Junior Academy members only. This is the Science Discussion Event, in which students present papers they have prepared. These papers are not associated with a prepared exhibit. Forty-eight students participated in this event in 1963 as compared with thirteen for the previous year. This increase reflects an emphasis placed by the governing committee upon this important event. It is anticipated that this will soon become one of the most important phases in the fair in future years.

Concurrently with the National Science Fair, a National Science Seminar and a National Science Exposition were held in Albuquerque, New Mexico. The seminars were conducted on a variety of topics by scientists, many of whom were Nobel laureates, who came to Albuquerque from throughout the United States. The Junior Academy is proud to have been the sponsor of a group of twenty Kentuckians; eight teachers and twelve students, who traveled to New Mexico at their own expense and participated in the seminars.

The greatest deficiency of the Junior Academy at present is *The Junior Science Bulletin*. This publication has declined from the position of prominence which it occupied during the tenure of Dr. Anna

#### Thomas A. Hutto

Schneib. After her retirement it was usually published twice each year, the minimum set forth in the Junior Academy Constitution. During the past three years, the *Bulletin* has been published only once, and calls for papers meet with a very limited response. There is a great need for an editor to rebuild this publication.

## The Future of the Junior Academy

In August, 1962, a Conference on Science Youth Activities under the joint sponsorship of Science Clubs of America and the Atomic Energy Commission was held on the University of Kentucky Campus. Out of this conference there came the expressed desire to coordinate the various science vouth activities in the state and to extend the science fair program to every county in the state. Dr. Frank Dickey formed a Committee on Science Youth Activities and appointed Dr. Ernest M. Spokes of the University of Kentucky, Chairman Pro-Tem. Dr. Clarence Chesnutt, Director of a Regional Science Fair previously organized in Campbellsville, and the writer were also asked to serve on the committee. Under the leadership of Dr. Spokes, the state was organized into ten regions in which science fairs would be held. Each region would be affliated with the National Science Fair-International and would be entitled to send two participants to the National Science Fair. Thus, the future potential is a group of twenty two students from Kentucky participating annually in the national fair.

The regional science fair pattern is not a new idea. It has been adopted by most of the Southern states and the states bordering Kentucky. In the past, the only Kentucky students who had an opportunity to compete in the national fair were those who participated in a fair in West Virginia, Tennessee, or Indiana. The first regional science fair in Kentucky and the first Kentucky fair to affiliate with the NSF-I was the Campbellsville fair in 1962. In 1963, a regional fair was organized in Middlesboro. This fair previously was part of the Knoxville fair. The Middlesboro Fair has been greeted with spontaneous enthusiasm by the local community. The greatest problem the Director of this fair has had is deciding from whom to accept the financing for the fair. At least two colleges are competing for sponsorship of the fair. One important aspect of the regional fair is the involvement of the local community.

The regional fair idea has not met the same response everywhere. However, competent people have assumed the Directorship of most of the regions and there are plans for a considerable increase in the number of regional fairs in 1964. If the Junior Academy is to continue to play an influential role in the direction of science youth activities in the state, it must assume leadership in the area of regional fairs. The regional fairs are looking to the Junior Academy for coordination and guidance in the establishment of policies. Each fair is an autonomous body but it provides the framework of a grass roots organization through which science opportunities can be extended to high school students to whom these opportunities were previously denied. Participation in the activities of the Junior Academy is one such opportunity which can be extended to these students through the regional fairs.

Further, a closer liasion can be developed with the Kentucky Science Teachers Association which will help to make the teachers more aware of the Junior Academy and its functions. Other closely related science youth activities which complement and reinforce the work of the Junior Academy include the Science Talent Search, directed by Dr. H. H. LaFuze of Eastern Kentucky State College, and the Visiting Scientist Program, which is now under consideration by the administration of the Kentucky Academy.

## Financial Status of the Junior Academy

One of the most pressing problems faced by the Junior Academy is financing. For several years, the major support for the organization has been provided by an industrial membership in the Kentucky Academy on the part of the Philip Morris Company. This has come about largely through the efforts of Mr. Spencer Jones of that company. Additional income is provided through the dues paid by club members. The dues are currently set at five dollars per club plus twenty five cents per member. The University of Kentucky has helped to alleviate the financial problem through its joint sponsorship of the fair. However, the State Science Fair was able to send only one student to the National Science Fair while each of the Regional Fairs in the state sent two winners. While none of the members of the governing committee receive a salary, these members are entitled to reinbursement for expenses incurred as a result of Junior Academy activities. However, during the last year, no expenses for travel and similar activities have been requested because of a need for funds for other purposes. The high cost of printing has been, in part, responsible for the decline in the fortunes of The Junior Science Bulletin. Several thousand pieces of correspondence are mailed throughout the state, incurring considerable postage expense. As a result, there is a need for a budget which can be financed with a reasonable degree of certainty. An active growing organization such as the Junior Academy must be properly financed if it is to fulfill its stated purpose.

