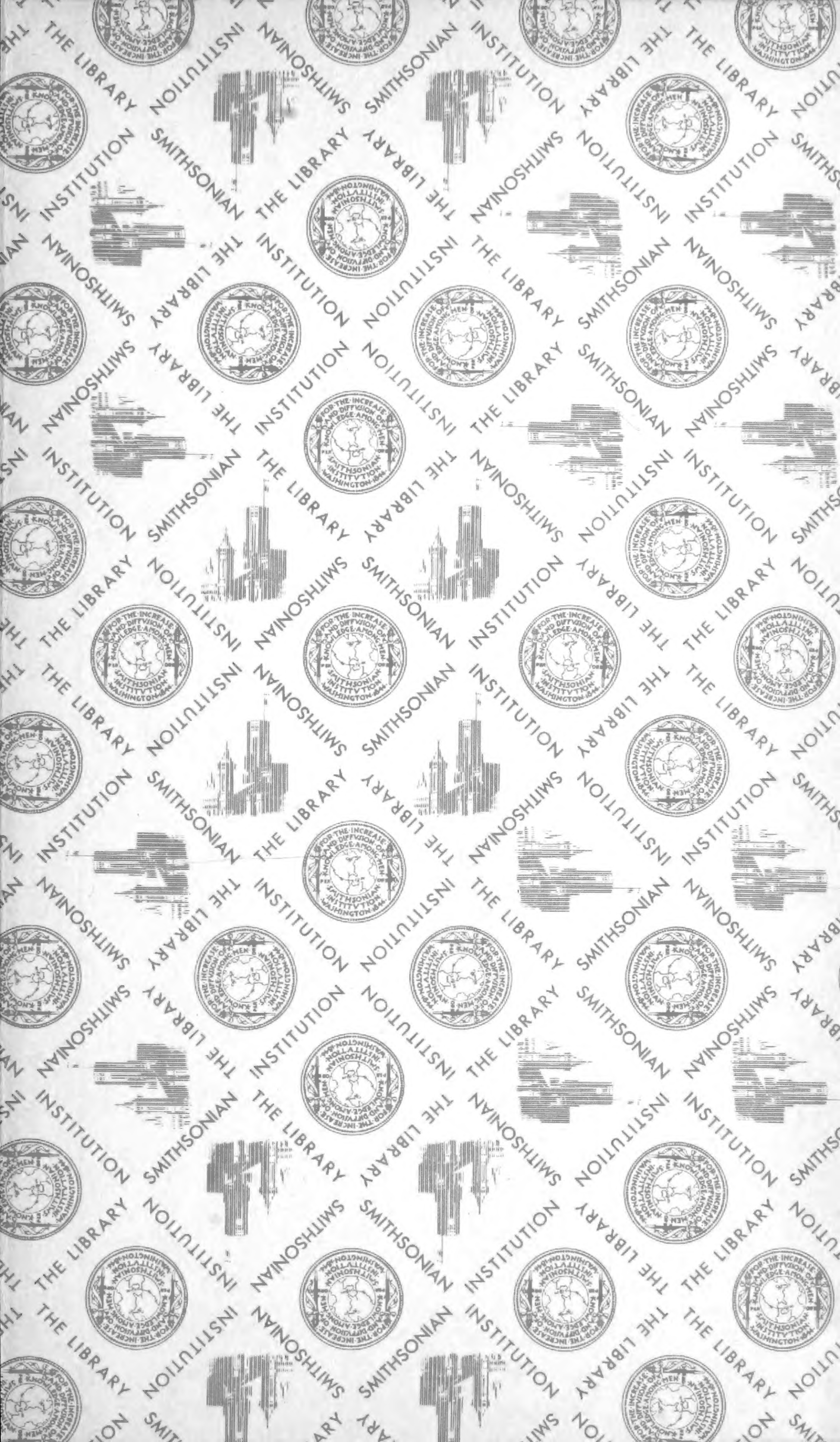
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TRANSACTIONS OF THE
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Volume 18 **February, 1957** **Number 1**

Gas Chromatography of C_2 to C_{11} Fatty Acids with Simplified Apparatus*

SISTER VIRGINIA HEINES, SISTER RODERICK JUHASZ,
SISTER MARY ADELINE O'LEARY AND GUENTER SCHRAMM†
Nazareth College, Louisville, Kentucky

In 1941 Martin and Synge (1) suggested the use of gas-liquid partition chromatography for analytical purposes. No other work along this line was reported until James and Martin (2) in 1952 followed up their suggestion by describing the application of this method for the separation of fatty acids, the microestimation of ammonia and the methyl amines (3), and later for the separation of volatile aliphatic amines and homologues of pyridine (4). The success of the technique is shown by the ever increasing number of publications from the industries (**5**, **6**) especially the application of gas-liquid chromatography to quantitative analysis of complex mixtures of hydrocarbon gases (**7**, **8**).

The new analytical tool is essentially parallel to the results obtained by the fractional distillation of mixtures, but better separation of very small quantities can be more easily achieved.

Briefly, the gas-liquid partition chromatography procedure involves fractionation of materials with a column consisting of a nonvolatile solvent supported on a finely divided inert solid. Movement of the sample through the column is promoted and controlled by the flow of an inert carrier gas (Figure 1). The purpose of the present study was to compare the efficiency of a more simply designed apparatus with the automatically controlled instrument of James and Martin for the separation of the fatty acids C_2 to C_{11} (C_8 was unobtainable by us at the time).

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* Built and owned by Institutum Divi Thomae Foundation, Cincinnati 6, Ohio. Construction supervised by H. G. Cassidy, Yale University.
† Present address: Proctor and Gamble, Research Laboratories, Cincinnati, Ohio.

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Apparatus

1. *Preparation of the Column.* The liquid phase consisting of BB-148 silicone (Dow-Corning) containing 10% (w/w) stearic acid is added to I. M. Hyflo-Supercel, (0.5 g. of liquid to 1 gram Supercel). The mixture is well stirred and let stand for several days in a well-stoppered Erlenmeyer flask. A 5 ft. length (6 mm. internal diameter) of pyrex glass tubing (Fig. 1, B) is drawn out at one end to form a short length of thick-walled capillary, C. A plug of glass wool, G_1, is pushed down the tube to the capillary to prevent blocking by the Supercel. The mixture is then added in small increments while gently tapping and rotating the tube. When the column is packed, a second plug of glass wool, G_2, is pushed down against the packing.

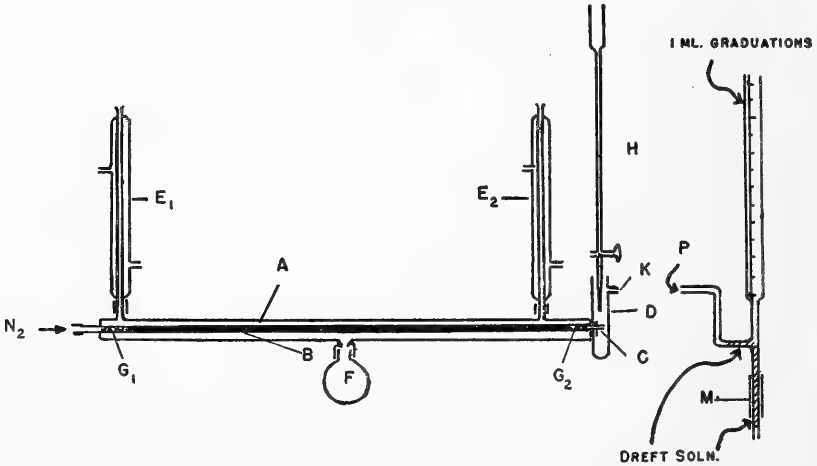

Fig. 1. Simplified Apparatus for Gas Chromatography.

2. *Jacket and Titration Cell.* A 4 ft. length (1 in. internal diameter) of pyrex glass tubing, A, is fitted to a titration cell, D, on the capillary end by a rubber gasket, and the other end attached to a tank of purified dry nitrogen gas, controlled by a two-way gauge. The jacket, A, holds two water condensers, E_1 and E_2, and a boiler, F, to heat the column. To the titration cell at K is attached a flow-meter device at P containing a Dreft solution. When the rubber tubing connection, M, on the flow meter is squeezed, bubbles of the Dreft solution rise into the side tube for timing the rate-flow of the gas through the column. The rise of a bubble through the burette (50

ml.) is timed with a stop watch and the accuracy has been found to be within 0.2% error. A Koch microburette, H, was used to deliver the 0.04N NaOH directly to the titration cell.

PROCEDURE

The chromatogram tube is first allowed to come to constant temperature by the boiling liquid. This requires about 40 minutes and can be recognized by the height of vapor rising into condenser tubes. Water (b.p. 100°) is used in the boiler for separation of a mixture of fatty acids from acetic to n-valeric (inclusive). When the column has reached a constant temperature, a mixture of the free fatty acids is applied with a micropipette to the glass plug at the end of the column, G, and connected to the nitrogen source. The acid mixture of acetic to n-valeric was prepared by mixing well one ml. of each acid and introducing 5 µl. of this mixture to the chromatogram column. At once 8 ml. of distilled water are introduced into the titration cell, D, and a drop of phenolphthalein indicator added. A few drops of the 0.04N NaOH from the microburette, H, are added to the titration cell to impart a pink color to the water.

The acids in vapor phase emerge from the column according to increasing molecular weight (2) and are absorbed in the water as can be detected by the fading of the pink color. Titration is continued with the base every minute until the pink color again persists. Acetic acid emerged from the column eight minutes from the start and required 0.34 ml. 0.04N NaOH for neutralization, which was completed in three minutes. The total volume of base required for acids from acetic to n-valeric (inclusive) was 1.52 ml., and separation of the six acids took less than two hours. The time of emergence for other acids used in this mixture is given on Table I.

At the end of this determination, the nitrogen gas was let flow through the tube for at least 30 minutes before changing the water for the higher boiling Cellosolve. The condensers were drained of the water and the column was heated with boiling Cellosolve before applying the mixture of acids C_6 to C_{11}. Although a fairly good separation of the components was attained in this series, it took five hours for emergence even though the pressure of the carrier gas was increased. The flow rate of the nitrogen gas may be measured at the start or at the end of the experiment. The flow rate for separation

TABLE I. RECOVERY OF C_2 TO C_{11} FATTY ACIDS FROM THE COLUMN

Acids	Boiling Points C.°	Emergence from start time—min.	Present in sample mg.	Recovered mg.
Acetic	118.1	8	0.82	0.80
Propionic	141.1	13	0.77	0.81
Iso-butyric	154.4	17	0.75	0.88
N-butyric	163.5	27	0.76	0.88
Iso-valeric	176.7	47	0.75	0.80
N-valeric	187	70	0.73	0.60
N-hexanoic	205	6	0.85	0.83
N-heptanoic	223.5	36	0.83	0.75
Nonanoic	254	100	0.81	0.75
Decanoic	268-270	180	0.80	0.75
Hendecanoic	225	310	0.82	0.71

of acids hexanoic to hendecanoic averaged 120 ml. per minute. The total volume of base required for these acids was 0.64 ml. 0.04N NaOH (see Table I).