The National Science Foundation is now making funds available to the Academies of Science in various states, for projects, in science education. Grants have been made to states for such programs as Visiting Scientists, Student Seminars, Mobile Exhibits, Short Term Teacher Courese, Student Workships, and Expansion of Junior Academy Programs. In the fall of 1962, a proposal was submitted for funds in the amount of \$10,135.35 for the expansion of the Junior Academy Program. Such funds would have covered travel expenses for the Governors, salary for a secretary, publication of *The Junior Science Bulletin*, and the State Science Fair program, postage and stationery expense, and funds for travel and per diem for students and teachers traveling to the state fair. The proposal did not receive support, however, due to the Foundation's limited funds.

The National Science Foundation recently announced that during the current year, 57 grants totaling \$526,445 were made to 36 state Academies of Science for the support of activities aimed at improvement of science education. It is interesting to note that all of the eight states which touch Kentucky's borders received grants for one or more of these programs. The amount of these grants were as follows: Tennessee, \$27,685, Missouri, \$10,900, Illinois \$8,000, Indiana, \$14,295, Ohio, \$22,000, West Virginia, \$6,100, and Virginia, \$9,725. There is reason to believe that a carefully considered and planned proposal from Kentucky will also be supported. This is a matter currently under consideration by the administration of the Kentucky Academy.

#### Conclusions

It is the observation of the writer that there has been a tendency to appoint a Counselor to the Directorship of the Junior Academy and then to neglect the role of the Junior Academy as long as the Counselor agreed to serve. It is the opinion of the writer that the Junior Academy must be the concern of all the members of the Kentucky Academy and that they must contribute more than moral support to the organization. The affairs of the Junior Academy have expanded to the point that one individual can not adequately serve them all. With this in mind, the following recommendations are made to the members of the Kentucky Academy of Science:

1. That the Constitution of the Kentucky Academy of Science as it relates to the Junior Academy be fully implemented and that three Counselors be appointed, one to be designated the Director, the second, Membership Chairman, and the third, Editor of *The Junior Science Bulletin*.

- 2. That a Committee on Financing be appointed within the Kentucky Academy to work with the Director in establishing a realistic budget which will meet the needs of the Junior Academy, and that said committee assume the burden of responsibility in obtaining financing for the budget.
- 3. That a part time secretary be hired and placed at the disposal of the Director of the Junior Academy.
- 4. That the Kentucky Academy extend its activities to include as many of the programs which advance science education as is deemed feasible.
- 5. That the Kentucky Academy submit proposals to the National Science Foundation for support of as many of these programs as is possible.
- 6. That the individual members of the Kentucky Academy continue to offer material and moral support to the Junior Academy.

Finally, the writer is indebted to many persons who have given encouragement and support during the two years of his tenure. These persons range from college presidents to high school students and are too numerous to mention individually. Grateful acknowledgment is hereby made to all who have contributed.

## A RECORD OF THE ALLIGATOR GAR IN KENTUCKY

ROGER W. BARBOUR

Department of Zoology, University of Kentucky, Lexington

The Alligator Gar, *Lepisosteus spatula* Lacepede, is known to occur in the Ohio River, but valid records are scarce. Records from streams tributary to the Ohio are rarer still. I recently found a newspaper clipping and two faded snapshots in the back of an old desk in the Zoology Department of the University of Kentucky that document the occurrence of the alligator gar in the Cumberland River.

The photographs, reproduced herewith as Fig 1, although of poor quality, clearly reveal the subject to be a large alligator gar. The back of one of the snapshots bears the message, "This fish was found dead in Cumberland River near Dycusburg. Name him. Yours Truly. T. Atchison Frazer."

The newspaper clipping is from the Crittenden Press, Marion, Crittenden County, Kentucky, under date of July 24, 1925. The article is quoted below in its entirety:

"A monster fish, possibly of an ocean species, was found dead in the Cumberland river near the farm of Gordon Sunderland, three miles below Dycusburg, in Livingston county on Wednesday morning of last week. Mr. Sunderland and his sons went out in a skiff and towed the huge fish to the river bank. Then by means of a rope fastened through a hole cut in the throat of the fish it was dragged by a mule up the bank and out into the field where some hogs were pastured. It is said the fish was intended as food for the hogs, but it seemed not to meet their fancy and they refused to touch it.