RESULTS

Quantitative estimations (Table I) are based on the amounts of each acid received and recovered by the column. A calibrated micropipette was used to deliver the mixture of acids to the column. A volume of 5 ul. gave a reproducibility of ± 6%. Retention time was easily reproducible in three runs made on the same series of acids. Paper chromatograms can be run on the fatty acids absorbed in the titration cell and thus establish identity of each acid in the mixture separating on the column. Such chromatograms were not made in this study.

From a consideration of Figure 2 and Figure 3, acetic acid shows shorter tailing than hexanoic or heptanoic acids. The upper curves (B) are graphical representation of the lower curves (A); all the acids show a fairly good resolution. A study of these results indicate that many factors enter into the efficiency of operating a column. James and Martin (1) state that a 4 ft. column (4 mm. internal diameter) has a "maximum permissible loading of approximately 1 mg. of each acid for the lower acids," and that the "minimum amount of acid detectable is 0.02 mg. of acetic acid." In the separation of fatty acids the mobile phase (fatty acid) is in equilibrium with the stationary phase (silicone-stearic). The stearic

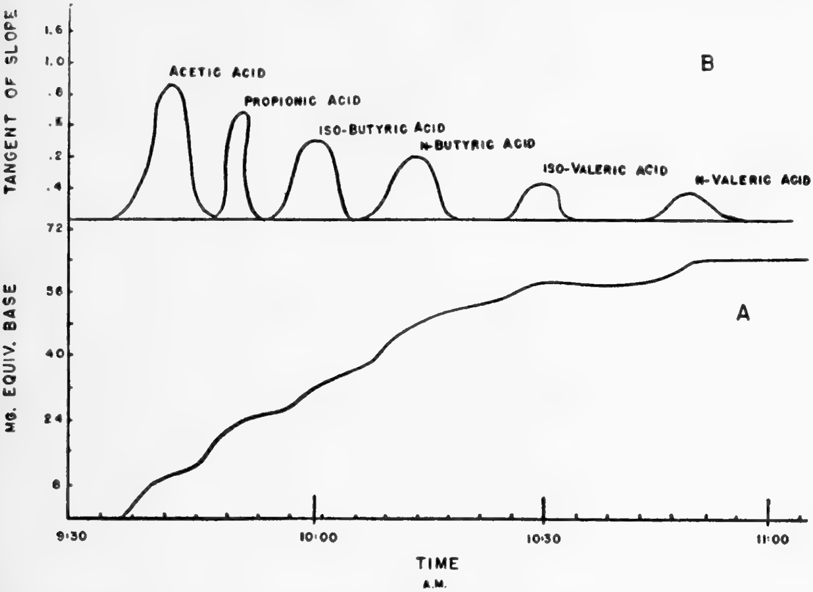

FIG. 2. The separation of acetic, propionic, isobutyric, butyric, isovaleric and valeric acids. **A** is the experimental curve. **B** is the graphical representation of the experimental curve. Nitrogn pressure, 57 ml./min. Temperature, 100° C.

acid is added for three reasons: first, to decrease the association of the monomer with the liquid phase; second, to prevent self-dimerization of acids; and third, to facilitate the association of the high boiling stearic acid with the fatty acid. The acids are then released at the column temperature in gas phase and emerge in order of their increased boiling points. Thus stearic acid effectively suppresses absorption in the liquid phase and prevents over loading of the column which would show up in linear isotherms. The high temperature of the jacket speeds the flow-rate of the vapors but increases self-dimerization of the fatty acids.

The chief advantages of using this simplified gas-liquid partition apparatus may be listed as follows: (1) all steps of operation are clearly visible; (2) recovery of microquantities of mixtures and time of emergence are reproducible; and (3) temperature control and flow-rate of carrier gas are easy to maintain. The good reproducible separations of fatty acids from a small volume mixture

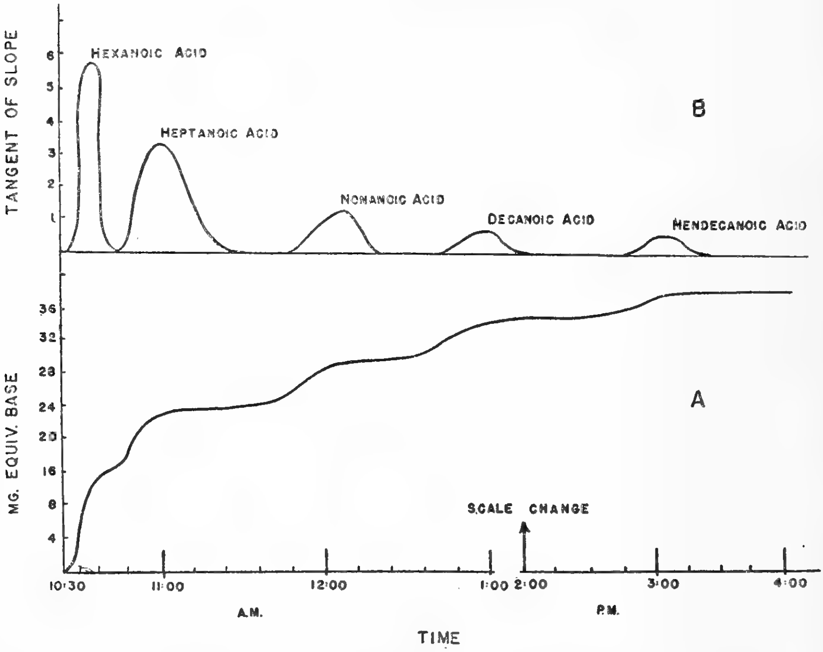

FIG. 3. The separation of hexanoic, heptanoic, nonanoic, decanoic and hendecanoic acids. **A** is the experimental curve. **B** is the graphical representation of the experimental curve. Nitrogen pressure, 120 ml./min. Temperature, 137° C.

predict the versatility of this set-up for the microestimation of organic mixtures other than the fatty acids.

SUMMARY

1. A simplified apparatus for determination of C_2 to C_{11} fatty acids by gas chromatography method is described.

2. Good separation of a mixture (5µl.) of fatty acids, acetic to n-valeric was attained in two hours; a mixture (5µl.) of fatty acids, n-hexanoic to hendecanoic in five hours.

3. Dry, purified nitrogen gas was used as carrier gas. A flow-rate of 56 ml./min., temperature 100° (water) for acids C_2 to C_5, and a flow-rate of 120 ml./min., temperature 137° (Cellosolve) for acids C_6 to C_{11}, were maintained.

4. A rough quantitative estimation of recovery of acids from the column showed a value within ± 6%.

5. This simplified apparatus is recommended because operation steps are visible and reproducible results predict separations of mixtures other than the fatty acids.

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Self-Diffusion Coefficients of the Sodium Ion in Solutions of Sodium Chloride in Methanol at 25°*

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The self-diffusion coefficients of water and of the sodium ion in aqueous sodium chloride solutions have been reported by Wang (**1, 2**). Numerous other diffusion studies have been made with water solutions but little attention has been given to diffusion in non-aqueous solvents.

Methanol was chosen to be used as the solvent in this study because of its similarity to water. Sodium chloride was chosen as the solute because the literature contains reliable conductance and transference data for sodium chloride in methanol (**3,4**). This is the first diffusion study in non-aqueous solvents for which comparable ionic mobility data are available.

Experimental

Reagent grade absolute methanol was purified further by refluxing for several hours over activated alumina then fractionating through an efficient column. The portion used in this study had a specific conductance of 4×10^{-7} ohm^{-1} cm^{-1}.

Reagent grade sodium chloride was recrystallized and fused.

Radioactive sodium was obtained as a aqueous sodium chloride solution from the Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

The capillary method was used for measuring self-diffusion. Samples of the solution containing radioactive sodium (Na-22) were held in capillary tubes constructed from precision bore tubing 0.05 cm. in diameter and ranging from 2.0 to 4.0 cm. in length. One end was sealed with a flat glass plate and the other end was ground smooth and square. The lengths were measured with a calibrated micrometer microscope and the volume was determined by filling with mercury and weighing. The capillary cells were held in a four-pronged glass holder suspended in the solvent in a one-liter flask. Agitation of the solvent was accomplished by a paddle stirrer the shaft of which passed through a mercury seal. The flask was immersed in a large oil-filled bath maintained at 25 ± .02° C.

Because of the volatility of the solvent extreme precautions were necessary to prevent changes in concentration of the tagged solution by evapora-

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tion. The solutions were prepared in a small "vaporstat" consisting of a beaker lined with absorbent paper which had been saturated with methanol and allowed to come to equilibrium. The beaker was fitted with a rubber stopper provided with a stoppered access hole. All solutions were stored with frequent shaking over an isopiestic bath solution.

In preparing a tracer solution 100 µl. of the stock radioactive sodium chloride solution was evaporated to dryness in a 3 ml. vial. Then the radioactive residue was dissolved in 0.5 to 1.0 ml. of the inactive bath solution. A calculated amount of solvent was added to each to compensate for the concentration change resulting from the weight of the solid tracer.

Diffusion was allowed to proceed until from 70 to 75% of the original tagged solution had been removed from the capillary tube. The approximate time was determined from a series of preliminary runs. The stirring rate was adjusted to 75 r.p.m. which yielded a Δl value (**5**) of zero.

Upon removing the capillary tube its contents were washed into a centrifuge tube and a saturated solution of hydrogen chloride in 60% aqueous methanol was added. This procedure gave a very fine uniform precipitate. The precipitate was centrifuged, washed with acetone, then suspended in acetone from which it was plated by a device similar to that described by Ames and Willard (**6**). The sample was counted on a methane-flow proportional counter and its specific activity was determined using a self-absorption correction curve.

Preliminary experiments showed some loss of tracer ions by exchange or absorption at the capillary walls. Errors resulting from this effect were minimized by prolonged equilibration with the active solution prior to the measurements.

RESULTS

Table I contains values of the self-diffusion coefficients of sodium ion determined by using tracer quantities of radioactive sodium at several concentrations of sodium chloride. The values were calculated from the measured ratios of $C_0/Cav.$ (**5**) by the equation:

$$\frac{Dt}{l^2} = \frac{4}{\pi^2} \ln \left(\frac{8}{\pi^2} \times \frac{C_0}{Cav.} \right)$$

Each coefficient reported represents the mean of from four to eight measurements. The standard deviations of the data vary from 0.9 to 1.5%.

DISCUSSION

Using the conductance and transference data reported by Gordon and co-workers (**3, 4**) with the Onsager limiting diffusion equation (**7**), the self-diffusion coefficient of sodium ion in sodium chloride

TABLE I. Self-diffusion Coefficients of Sodium Ion in Methanol Solutions of Sodium Chloride at 25°

Molal Concentration	D x 10^5 (cm.^{2}/sec.)
0.001	1.206 ± .014
0.0025	1.202 ± .018
0.005	1.197 ± .010
0.020	1.164 ± .010
0.040	1.151 ± .010

solutions in methanol at low concentrations may be represented by the following equation:

$$D_{Na+} \times 10^5 = 1.204 - 0.984 \sqrt{C}$$

The results of this study indicate that the limiting value predicted by this equation (1.204) is approached at low concentrations. However the slope of the $D_{vs.} \sqrt{C}$ curve is less negative than that predicted by theory; hence, the limiting slope would be approached from above. In addition the data obtained over the experimental range studied suggest the probability of a slight maximum in the region 0.0001 to 0.001 molal. Limitations of specific activity of the active sample prevented obtaining reliable measurements below 0.001 molal. There is no evidence of the possibility of a very pronounced maximum in the D vs. $C^{1/2}$ curve up to the limit of the solubility of the salt which is about 0.1 molal.

At equal molal concentrations of solute, the diffusion of sodium ion is about 10% greater in water than in methanol. In 0.005 molal aqueous sodium chloride solution the diffusion coefficient is 1.32 x 10^{-5} cm.^{2}/sec. It is of interest to note that in the diffusion of sodium ion in aqueous sodium chloride solutions Wang (2) found a very pronounced maximum in the plot of D *versus* $\sqrt{C}$ at a concentration of approximately 1/20 of the saturation value. Also, at low concentrations the slope of the plot is less negative than the theoretical limiting Onsager slope.

Wang has pointed out that distortion in the molecular structure of the solvent about a solvated ion is less for ions with greater "effective" sizes. With increasing concentration of the solute this distortion increases, the activity coefficient of the ion along the path of diffusion increases (8), and the energy of activation for diffusion becomes less. Changes in the "distortion" of the molecular structure of the solvent about an ion probably include changes in the degree of "solvation" of the ion. This results in more rapid diffusion leading toward a maximum in the diffusion curve where this effect finally is counteracted by the increasing relaxation force.

Since the limiting conductance-viscosity product for the sodium ion in methanol (3) is very small (0.246 at 25°), the solvated ion must be relatively large. Because of the considerations set forth above a very pronounced maximum in the value of the self-diffusion coefficient might be expected as the concentration is increased. However, in methanol which has a dielectric constant of only 32.6, the relaxation effect produced by the mutual attraction between oppositely charged ions increases so rapidly with increasing concentration that the maximum in the diffusion rate is nearly eliminated.

SUMMARY

1. The self-diffusion coefficients of sodium ion in methanol solutions of sodium chloride have been determined at 25° C. for concentrations ranging from 0.04 to 0.001 molal.
2. Comparison of the experimental values of the diffusion coefficients with the theoretical limiting value of 1.204 cm.^{2}/sec. indicates the probability of only a slight maximum in the diffusion rate as it changes with increasing concentration.
3. An explanation is offered for the contrast in the diffusion behavior of the sodium ion in water and in methanol.

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An Albino Brook Lamprey, *Lampetra aepyptera* (Abbott), in Kentucky*

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AN ALBINO individual was among a series of seven specimens of the brook lamprey *Lampetra aepyptera* (Abbott) taken with the use of cresol one mile east of Means in Bull Fork Branch of Slate Creek, Licking River drainage, Menifee County, Kentucky, by the writers on March 29, 1956.

The specimen is a male of 131 mm. total length. Some other measurements, given as thousandths of the total length, follow: tail, 275; length of disc, 97; eye, 18; eye to tip of "snout," 87; eye to posterior margin of last gill opening, 118; depth, 63; thickness, 55; height and length of first dorsal fin, 35 and 159; height and length of the second dorsal fin, 60 to 260. There are 62 trunk myomeres.

The supraoral lamina is deeply notched and bears a pronounced rounded cusp at either end. The infraoral lamina is long, with about nine crenulations. Three small laterals lie to either side of the oral aperture; the posterior two on the left and the posterior one on the right are bicuspid. The small marginals comprise a single row on the posterior field of the disc and about three to five rows on the anterior field. The posterior field bears no other teeth, while the anterior field of the disc bears a few very small teeth. The entire oral structure is in good condition, neither teeth nor oral fimbriae showing the wear and abrasion which commmonly result from nest-building activities.

In life the animal was a warm, ruddy flesh-color dorsally, paler on the lower sides, and largely white on the belly. The pupil was red. Shortly after the animal was killed in formalin its colors were compared with plates in Maerz and Paul (1930) and determined to be approximately as follows: dorsum Roe (plate 12, 6 H); belly paler, to white; caudal fin Sulphine Y (pl. 12, 4 L). At the time

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* This report is a result of an in-progress survey of Kentucky fishes sponsored by the U. S. Fish and Wildlife Service (Federal Aid Project F-7-R), the Kentucky Department of Fish and Wildlife Resources, and the University of Louisville.

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of capture the general hue was much redder, due probably to capillary dilatation in the presence of cresol.

No melanophores or other chromatophores were visible in microscopic examination. Seversmith (1939: 227) stated that the coloration of the adult *L. aepyptera* is due to four causes: (1) Stellate melanophores below the epidermis. (2) "Very small cellular inclusions which reflected a coppery sheen. These are extremely minute and more or less evenly distributed in the cells just under the epidermis." (3) "Scale-like cell inclusions" which result in the white of the belly. (4) The "imperfectly transparent epidermis" which may break up the light and impart a bluish tone. Of these four factors, only the first appears to be absent in the specimen under consideration.

The stream where this collection was made is about 20 feet wide and comprises a series of riffles (about 75%) and flowing pools. The bottom consists of rubble, gravel and sand with limestone bedrock exposed occasionally in the pools. In the riffles the depth was about 8 to 12 inches and the velocity about 1 ft./sec.; in the pools the maximum depth was about 4 ft. and the velocity ranged from 0.2 to 0.5 ft./sec. The water temperature was 15.5° C., the air temperature 21°. The water was relatively clear, with an estimated Secchi disc transparency of 30 inches. The time of capture was 1:00 p.m. The sky was mostly clear. The lamprey was taken with a dip net while it was vigorously swimming near the surface at the head of one of the pools.

Although this individual may not have spawned (its teeth were not worn and it contained milt at the time of capture), there is evidence that some of the other specimens taken at the same time had done so.

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Effect of Soft X-Rays on the Luminescence Excited in Solutions of Biological Compounds by Co-60 Radiation

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Introduction

Discussions on the role of excitations on the biological effects of radiation indicate a need for additional knowledge of energy transport mechanisms in irradiated biological systems. Opportunities for an understanding of these mechanisms in liquid scintillators are offered by the studies of Kallman and Furst (**1**), Reynolds (**2**), Birks (**3**), Bowen (**4**), Harrison (**5**), Swank (**6**), and others. These considerations stimulated studies on the effect of 50 kv x-rays on the luminescence efficiency of biological compound solutions. Previous work on irradiated biological systems generally has been concerned with chemical changes, enzyme activity, etc., with little attention given to the radiation-induced decomposition products. Maxwell and co-workers (**7**) made a systematic study of the decomposition of a single-solute system, glycine, by 50 kv x-rays. In this work they identified at least eight decomposition products. The findings presented here involve a study of the luminescence efficiency of different biological compounds under high-energy gamma bombardment before and after 50 kv x-irradiation.

Experimental

The experimental arrangement used for the luminescence efficiency measurements in these studies consisted of a lead shield containing a Co^{60} gamma source, a sample changer with 4 ml of the solution or solvent in a porcelain crucible, and a 1P28 photomultiplier tube with a commercial photometer as a recording instrument. Further details of this experimental setup are discussed in a previous report (**8**). The biological compounds studied were generally dissolved in plain distilled water. The luminescence efficiency of the irradiated solution was measured relative to the luminescence of the non-irradiated solu-

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tions. This was repeated for various solution concentrations. Determinations were also made of the chemical concentration of the biological compound in the irradiated solutions. These results were correlated with the luminescence changes in these solutions for the same x-ray dose value.

The x-rays were delivered by a thin beryllium-window tube operated at 50 kv and 40 ma without added filtration. An open shallow dish containing 5 ml of a solution of the compound was irradiated at a distance of 5 cm. Under these conditons the dose rate applied was approximately 110,000 r/min. The dose rate was determined by measuring spectrophotometrically (Beckman DU-320 mμ) the reduction of ceric sulfate solution ($\frac{200\mu M\ Ce^{+4}\ in}{liter}$ 0.8 N H_2SO_4) assuming 2.58μM Ce^{+4}/liter·1000 rep as the reduction yield. The solution was magnetically stirred during the irradiation in order to continually replace the irradiated surface layer and thus increase the yield.

RESULTS AND DISCUSSION

The luminescence efficiency of various biological solutions under Co^{60} gamma radiation bombardment in relation to the luminescence of the solvent was reported previously by Kereiakes, et al. (**8**). All biological compounds studied quenched the luminescence of the distilled water solvent.

Results of the dose-effect on the luminescence efficiency for two biological compound solutions exposed to 50 kv x-irradiation is presented in Figure 1. Irradiated tryptophane solutions showed a decrease in luminescence, while x-radiation resulted in an increase in light emission of ascorbic acid solutions. These radiation effects on the luminescence demonstrate the complexity of the problem. In each case the solutes are decomposed by x-rays as shown by Kinsey (**9**), Proctor and Goldblith (**10**), Barron (**11**), Alder and Eyring (**12**), and other investigators. For tryptophane, where the luminescence decreased, it may be considered that the decomposition products were able to increase the quenchng properties of the solution. With ascorbic acid, the decomposition products stimulated the light emission (Figure 1).