"Dr. T. A. Frazer, who is always interested in such things, visited the spot and reported the following measurements:

"The fish was seven and one half feet in length, and about sixty inches in circumference at the largest place, which was about a foot back of the gills. The eyes were about ten inches apart and about four inches back of the angle of the jaw. The nose measured three and a half inches across and there were four nostrils. The fish had large jaws filled with about forty large sharp teeth.

"The head was about eighteen inches long, with the ears about three inches in front of the gills.

"The fins were about eight inches from the tail, one under and one over the fish. The tail fan was about ten inches across.

"The fish was judged to be an old one from the development of the teeth.

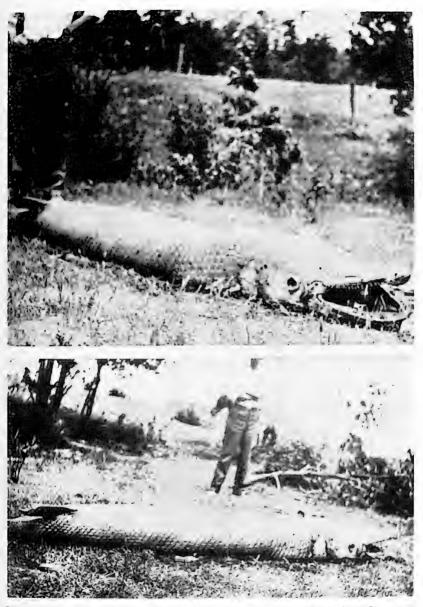


Figure 1.—Two views of an alligator gar found dead in the Cumberland River near Dycusburg in 1925.

"The body was rather round and was not covered with scales but with a kind of armor. The estimated weight is about 350 to 600 pounds.

"This is the largest fish story ever told of this section of the country, and created a great deal of excitement. Many people visited the place and viewed the monster fish."

There are some obvious errors in the story, particularly the portion dealing with the "ears," and the weight estimates are overly high. Nonetheless, they do not detract from the authenticity of the record of a large alligator gar in the Cumberland River in 1925.

## ACADEMY AFFAIRS

The annual business meeting of the Kentucky Academy of Science was called to order by Lyle Dawson on Friday afternoon, October 18, at 3:00 p.m. in the Chemistry-Physics Building on the campus of the University of Kentucky.

The minutes of the 1962 business meeting were read and approved. The treasurer's report was given by J. H. B. Garner. It was moved that the report, previously audited by R. Barbour, J. Edney, and M. Moosnick, be accepted. The motion carried.

R. Jordan reported a membership exceeding 850 in thirty Junior Academy Clubs in Kentucky. The fall meeting of the Junior Academy was held at Model High School in Richmond and the spring meeting, the science fair, was held at the University of Kentucky. The decision was made to hold the spring meeting in Lexington every year. The Junior Academy is now affiliated with Science Fair International. Fred Gittes of the Fort Knox High School was the winner of the science fair at the University of Kentucky and was awarded expenses for his trip to the national fair at Albuquerque, New Mexico. His project was a study of blood coagulation of x-ray irradiated mice. The National Science proposal for a grant to the Junior Academy was turned down and another proposal has been sent to the Foundation. Phillip Morris has again supported the Junior Academy with a \$500 grant.

The research grant committee reported two proposals for research grants were received just prior to the business meeting. It was moved and seconded that the Academy accept the decision the executive committee might make at their meeting regarding the proposals. The motion carried.

A list of new members was read. They are as follows: James Haynes, Alan Maxwell, Milton Campbell, John Walkup, Virgina Barrett, H. L. Stephens, H. B. Dubey, Edward Morris, Darrell D. Dobbs, Malcolm K. Miller, William C. Sherer III, Edward W. L. Smith, Robert R. Carkhuff, William M. Chambers, Larry Chasin, Mark Cunningham Jr., Boice N. Dougherty, Walter Dickenson, Theodore B. Dixon, Charles F. Elton, Stanley Goldman, Thomas Greenland, Jesse G. Harris, Spencer Leiterman, Walter I. Lindley Jr., Andree J. Lloyd, A. Dudley Roberts, John Rosell, Leon David Silber, Roger C. Smith, Richard Thomas, Charles B. Truax, Selwyn Zerof.

It was moved that the new members be accepted upon payment of the membership dues. The motion was seconded and carried. L. Lancaster moved that a membership committee be made up with a member at each school to aid in the soliciting of new members. He suggested that the President appoint the committee. The motion was seconded and carried.