Figure 2 shows the effect of concentration on the x-ray-induced changes in the luminescence of biological compound solutions. The following reactions were observed: a change increasing with concen-

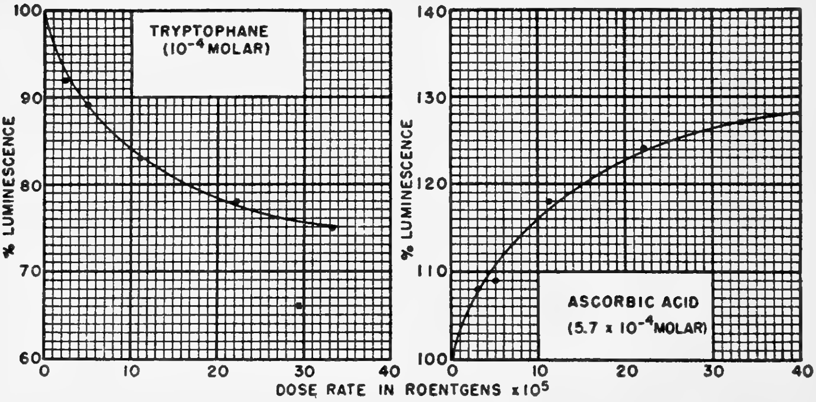

FIGURE 1: EFFECT OF 50 KV X-RAYS ON THE LUMINESCENCE OF BIOLOGICAL COMPOUND SOLUTIONS

tration and approaching a saturation value (glutathione); and a change passing through a maximum with increasing concentration (ascorbic acid). With ascorbic acid, which showed an increase in luminescence (yet never surpassing that of the solvent), the decomposition products may be considered to have less efficient quenching properties at certain concentrations.

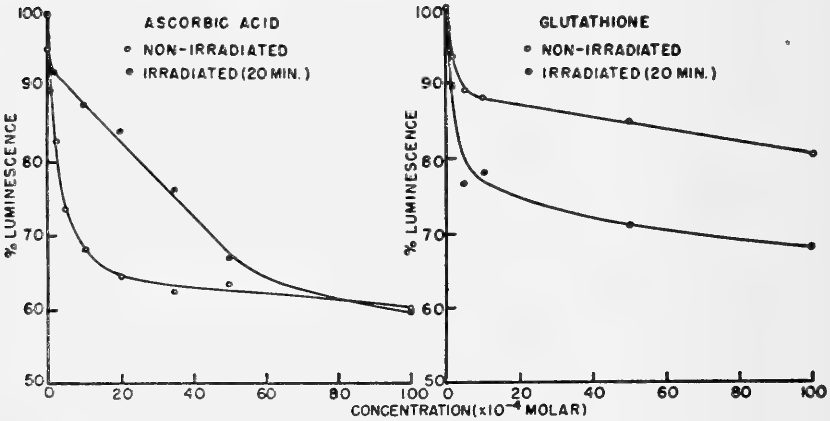

FIGURE 2: EFFECT OF CONCENTRATION ON THE X-RAY INDUCED CHANGES IN THE LUMINESCENCE OF BIOLOGICAL COMPOUND SOLUTIONS (DOSE RATE ~ 1.1×10^5 r / MIN.)

If the luminescence change is to be attributed to the decomposition of the solute by the x-rays, one would expect the luminescence curve changes to follow closely the decomposition curve. This did not seem to be the case. The results of parallel studies on the change in luminescence and in solute concentration of the solutions after x-irradiation are presented in Figure 3. The values for the observed changes do not coincide at the same dose. Ascorbic acid, after 500,000 r of 50 kv x- irradiation, showed a luminescence change of about 60%, whereas the change in the solute concentration was about 92%. The same trend, only on a smaller scale, was observed for glutathione. After an extended period of irradiation, both the luminescence and the concentration showed a 100% change.

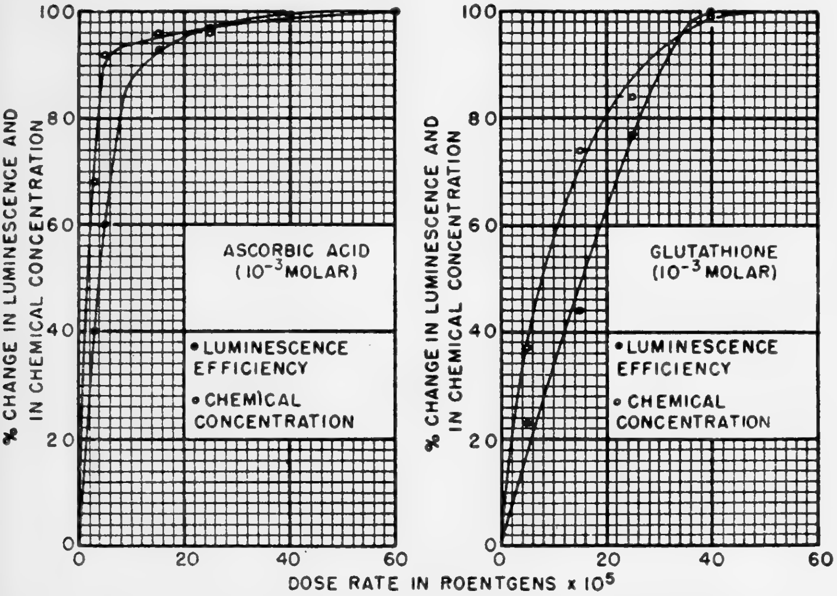

FIGURE 3: PARALLEL STUDY OF CHANGE IN LUMINESCENCE AND IN CHEMICAL CONCENTRATION AFTER 50 KV X-IRRADIATION.

The complexity of the problem is indicated by Maxwell's experiments with glycine solution (7). In his investigations, glycine solutions irradiated with 50 kv x-rays produced primary decomposition products which undergo secondary reactions. In the course of these reactions, the original simple one-solute system was changed into a several-solute complex system. The differences observed in the parallel studies on luminescence and solute concentration changes in x-irradiated biological compound solutions indicate the importance of considering the role of the radiation-induced decomposition products in energy transfer processes and in the overall effect in irradiated biological systems.

SUMMARY

Ascorbic acid solutions showed an increase in the luminescence after x-irradiation, while irradiated glutathione and tryptophane solutions showed a decrease. Ascorbic acid, after 500,000 r of 50 kv x-irradiation, resulted in a 60% change in luminescence, whereas the corresponding change in solute concentration was 92%. The same trend, only on a smaller scale, was observed for glutathione. These observed differences indicate the importance of the radiation-induced decomposition products when discussing energy transfer processes and overall effects in irradiated biological systems.

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The following resolution has been presented by the Resolutions Committee: "Be it *Resolved*, That the Kentucky Academy of Science expresses its sincere appreciation in tribute to the late Austin Ralph Middleton for his long years of service as the Academy's representative to the AAAS."

A change has been made in the plans for the 1957 Spring Meeting of The Kentucky Academy of Science. Registration will take place at the Paul L. Garrett Student Center, Western State College, Bowling Green, on April 26. The program for that day and the evening is scheduled on the campus of Western State College. The following day field trips and luncheon are planned for Mammoth Cave National Park.

Treasurer's Report

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	$1362.71
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News and Notes

Dr. Richard H. Wiley, Chairman of the Chemistry Department, University of Louisville, has been awarded a National Science Foundation Senior Post-Doctoral Fellowship for the academic year 1957-58. He will study in the Imperial College of Science and Technology, London, England, under the sponsorship of Dr. R. P. Linstead, Rector of the College. Dr. Wiley will be doing research on the synthesis and geometric isomerism of certain unsaturated acids which are of interest as intermediates in the biosynthesis of cholesterol.

Dr. Roger W. Barbour of the Zoology Department, University of Kentucky, will spend the next two years as a visiting professor at the University of Indonesia. This arrangement was made possible through the cooperation of the University of Kentucky and the ICA.

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The TRANSACTIONS OF THE KENTUCKY ACADEMY OF SCIENCE is a medium of publication for original investigations in science. Also as the official organ of the Kentucky Academy of Science, news and announcements of interest to the membership are published therein. These include programs of meetings, titles, abstracts of papers presented at meetings, and condensations of reports by the Academy's officers and committees.

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TRANSACTIONS of the KENTUCKY ACADEMY of SCIENCE

Official Organ
KENTUCKY ACADEMY OF SCIENCE

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Some Epigean Isopods and Amphipods from Kentucky*

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INTRODUCTION

ISOPODS AND AMPHIPODS from Kentucky have been treated by several authors, such as Packard (1871) and Hay (1902), and original descriptions of a few species have been based on material collected from that state. Many of the older papers were based on data from relatively hurried collecting trips, often to the Mammoth Cave region, and, furthermore, are now of limited value for purposes of identification. However, the papers of Hubricht (1943) and Hubricht and Mackin (1949) have served to clarify many of the taxonomic details. Nevertheless, there are still many important distributional and systematic data to be published, particularly with respect to the isopod genus *Asellus*.

Preliminary collections of fresh-water invertebrates near Louisville revealed an abundance of isopods and amphipods, and the present study, begun late in 1953, is based on samples of those malacostracans from more than 140 collections in which 85 stations were represented.

In as much as this is not intended as a comprehensive study of these two orders of Crustacea for Kentucky, there are certain limitations in the data: (1) the majority (72) of the collecting stations were in Jefferson County; (2) although there are several species known from Kentucky, the subterranean forms are not discussed; and (3) no collections were made during July or August. However, on the basis of reports in the literature, the collections probably include representatives of most of the epigean species that occur in the state. One noteworthy exception is *Crangonyx anomalus* Hubricht. Origi-

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nally named and figured from Kentucky specimens, this species has been collected from Fayette, Mason, and Bourbon Counties and may be restricted to waters of the Cincinnati Anticline (Hubricht, 1943).

ISOPODA

Asellus and *Lirceus*, the two genera of isopods represented in the collections, are readily separated by differences in the ventral surface of the abdomen. The two-segmented exopodites of the third pleopods cover the gills and almost the entire abdomen. Each exopodite has a transverse suture which marks the junction of the basal and distal segments. In *Asellus*, the line of the suture is only slightly oblique and divides the exopodite into two subequal parts (Fig. 1, A). In *Lirceus*, on the other hand, the suture is markedly oblique and extends diagonally from near the medio-distal corner to a point about midway on the lateral surface (Fig. 1, B). Apparently this character serves to distinguish the two genera in all instars.

Among females, only the first pair of pleopods is found anterior to the third pair; the second pleopods are wanting. Among the males, the first three pairs of pleopods are present. In mature males of the genus *Asellus*, the first two pairs (Fig. 1, C, D), although miniature when compared with the third pleopods, have become increasingly important in specific determination (*cf.* Mackin and Hubricht, 1938).