M. Wharton, the Academy A.A.A.S. representative, reported on the A.A.A.S. meeting. She reported on the concern of A.A.A.S. regarding the preservation of natural life areas. There are about 80 to 90 such college areas many of which have been acquired during the last five years due to the urgency for such action. She suggested that an additional representative, possibly a Junior Academy representative, be sent to the next conference.

R. Chapman reported that he had headed a ten member committee (L. Alexander, J. Black, J. Conkin, E. Hammaker, L. Lancaster, D. Lindsay, H. Nollau, W. Read, C. Whittle) for accumulating data and drawing up a proposal for a visiting Scientists program financed by the National Science Foundation. The decision of the Foundation will be reported by March 1, 1964.

The Nominating committee (J. Carpenter, J. Rodriguez, G. Shoemaker) gave the following nominees:

> President Elect: C. B. Hamann Vice President: F. Kodman Secretary:G. Levey Treasurer: J. H. B. Garner A.A.A.S. Representative: M. Wharton Board of Directors: J. Conkin, W. Ehmann

H. Eckstrom moved that the slate be elected unanimously. The motion was seconded and carried. The nominating committee gave three suggestions: 1. The vice-president of the Academy should be given the assignment of new member solicitation. 2. The board of directors should be required to meet at least once each year. 3. The constitution and by-laws should be examined carefully and the necessary additions and changes made in the near future.

A discussion of Junior Academy activities followed during which it was moved that Mrs. H. Williams be the second representative to the Academy Conference and that her expenses be paid by the Academy. She suggested that R. Jordan be appointed. R. Jordan then indicated that T. Hutto is to be one of the speakers at the conference and that he was planning to attend the conference whether he was appointed an Academy representative or not. It was moved that he be the second representative to the conference. The motion was seconded and carried. The question regarding expenses was referred to the executive committee. The meeting adjourned at 4:15 p.m.

Meetings of the sections were then held the following morning at 9:00 a.m. with contributed papers in Zoology, Chemistry, Botany, Geology, Psychology, and Physics.

The officers who were elected at the sectional meetings are as follows:

Microbiology (no sectional meeting held so officers carried over to next year)

Emil Kotcher, chairman Lucia Anderson, Secretary

#### Botany

Edward T. Brown, Jr., chairman Robert Larance, Secretary

#### Geology

James Chaplin, chairman John Philley, Secretary

Chemistry

Ellis Brown, chairman Darnell Salyer, Secretary

Psychology

Alice Van Krevelen, chairman Mary Ellen Curtin, Secretary

Zoology

John C. Williams, chairman Marilyn Cole, Secretary

## Sectional Meetings

#### MICROBIOLOGY SECTION

Emil Kotcher, Chairman Lucia Anderson, Secretary

Due to the conflict in meeting time with the Southeastern Microbiology group there will be no sectional meeting.

#### ZOOLOGY SECTION

Chemistry-Physics Building, Room 103 Roger W. Barbour, Chairman J. G. Rodriguez, Secretary

On the daily activity of *Microtus ochrogaster* in February. Roger W. Barbour, Department of Zoology, University of Kentucky.

Some studies on Rhoad's cotton mouse, *Peromyscus gossypinus megacephalus*, in Alabama. Jackie Batson, Department of Zoology, University of Kentucky.

Modification of developmental patterns of pea beetle embryos. Alfred Brauer, University of Kentucky.

Some effects of ultraviolet irradiation of the viability and infectivity of *Trichinella spiralis* larvae placed in mice. J. M. Edney and Karen J. Ott, Department of Zoology, University of Kentucky.

Biological Studies in the Genus Androchirus (Coleoptera: Alleculidae). J. M. Campbell, Department of Biology, Eastern State College.

Home range of the prairie vole, *Microtus ochrogaster*. Michael J. Harvey, Department of Zoology, University of Kentucky.

Observations on home range and movements of *Natrix septemvittata*. Marion D. Hassell, Department of Zoology, University of Kentucky.

Some notes and observations on the evolution of Troglobitic invertebrates in the Central Appalachians. John R. Holsinger, Department of Zoology, University of Kentucky.

The results of a five year fish management experiment in a small limestone quarry. Henry H. Howell, Asbury College.

Preliminary studies on the reproductive potential of *Drosophila affinis*. Willard Jinks, Department of Zoology, University of Kentucky.

Preliminary studies in the behavior of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) in its natural habitat. W. E. King and J. G. Rodriguez, Department of Entomology and Botany, University of Kentucky.

Chloride differences between polluted and unpolluted streams near Lexington, Kentucky. Robert A. Kuehne, Department of Zoology, University of Kentucky.