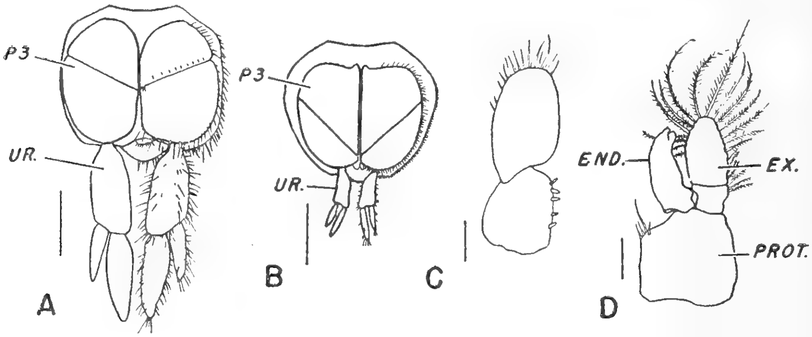

FIGURE 1

A. *Asellus militaris*, male: ventral aspect of abdomen and uropods. *P3*, 3d pleopod; *UR.*, uropod. Scale line, 2 mm. in **A** and **B**.

B. *Lirceus fontinalis*, male: ventral aspect of abdomen and uropods. Symbols as in **A**.

C. *Asellus intermedius*, male: 1st pleopod. Scale line, 0.2 mm. in **C** and **D**.

D. *Asellus intermedius*, male: 2d pleopod. *END.*, endopodite; *EX.*, exopodite; *PROT.*, protopodite.

Microscopic details of these structures certainly should be stressed in future taxonomic publications about the American species of *Asellus*. Unfortunately, adequately detailed figures of these appendages for the species discussed here are not available in the literature. However, other contrasting anatomical features of these pleopods, as listed in Table I, can be used to separate the males of the four species under consideration, but might be inadequate when additional species are considered. No detailed diagnoses have been attempted and all fine microscopic details have been omitted.

TABLE I. DIFFERENTIAL CHARACTERISTICS FOR THE FIRST TWO PLEOPODS IN THE MALES OF FOUR EPIGEAN SPECIES OF *Asellus* FOUND IN KENTUCKY.

	First Pleopod	Second Pleopod
A. brevicaudus	Broadly truncate with long plumose setae.	Protopodite 3 times as long as either endopodite or exopodite.
A. intermedius	Broadly truncate with bristles.	Protopodite subequal in length to the exopodite; exopodite and endopodite subequal in length; tip of endopodite bifid.
A. militaris	Tip narrow & rounded with short bristles.	Protopodite shorter than exopodite; exopodite extends one-third or more of its length beyond endopodite; tip of endopodite curved outward and bluntly rounded.
Undescribed species	Broadly truncate with bristles.	Protopodite and exopodite subequal, or protopodite longer; exopodite extends about one-third of its length beyond endopodite; tip of endopodite tapered and narrow; endopodite with basal extensions.

Asellus brevicaudus Forbes

This small isopod is characterized by stubby uropods which are about half as long as the abdomen. The distal segment of the male first pleopod bears several plumose, terminal setae, slightly shorter than, or subequal in length to the segment (not clearly figured by Richardson, 1905: 424). In the other species listed here, the segment is tipped with shorter "bristles" (Fig. 1, C).

The protopodite of the second pleopod is about three times as long as either the endopodite or the exopodite. The differences in the relative lengths of these segments are not great in the other Kentucky species (Fig. 1, D).

A. brevicaudus was collected from Harrod's Creek (Oldham Co.), Goose Creek (Jefferson Co.), and from the stream that issues from the mouth of Morgan Cave in Otter Creek Park (Meade Co.). A pair taken on February 18, 1955, remained coupled for 48 hours after being taken into the laboratory. Gravid females were encountered during May of the same year.

Asellus intermedius Forbes

A. intermedius (Fig. 1, C, D) is different from the following two species in that the exopodite and endopodite of the second pleopod are subequal in length, and the tip of the endopodite is bifid. In those other two forms at least one-third of the exopodite extends beyond the distal surface of the endopodite.

This species, common in springs and streams, was not collected from temporary ponds or from the shallow stream headwaters. Pairing was observed in late February, and gravid females were found in March, April, and May.

Asellus militaris Hay

In *A. militaris* (Fig. 1, A) the tip of the endopodite of the male second pleopod curves outward and is bluntly rounded, whereas in the following form it tapers to a narrow distal extremity, and in *intermedius* it is distinctly bifid (Fig. 1, D). Another contrast exists with respect to the first pleopod: in *intermedius* (Fig. 1, C) and the following form, the terminal segment is broadly truncate whereas in *militaris* the distal tip is narrow and rounded.

This isopod is an inhabitant of temporary ponds, and is almost completely restricted to such habitats. The only exception in these collections was some specimens taken from Mill Creek, Jefferson County, during November. Mill Creek is located near several temporary ponds by the Ohio River. It is quite possible that the asellids gained access to the creek from one of these ponds during periods of high water.

Many males carrying females were found under the ice on December 26, 1954. Pairing was also observed during the first five months of the year, and gravid females were taken in collections between early January and the end of April.

Asellus sp.

This species is known to Mackin and Hubricht (personal communication), who have selected a name for it and have many data on its distribution and morphology.

In addition to the comparisons made above, the presence of conspicuous basal extensions (one lateral, one medial) on the endopodites of the second pleopods serves to separate males of this form from *militaris* and *intermedius*.

Several specimens were collected from one short stretch of the Middle Fork of Bear Grass Creek, Jefferson County. In that area the water was slow-moving, and the bottom was muddy and strewn with dead leaves. There were gravid females in the late-winter collections.

Lirceus fontinalis Rafinesque

L. fontinalis (Fig. 1, B) is probably the most abundant aquatic malacostracan in Jefferson County. It lives in a wide variety of habitats and was present at 85% of the collecting sites. Although it is extremely common in intermittent streams and ditches, and a few were found in temporary ponds with *Asellus militaris,* it did not occur in relatively large numbers in permanent ponds. It was the only isopod collected from the shallow, rocky, upper reaches of the creeks in Jefferson County where *Asellus intermedius* frequently could be found farther downstream.

Reproduction usually takes place during the spring, but a few gravid females were collected each year during November and December.

AMPHIPODA

At least six species of amphipods, referable to the four genera, *Crangonyx, Gammarus, Hyalella,* and *Synurella,* were collected from surface waters in and around Louisville.

Each of these genera is rather easily identified by external morphological characteristics as shown in Table II. In *Hyalella* the antennules (first antennae) are markedly shorter than the second antennae, but in the other three genera they are longer. This characteristic, together with the presence of an uncleft telson serve to identify *Hyalella.* In some of the available keys to the fresh-water Amphipoda (*e.g.* Weckel, 1907; Ortman, 1918; Mackin, 1941; Pennack, 1953) the presence or absence of an accessory flagellum arising from the third segment of the first antenna is of fundamental importance. In the genera discussed here this flagellum is absent only in *Hyalella.* However, it can not always be observed readily in *Synurella* and *Crangonyx* because it is somewhat rudimentary and held closely against the 4th segment (Fig. 2, A). In *Gammarus* the accessory flagellum consists

TABLE II. DIFFERENTIAL MORPHOLOGICAL CHARACTERISTICS WHICH SERVE TO DISTINGUISH BETWEEN FOUR GENERA OF EPIGEAN AMPHIPODA FOUND IN KENTUCKY.

	Hyalella	*Gammarus*	*Synurella*	*Crangonyx*
Antennae	First pair shorter than second.	First pair longer than second.	First pair longer than second.	First pair longer than second.
Accessory flagellum	Absent.	Large and conspicuous.	Small and inconspicuous.	Small and inconspicuous.
Telson	Uncleft.	Cleft.	Cleft.	Cleft.
Abdominal terga	Some with median spines caudally directed. Conspicuously imbricated.	Without spines. Imbrication not conspicuous.	Without spines. Imbrication not conspicuous.	Without spines. Imbrication not conspicuous.
Third Uropods	Slightly longer than telson; smaller than first two uropods.	Much longer than telson; slightly longer than the second uropods.	Shorter than telson; smaller than first two uropods.	Longer than telson; smaller than first two uropods.

of more segments and is quite readily noted because its tip extends well beyond the distal margin of the 4th segment (Fig. 2, B).

A simple method for identifying the genus at hand is based on observation of the lateral aspect of the abdomen and its appendages. The last abdominal segment bears the telson as well as the third (last) pair of uropods, and relative lengths of these two structures are of diagnostic value. In *Gammarus* the last uropod is much longer than the telson, and is clearly biramous (Fig. 2, C). Furthermore, the third uropod is not markedly smaller than the first two, as is the case is the other genera. It is actually larger than the second.

In *Synurella* the telson is usually slightly longer than the third uropod (Fig. 2, D), but in *Crangonyx* (Fig. 2, E, F) and *Hyalella* the reverse is true. The endopodite of the last uropod of *Crangonyx* is small, when compared to that of *Gammarus,* and in *Synurella* it is so rudimentary that the appendage appears uniramous. The third uropod is uniramous in *Hyalella.* Furthermore, in *Hyalella* the posterior margins of some abdominal terga bear single, median points which project caudally. These give the dorsal margin of the abdomen an imbricated appearance, not shown so conspicuously in the other genera.

The three species of *Crangonyx* can be separated by the characteristics given in Pennak (1953: 445) or Hubricht and Mackin (1940).

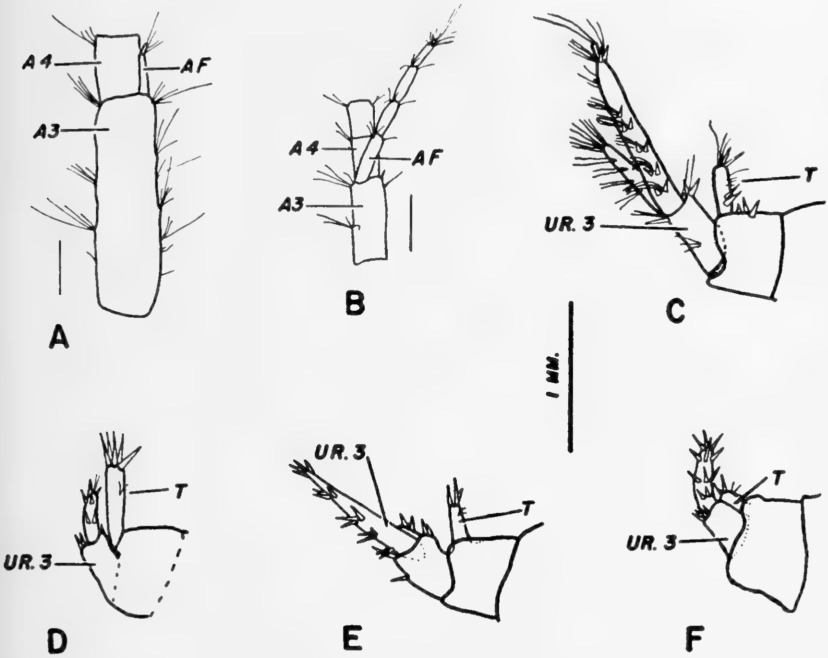

FIGURE 2

A. *Synurella dentata,* male: portion of first antenna. *A3,* 3d antennal segment; *A4,* 4th antennal segment; *AF,* accessory flagellum. Scale line, 0.2 mm.

B. *Gammarus minus,* male: same as in **A.**

C. *Gammarus minus,* male: right lateral aspect of last abdominal segment and its appendages. *T,* telson; *UR. 3,* 3d uropod. Scale line, 1 mm., applies to **C, D, E, F.**

D. *Synurella dentata,* male: same as in **C.**

E. *Crangonyx obliquus,* female: same as in **C.**

F. *Crangonyx shoemakeri,* female: same as in **C.**

Because immature species of *gracilis* and *shoemakeri* resemble one another rather closely, adult females are the best for purposes of identification. *C. obliquus* superficially resembles *anomalus,* but is separated easily from *gracilis* or *shoemakeri* (for a good description of *C. anomalus* see Hubricht, 1943). *C. obliquus* was originally described as *Eucrangonyx obliquus* by Hubricht and Mackin (1940) and as *Crangonyx richmondensis* by Ellis (1940). Both papers contain useful figures for identification.

Crangonyx gracilis S. I. Smith

This widely distributed species ranked third in frequency among the amphipods in the collections, although large populations were never found. It was taken from a variety of habitats, including permanent ponds where the other animals discussed in this paper were rarely present. Most collections from temporary ponds and springs, and many from streams contained a few individuals of this species. Gravid females were noted from late February until the latter part of April.

Crangonyx obliquus (Hubricht and Mackin)

C. obliquus (Fig. 2. E) was collected from seven stations (Jefferson Co., 6; Oldham Co., 1). Its typical habitats were springs, spring brooks, and temporary ponds. Gravid females were taken between the early part of February and the middle of April.

Crangonyx shoemakeri (Hubricht and Mackin)

Large populations of this species (Fig. 2, F) were found on several occasions in intermittent, grassy ditches, temporary pools, and the more mature sections of the local creeks. It was second in frequency of occurrence among the Amphipoda collected. The peak of its reproductive activity in Jefferson County was reached in March and April, although gravid females were encountered from late December until the middle of May.

Gammarus minus Say

This species (Fig. 2, B, C) was found in large numbers in the stream issuing from Morgan Cave in Otter Creek Park, Meade County, where the males were carrying females on December 31, 1956. None were taken from within the cave, although just outside they were abundant. A few were found also in Goose Creek, Jefferson County.

Synurella dentata Hubricht

S. dentata (Fig. 2, A, D) was the most frequently encountered amphipod during the collecting period. It is an inhabitant of streams and springs, but was absent in the temporary and permanent pond samples. One of the largest populations was found in thick masses of water cress in a spring brook. Males were observed carrying females in both the autumn and spring.

A commonly observed phenomenon was the presence of *Lirceus fontinalis* and *Synurella dentata* in the shallow, rocky headwaters of the creeks where other isopods and amphipods usually did not occur.

Hyalella azteca (Saussure)

This well-known species seems to be very rare in Jefferson County. It was found in only one station, a permanent pond situated in the flood plain of the Ohio River. This pond is flooded by the river almost every spring. The scarcity of this amphipod may be due to the relatively few ponds here which have extenseve weed growths.

DISCUSSION

The generalization has been made that usually no more than one species of aquatic isopod is found in any particular habitat (Hatchett, 1947; Pennak, 1953). However, these Kentucky collections, which contain so many of the locally abundant and euryokous *Lirceus fontinalis,* do not bear this out. *Lirceus* was found living with all the other species of Isopoda, and with all the Amphipoda except *Hyalella. Asellus militaris* was not observed with other members of its genus because of its almost complete restriction to temporary ponds. Furthermore, the undescribed species of *Asellus* shared a habitat only with the ubiquitous *Lirceus.* However, *Asellus intermedius* and *A. brevicaudus* were taken together in at least one stream collection. Thus, in these Kentucky samples, it appears that the association of two species of *Asellus* in one habitat is rare, but *Lirceus* coexists with all four.

In the amphipods, as might be expected, when different species were present at a collecting station, one was markedly predominant. In the majority of the stream samples *Synurella* was most abundant, but in springs, grassy ditches, and most of the spring brooks, a species of *Crangonyx* (usually *shoemakeri*) outnumbered it. In certain habitats two or more species were found living together, whereas in different ecological niches a similar association was not seen. Thus, *Synurella* occurred with three species of *Crangonyx* in brooks and springs, but was absent in temporary ponds. Neither *Hyalella* nor *Gammarus* were found with other amphipods, but since they were collected so infrequently, little significance can be attached to this phenomenon. However, it may well be, that in this region, with its relatively rich and diversified amphipod fauna, *Hyalella* and *Gammarus* are restricted in distribution by competition with the other forms.

Van Name (1936) described *Asellus communis* as "the most abundant and widely distributed fresh-water isopod in the eastern half of the United States." Now, more than twenty years later, it is apparent that the *Asellus communis* referred to by Van Name applies to several distinct species. Still, it is the custom among biologists, not familiar with the Isopoda, to call any aquatic isopod "*Asellus,*" and furthermore, they are more than likely to call the amphipods in their collections, "*Gammarus*". Around Louisiville, Kentucky, at least, the probability that such identifications are correct is rather slight.

SUMMARY

1. Epigean species of aquatic isopods and amphipods were collected from 85 stations in Kentucky over a period of approximately three years. Most collections were made in Jefferson County.
2. The Isopoda, listed in order of frequency in the samples, were: *Lirceus fontinalis, Asellus intermedius, A. militaris, A. brevicaudus,* and an undescribed species of *Asellus.*
3. The Amphipoda, similarly listed, were: *Synurella dentata, Crangonyx shoemakeri, C. gracilis, C. obliquus, Gammarus minus,* and *Hyalella azteca.*
4. Some characters useful in identification, and certain ecological observations are presented.

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2-Propanol as an Electrolytic Solvent*

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A RECENT PAPER (1) from this Laboratory contains a discussion of the properties of solutions of some alkali metal and tetraalkylammonium salts in 1-propanol. All of the salts which were studied have dissociation constants in the range of 1-6 x 10^{-3}. The results provide evidence that bromides and sodium salts are more solvated and more dissociated than corresponding iodides and potassium salts respectively in 1-propanol. Also, in contrast to an earlier study (2), the additivity of limiting ionic equivalent conductances in 1-propanol was confirmed.

2-Propanol and 1-propanol have dielectric constants of 19.1 and 20.4 and viscosities of 0.0209 and 0.0193 poise respectively at 25°C. On the basis of these data, electrolytes should be slightly less conducting and less dissociated in 2-propanol; nevertheless, owing to the similarities in both the physical and chemical properties of these isomeric alcohols, pronounced parallelism in the behavior of electrolytes in these media should be manifested. Previous studies concerning the use of 2-propanol as an electrolytic solvent (2, 3) are brief and inconclusive. The objectives of this research, therefore, are to investigate more thoroughly the conductance behavior of electrolytes in 2-propanol and to compare the results for 2-propanol with those for 1-propanol.

EXPERIMENTAL

1. *Purification of Solvent.*—2-Propanol ('Baker Analyzed' Reagent) was refluxed overnight in contact with Activated Alumina (previously dehydrated by heating for several hours at 175°) and then was distilled through a 100-cm., vacuum-jacketed fractionating column packed with glass helices. The retained middle fractions had conductivities in the range of 1-3 x 10^{-8} ohm^{-1} $cm.^{-1}$ as well as other desired physical properties.

2. *Salts.*—The preparation and/or purification of the salts have been described previously (**4, 5, 6, 7**). The length and the temperature of the drying *in vacuo* of each salt to constant weight depended upon the nature of the salt.

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* Taken from a thesis submitted by Harry M. Smiley in partial fulfillment of the requirements for the degree of Master of Science.

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3. *Procedure and Apparatus.*—The dielectric constant of each of several fractions of 2-propanol was measured at a frequency of ten megacycles using the equipment and the procedure which have been described by Leader (**8**).

The viscosity of each of several fractions of the solvent was determined using size —50 routine Cannon-Fenske viscometers which were calibrated

TABLE I. EQUIVALENT CONDUCTANCES OF SOME SALTS IN 2-PROPANOL AT 25°C.

C x 10^4	Λ	C x 10^4	Λ	C x 10^4	Λ
(a)	KI	(b)	KSCN	(c)	NaI
1.223	20.22	2.936	18.82	3.510	17.77
2.728	18.24	5.188	17.09	7.106	16.11
4.215	16.89	10.70	14.62	10.32	15.10
6.045	15.70	14.98	13.44	15.87	13.95
8.306	14.61	21.87	12.15	22.22	12.88
10.15	13.90	29.04	11.22	28.40	12.15
(d)	NaSCN	(e)	Pr_4NBr	(f)	Pr_4NI
1.315	19.97	1.926	19.65	2.337	19.81
5.033	17.36	4.401	17.55	4.402	17.87
12.72	14.73	7.894	15.74	7.999	15.79
18.96	13.50	12.63	14.18	11.91	14.34
26.57	12.44	17.26	13.12	17.10	13.01
35.50	11.56	23.45	12.08	22.53	12.00
(g)	Bu_4NBr	(h)	Bu_4NI	(i)	Me_3PhNBr
1.545	18.56	1.101	19.99	2.541	17.85
2.795	17.29	2.036	18.59	4.569	16.02
4.767	15.91	3.191	17.32	8.434	13.89
6.671	14.92	4.652	16.14	14.15	12.10
9.217	13.94	6.231	15.17	18.78	11.16
11.30	13.30	7.976	14.28	24.79	10.24
(j)	Me_3PhNI	(k)	Me_3PhNO_3SPh	(i)	Et_3NHBr
0.4865	23.34	1.942	17.52	1.394	18.15
1.599	21.12	3.674	15.50	2.052	16.27
2.709	19.64	6.702	13.44	4.747	13.34
5.437	17.27	10.14	12.00	7.514	11.66
8.308	15.70	13.64	10.99	12.28	9.95
11.26	14.55	17.86	10.11	17.07	8.89
......	.....	......	.....	23.34	7.96
......	.....	......	.....	31.92	7.11
......	.....	......	.....	38.73	6.66
(m)	Et_3NHPi				
1.279	21.24				
2.465	19.31				
3.850	17.69				
4.985	16.71				
7.483	15.10				

Symbols Pr—propyl, Bu—butyl, Me—methyl, Ph—phenyl, Et—ethyl, Pi—picrate.

by the Cannon Instrument Company. Kinetic energy corrections were considered to be negligible.