Insectivora of Kentucky. Charles L. Rippy, Department of Zoology, University of Kentucky.

Studies on the development of a chemically defined diet for the two-spotted spider mite, *Tetranychus telarius* L. (Acarina: Tetranychidae). T. N. Seay, Carolyn D. Durham, and J. G. Rodriguez, University of Kentucky.

A study of the reproductive ability of *Drosophila affinis* using the yeast *Kloeckera apiculata* as a nutrient source. B. A. Semp and J. M. Carpenter, Department of Zoology, University of Kentucky.

#### CHEMISTRY SECTION

Chemistry-Physics Building, Room 148 Thomas Kargl, Chairman

#### Ellis Brown, Secretary

"The structure of Dimethyl Sulfone," Donald E. Sands, University of Kentucky,

"Radiolysis of Alkylated Benzenes," N. T. Lipscomb, R. H. Wiley and *Charles McKnight*, University of Louisville.

"Some Heterocyclic Ring Systems with Bridgehead Nitrogen Atoms," Kevin T. Potts and *Harold Burton*, University of Louisville.

"A Study of the Oxygen Uptake of Ascites Tumor, S-37," Sister Virginia Heines, Catherine Spalding College, Louisville, Kentucky.

"The Synthesis of Some 1,2,3-Diazaphosphorinane-2-oxides as Potential Antitumor Agents," John L. Meisenheimer, Eastern Kentucky State College. Research performed in Association with Professor John H. Billman, Indiana University.

(10-Minute Break)

"New Meso-ionic Ring Systems," Kevin T. Potts, University of Louisville.

"Preparation of Azo Compounds in the Diazine Series," *Donald R. Kelley* and Ellis V. Brown, University of Kentucky.

"Polarographic Studies of the Interaction of Hemin with Organic Mercury Compounds," Sister M. Angelice Seibert, Ursuline College, Louisville. "Activation Analysis Using a Neutron Generator," J. R. Voght and W. D. Ehmann, University of Kentucky.

"Determination of Gold, Platinum, Mercury, and Antimony in Meteorites by Activation Analysis," P. A. Baedecker, J. T. Tanner, and W. D. Thumann, University of Kentucky.

All papers ran approximately 15 minutes.

The name in italic type presented the paper.

#### BOTANY SECTION

#### Chemistry-Physics Building, Room 111

#### Carl E. Hendrickson, Chairman

Robert Larence, Secretary

Studies on the Vascular Flora of Kentucky Point, Kentucky. Edward T. Browne, Association Professor of Botany, University of Kentucky. 15 minutes.

Chemically-induced Synchronization of Meristematic Cells in Vicia faba. Sister Augustine Mattingly, Biology Department, Brescia College.

Rate of DNA syntheseis in onion root meristem during X irradiation. Richard Oliver (Introduced by C. B. Hamann), Biology Division, Oak Ridge National Laboratory, and Asbury College.

#### GEOLOGY SECTION

Chemistry-Physics Building, Room 211 James E. Conkin, Chairman John C. Philley, Secretary

Devonian Foraminifera of North America. James E. Conkin and Barbara M. Conkin, University of Louisville.

The Louisiana Limestone of Missouri and Illinois. James E. Conkin and Barbara M. Conkin, University of Louisville.

Cold Wave Patterns in Relation to Lexington, Kentucky and Orlando, Florida. J. R. Schwendeman, Geography Department, University of Kentucky.

#### PSYCHOLOGY SECTION

Joan Lee, Chairman Mary Ellen Curtin, Secretary Room M122–Medical Center–9:00-1:00

#### A SYMPOSIUM

"Statistical significance tests-are they sensible or slippery?"

#### PARTICIPANTS

Dr. James S. Calvin, Professor of Psychology

Dr. Charles F. Elton, Dean of Admissions

Mr. Selwyn Zerof, Computing Center, University of Kentucky

Dr. John R. Binford, Associate Pprofessor of Psychology, University of Louisville

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Ledley, R. S. and Lusted, L.B. Reasoning foundations of medical diagnosis, *Science*, 3 July 1959, 130, 9-21.

Rozeboom, W. W. Null-hypothesis significance test. *Psychological Bulletin*, 1960, 57, 416-428.

# Academy Affairs

#### PHYSICS SECTION

(Kentucky Association of Physics Teachers) Chemistry-Physics Building, Room 155

10:00 a.m. Invited Paper

The New 5.5 Mev Van de Graaf Accelerator. Bernard D. Kern, University of Kentucky Contributed Papers

12:00 Lunch

1:30-2:30 p.m. Tour of the Van de Graaf Accelerator Facilities

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