The bridge, conductance cells, temperature bath and control, and the procedures involved in the preparations and transfers of solutions and in the measurements of resistances have been discussed in earlier papers (**4**, **5**).

In converting concentrations from a weight to a volume basis, it was assumed that the densities of the solutions were equal to that of the solvent. All weights were corrected to vacuum. The conductivity of a salt was obtained by subtracting the conductivity of the solvent from that of the solution.

The following data for 2-propanol at 25° were used in the calculations: density, 0.7807 g./ml.; viscosity, 0.0209 poise; dielectric constant, 19.1. The value for the density compares excellently with the value of 0.7808 g./ml. which has been obtained by several other investigators (**9**). The value for the viscosity is slightly greater than the values of 0.0206 and 0.0207 which have reported by Hovorka and Simms (**1**) and Brey (**10**) respectively. The dielectric constant of 19.1 which was obtained in this study is intermediate in comparison to the values of 18.0, 18.6 and 19.6 which have been reported by Åkerlöff (**11**), Larson and Hunt (**12**) and Berlinger and Rüter (**13**) respectively.

RESULTS AND DISCUSSION

At the beginning of this study, the conductance of potassium chloride in water at 25° was determined for several concentrations in the range of 1-25 x 10^{-4} N. The results showed agreement within 0.1 per cent with the widely accepted data of Shedlovsky, Brown and MacInnes (14) and therein indicated satisfactory experimental technique.

TABLE II. TEST OF THE APPLICABILITY OF THE ONSAGER EQUATION TO THE CONDUCTANCE OF ELECTROLYTES IN 2-PROPANOL AT 25°C.

Salt	Experimental Slope ($-S_E$)	Theoretical Slope ($-S_T$)	$100\frac{(S_E - S_T)}{S_T}$
NaI	219	94	133
NaSCN	250	96	161
KI	341	98	248
KSCN	380	101	276
Pr_4NBr	340	99	243
Pr_4NI	390	102	282
Bu_4NBr	295	95	211
Bu_4NI	380	98	288
Me_3PhNBr	425	99	329
Me_3PhNI	408	102	300
Me_3PhNO_3SPh	430	97	343
Et_3NHPi	525	104	409
Et_3NHBr	740	103	618

Corresponding values of the equivalent conductance, Λ, and the concentration in gram-equivalents per liter, C, for each of thirteen salts in 2-propanol are presented in Table I. The phoreogram (**15**) for each salt is catabatic. As shown by the data in Table II, the experimental slopes are 133 to 618 per cent numerically greater in comparison to the theoretical slopes calculated by the Onsager equation (**16**) which may be simplified as follows for a 1-1 electrolyte in 2-propanol at 25°:

$$1. \qquad \Lambda = \Lambda_o - [1.91\,\Lambda_o + 52.3]\,\sqrt{C}$$

Since this general pattern of behavior suggests that appreciable ionic association occurs in 2-propanol solutions, the Fuoss-Shedlovsky method (**17**) was selected for the analysis of the conductance data. The applicable equation, which permits the simultaneous evaluation of the limiting equivalent conductance and a dissociation constant, may be written as follows:

$$2. \qquad S\Lambda = \Lambda_o - \frac{Cf^2\,S^2\,\Lambda^2}{K\Lambda_o}$$

According to equation # 2, if a plot of $S\Lambda$ *versus* $Cf^2\,S^2\,\Lambda^2$ is linear, the value of the ordinate intercept corresponds to Λ_o, and the slope is equivalent to $-\dfrac{1}{K\Lambda_o}$, in which K is the dissociation constant. Utilizing successive approximations of Λ_o, this method produces a linear plot for each of the salts in 2-propanol. Data obtained from plots of equation # 2 are summarized in Table III.

TABLE III. DATA PERTINENT TO PLOTS OF THE FUOSS-SHEDLOVSKY EQUATION FOR SOME ELECTROLYTES IN 2-PROPANOL AT 25°C

Salt	Λ_o	K x 10^3
NaI	21.80	1.6
NaSCN	22.47	1.4
KI	23.65	0.75
KSCN	24.39	0.83
Pr_4NBr	23.57	1.0
Pr_4NI	25.16	0.75
Bu_4NBr	21.70	1.0
Bu_4NI	23.26	0.71
Me_3PhNBr	23.76	0.61
Me_3PhNI	25.37	0.74
Me_3PhNO_3SPh	23.20	0.47
Et_3NHPi	25.84	0.54
Et_3NHBr	25.11	0.26

The values of the dissociation constants for salts in 2-propanol are 2 to 4 times less than those for corresponding salts in 1-propanol (**1**). This is expected since the dielectric constant of 2-propanol is slightly lower; nevertheless, whereas the dissociation constant may be 2 to 4 times different, the fractions of associated solute differ very little. Parallelism with the behavior of salts in 1-propanol is evidenced by the ions of small size and concomitant large charge density being more dissociated as a result of solvation by ion-dipole interactions. Apparently the ionization and dissociation of the triethylammonium salts are complicated with hydrogen bonding.

The data in Table III pertinent to the limiting equivalent conductances are both interesting and revealing. In contrast to the results of Hovorka and Simms (**2**), the expected additivity of ionic conductances is confirmed. For example, the difference between the conductances of potassium and sodium salts was found to be 1.85 ± 0.08 and 1.95 ± 0.09 ohm^{-1} cm.^{2} equiv.$^{-1}$ respectively for the iodides and thiocyanates; the difference between the conductances of corresponding iodides and bromides was found to be 1.59 ± 0.08, 1.56 ± 0.08 and 1.61 ± 0.08 ohm^{-1} cm.^{2} equiv.$^{-1}$ respectively for the Pr_4N^+, Bu_4N^+ and Me_3PhN^+ salts. Our value of 23.65 for Λ_0 for KI agrees excellently with the corresponding value of 23.64 reported by Hovorka and Simms; however, our value of 21.80 for NaI differs greatly from their corresponding value of 18.96. The explanation for this discrepancy is not obvious and, unfortunately, no other direct

TABLE IV. DATA PERTINENT TO LIMITING IONIC EQUIVALENT CONDUCTANCES IN 2-PROPANOL AT 25°C. AS DETERMINED BY INDIRECT METHODS

Ion	Method 1	Method 2	Method 3
SCN^-	14.4	14.3	14.5
I^-	13.7	13.6	13.8
Pi^-	12.8	12.7	12.9
Br^-	12.1	12.0	12.2
$PhSO_3^-$	11.5	11.4	11.6
Et_3NH^+	13.0	13.1	12.9
Me_3PhN^+	11.7	11.8	11.6
Pr_4N^+	11.5	11.6	11.4
K^+	10.0	10.1	9.9
Bu_4N^+	9.6	9.7	9.5
Na^+	8.1	8.2	8.0

Method 1—Based upon the limiting conductance-viscosity product for the Bu_4N^+ ion as 0.201 ohm^{-1} cm.^{2} equiv.$^{-1}$ poise. (**18**)
Method 2—Based upon the limiting conductance-viscosity product for the Pi^- ion as 0.267 ohm^{-1} cm.^{2} equiv.$^{-1}$ poise. (**18**)
Method 3—Based upon the assumed equality of the conductances of the Me_3PhN^+ and the $PhSO_3^-$ ions. (**19**)

comparisons are possible. Similar differences were observed when comparing the results for salts in 1-propanol (1). It is believed that the use of more soluble and carefully dried salts eliminated many of the potential experimental and extrapolation errors inherent in the results which were reported by the other investigators.

A direct proportionality does not appear to exist between the magnitude of Λ_0 and the viscosity of the solvent when comparing results for 2-propanol with those for 1-propanol. Whereas 2-propanol is 8 per cent more viscous, salts are characterized by limiting equivalent conductances in 2-propanol which are 3 to 9 per cent less than those in 1-propanol. Varying extents of solvation of the smaller ions probably account for this behavior.

It is generally believed that large ions having concomitant small charge densities are essentially nonsolvated in solution. Several such ions are the tetra-n-butylammonium, the picrate, the trimethylphenylammonium and the benzenesulfonate ions. The limiting equivalent conductance—viscosity products of these ions are used frequently to determine ionic limiting equivalent conductances indirectly. Data determined in this manner are presented in Table IV. It is interesting to note that the results which were obtained by the three methods agree very well; nevertheless, it should be realized that these are approximation methods and may be accurate within perhaps only five per cent.

In reviewing the objectives of this study, virtually complete parallelism has been observed for the conductimetric behavior of electrolytes in 2-propanol with respect to that previously reported for 1-propanol. Also, in conformance with predictions, electrolytes are slightly more associated and less conducting in 2-propanol as a result of its lower dielectric constant and greater viscosity.

SUMMARY

1. The conductivity of each of 13 electrolytes in 2-propanol at 25° has been measured at several concentrations in the range of 1-35 x 10^{-4}N.
2. For each salt, the slope of the plot of Λ *versus* $\sqrt{C}$ is 1.3 to 6.2 times numerically greater than the slope predicted by the Onsager equation.
3. Values of the limiting equivalent conductances and dissociation constants which have been calculated by the Fuoss-Shedlovsky

method range between 21 and 26 ohm^{-1} cm.^2 equiv.^{-1} and between 0.2 and 1.6 x 10^{-3} respectively.

4. The most dissociated salts in 2-propanol contain ions which have the smallest crystallographic radii, but the largest effective sizes in solution as a result of ion-dipole solvation.
5. The additivity of limiting ionic equivalent conductances in 2-propanol has been confirmed.
6. Values of limiting ionic equivalent conductances have been determined by three indirect methods.
7. The results for solutions of electrolytes in 2-propanol show complete parallelism with those which have been reported previously for 1-propanol.

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Dipolar Ions in Nonaqueous Solvents. II. The Apparent Molal Volume of Sulfamic Acid in N,N-Dimethylacetamide, N,N-Dimethylpropionamide, N,N-Dimethylbutyramide, Dimethyl Sulfoxide and Water at 25°C. * †

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THE INITIAL PAPER in this series (1) presents dielectric increment data as the first supporting experimental evidence for the dipolar ionic (or zwitterionic) structure of sulfamic acid in solution. Sulfamic acid, which is known to exist in the solid state as a dipolar ion (2), is moderately soluble in N,N-dimethylacetamide, in N,N-dimethylpropionamide and in dimethyl sulfoxide forming stable solutions which are characterized by the almost complete absence of conducting species and by dielectric properties which are analogous to those exhibited by solutions of amino acids in water, therein indicating that sulfamic acid remains in the dipolar form after dissolution. This behavior necessitates the basicity or proton-accepting properties of the sulfamate ion to be greater than those of the three nonaqueous solvents which are named above. In contrast, owing to the greater basicity of water in comparison to that of the sulfamate ion, sulfamic acid is dissociated completely into solvated protons and sulfamate ions in aqueous solution (3).

Another source of evidence from solution supporting the existence of dipolar ionic species is electrostriction, that is, contraction due to electrostatic attractions between the solvent molecules and the ionized groups of the dipolar ion (4, 5, 6). The amount of electrostriction is determined by subtracting the apparent molal volume of the solute, Φ (V_2), from the calculated gram formula volume of the solute. The apparent molal volume of a solute in solution is very conveniently calculated using the following equation:

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$$1. \quad \Phi\ (V_2) = \frac{\dfrac{1000\ m\ M_2}{d} - \dfrac{1000}{d_o}}{m} = \frac{1000}{mdd_o}\ (d_o - d) + \frac{M_2}{d}$$

where $\Phi\ (V_2)$ is the apparent molal volume of the solute in ml. per mole, m is the molality (that is, the number of moles of solute per 1000 grams of solvent), M_2 is the gram formula weight of the solute, d and d_o are the densities of the solution and solvent respectively in g. per ml.

The objectives of the research described herein are to determine the apparent molal volume of sulfamic acid in N,N-dimethylacetamide (DMA), in N,N-dimethylpropionamide (DMP), in N,N-dimethylbutyramide (DMB), in dimethyl sulfoxide (DMSO) and in water, to ascertain the amount of electrostriction in each system, and to compare the results for the nonaqueous media with those for sulfamic acid in water and for amino acids in water.

EXPERIMENTAL

1. *Solute.*—Sulfamic acid which was obtained from the G. Frederick Smith Chemical Company having a purity assay of 99.98% was dried in a desiccator over anhydrous magnesium perchlorate prior to using without further purification.

2. *Solvents.*—DMA and DMP were synthesized similarly from the proper acid and dimethylamine as described in an earlier paper (**7**). DMB was synthesized by absorbing the proper weight of dimethylamine in a solution of butyryl chloride in benzene. After the dimethylamine hydrochloride was removed by filtration and treatment with a cold saturated aqueous potassium hydroxide solution, the DMB was recovered by fractional distillation. Each amide was rendered anhydrous by refluxing it for several hours in contact with pre-treated calcium oxide. The values of the physical properties of the retained fraction of the distillate in each case checked with corresponding data in the literature.

DMSO, which was donated by the Stepan Chemical Company, was rendered anhydrous also by refluxing it for several hours in contact with calcium oxide. Two fractional distillations at reduced pressure yielded a product having the desired physical properties.

Distilled water of good quality was available from a departmental supply.

Fisher Certified Reagent toluene and carbon tetrachloride were used without further purification as the inert displacement solvents for the determination of the density of solid sulfamic acid.

3. *Apparatus and Procedure.*—Five 25-ml. Reischauer pycnometers with ground-in glass stoppers were used for the determination of densities. The calibrations of these pycnometers were based upon the density of distilled water as 0.99707 g./ml. at 25°C.

Solutions were prepared on a weight basis with all transfers except those for the aqueous solutions being made in a dry box under a positive pressure of nitrogen. Weights which were used were compared against a set calibrated by the National Bureau of Standards. Necessary buoyancy corrections were applied.

The temperature of the contents of the pycnometers was maintained at 25.00 ± 0.02°C. using a Model S-84805 Sargent constant temperature water bath.

RESULTS AND DISCUSSION

The experimental and calculated data pertinent to the densities of solutions of sulfamic acid and to the apparent molal volume of sulfamic acid at various concentrations in the five solvents at 25°C. are presented in Tables I through V.

TABLE I. DATA PERTINENT TO THE DENSITIES OF SOLUTIONS AND TO THE APPARENT MOLAL VOLUME OF SULFAMIC ACID IN DMA AT 25°C

Molality	Density (g./ml.)	Φ (ml./mole)
0.1192	0.9432	40.2±2.4
0.2000	0.9478	39.4±1.4
0.3352	0.9556	38.4±0.8
0.4263	0.9604	39.1±0.6
0.5433	0.9671	38.4±0.5
0.6745	0.9743	38.5±0.4
0.7439	0.9782	38.2±0.4
0.9005	0.9865	38.5±0.3
1.0361	0.9936	38.6±0.3
1.0756	0.9957	38.6±0.3
1.2005	1.0023	38.6±0.2
1.3152	1.0080	38.8±0.2
	(Statistical mean)	38.8±0.4

TABLE II. DATA PERTINENT TO THE DENSITIES OF SOLUTIONS AND TO THE APPARENT MOLAL VOLUME OF SULFAMIC ACID IN DMP AT 25°C

Molality	Density (g./ml.)	Φ (ml./mole)
0.1426	0.9292	38.9±2.0
0.2645	0.9362	38.0±1.0
0.4069	0.9442	37.9±0.7
0.5239	0.9503	38.7±0.5
0.6462	0.9570	38.6±0.4
0.7131	0.9609	38.2±0.4
0.8742	0.9692	38.7±0.3
1.0118	0.9761	39.1±0.3
1.1679	0.9841	39.3±0.3
1.3076	0.9914	39.2±0.2
1.3439	0.9934	39.0±0.2
	(Statistical mean)	38.7±0.3

Table III. Data Pertinant to the Densities of Solutions and to the Apparent Molal Volume of Sulfamic Acid in DMB at 25°C

Molality	Density (g./ml.)	Φ (ml./mole)
0.1241	0.9124	40.1±2.4
0.2342	0.9182	41.0±1.3
0.3365	0.9241	39.4±0.9
0.5176	0.9341	38.9±0.5
0.5977	0.9386	38.5±0.5
	(Statistical mean)	39.6±0.9

Table IV. Data Pertinent to the Densities of Solutions and to the Apparent Molal Volume of Sulfamic Acid in DMSO at 25°C

Molality	Density (g./ml.)	Φ (ml./mole)
0.1015	1.1008	47.2±3.8
0.1969	1.1057	46.3±1.8
0.2979	1.1111	45.2±1.3
0.4007	1.1164	44.9±0.9
0.4771	1.1199	45.6±0.8
0.6362	1.1281	45.1±0.6
0.6771	1.1302	44.9±0.5
0.8055	1.1365	44.9±0.4
0.8790	1.1401	44.8±0.4
0.9890	1.1457	44.6±0.4
1.0563	1.1481	45.2±0.3
1.2904	1.1591	45.1±0.3
	(Statistical mean)	45.3±0.5

Table V. Data Pertinent to the Densities of Solutions and to the Apparent Molal Volume of Sulfamic Acid in Water at 25°C

Molality	Density (g./ml.)	Φ (ml./mole)
0.1054	1.0028	42.4±3.6
0.2096	1.0080	44.4±1.8
0.2855	1.0120	44.3±1.2
0.4068	1.0182	44.3±0.8
0.6237	1.0286	45.1±0.6
0.7111	1.0327	45.4±0.5
0.8057	1.0371	45.6±0.5
0.9122	1.0421	45.7±0.4
1.0282	1.0475	45.7±0.4
1.1999	1.0553	45.9±0.3
	(Statistical mean)	45.0±0.7

Equations of the form, $d = d_o + km$, have been deduced reproducing the density data for solutions of sulfamic acid in each solvent for concentrations not exceeding unit molality.

The results are summarized in Table VI. The data for the aqueous solutions coincide with those which appeared recently in a technical bulletin (**8**).

TABLE VI. PARAMETERS SATISFYING THE EQUATION, $d = d_0 + km$, FOR SULFAMIC ACID IN VARIOUS SOLVENTS AT 25°C

Solvent	d_O	$k \times 10^2$
DMA....................	0.9366	5.57
DMP....................	0.9212	5.52
DMB....................	0.9056	5.50
DMSO..................	1.0959	5.00
H_2O......................	0.9971	5.06

The accuracy of a value for the apparent molal volume of a solute is a function not only of the accuracy of the density data but also of the difference between the densities of the solution and the solvent as shown in equation No. 1. The maximum uncertainty in any given density value reported herein is estimated to be ± 0.0002 g./ml. When this magnitude of error is considered in the calculations based on equation No. 1, the values of the apparent molal volume have the corresponding inherent uncertainties which are indicated with the data in the tables. It is readily discernible that the accuracy of the value for the apparent molal volume of a solute increases about ten times as the molal concentration of the solute increases from 0.1 to 1.0. Since there are considerable variances of the values determined for the apparent molal volume, but all determined values appear to overlap, it appeared convenient to treat these data by a statistical method in order to determine an average value and an average error. This was accomplished by using the Student's-t distribution method (**9**) and the results appear with the other data in Tables I through V.

In order to determine the magnitude of electrostriction per mole of solute, it was necessary to know the gram formula volume of sulfamic acid which is calculated by dividing the gram formula weight by the density. Since the two literature values of 2.03 g./ml. (**10**) and 2.126 g./ml. (**11**) disagree, it was necessary to redetermine the density. Using toluene and carbon tetrachloride as inert displacement solvents, density values for sulfamic acid of 2.125 ± 0.001 and 2.127 ± 0.001 g./ml. respectively were obtained at 25°C. These agree excellently with the value of 2.126 g./ml. as reported by Cuprey (**11**). (It also seems worthwhile to mention that a maximum and reproducible value for the density can be obtained only if precautions are taken to remove entrapped air.) Using 2.126 g./ml. as the density, the calculated gram formula volume of sulfamic acid is 45.67 ml./mole.

From the data in Tables I, II and III it may be observed that statistically the apparent molal volume of sulfamic acid in DMA, DMP and DMB is constant and equal to about 39 ml./mole therein indicating an electrostriction of about 7 ml./mole in each of the amides. In contrast to this behavior, however, the apparent molal volume of sulfamic acid in water and in DMSO is equal within experimental error to the calculated gram formula volume and indicates no electrostriction.

The dielectric properties of solutions of sulfamic acid in DMSO in comparison to those for the amides (**1**) suggest that an intermediate amount of electrostriction, rather than none, might be expected for solutions of sulfamic acid in DMSO. However, as described very well by Bernal and Fowler (**12**), the volume of electrostriction is a function of the structure of the solvent and of the relative volumes of a given quantity of solvent molecules when existing in the role of solvent and when selectively orientated about a charged species as a result of electrostatic attractions. As evidenced by the high values for its density, dielectric constant, viscosity and boiling point, DMSO (**13**) has a closely-packed liquid structure as a result of dipole-dipole attractions since hydrogen bonding is not likely. Hence, a probable explanation for the lack of electrostiction for solutions of sulfamic acid in DMSO is that the volume of a given number of DMSO molecules is the same regardless of whether they exist as part of the solvent or as part of a dipolar ion—solvent dipole electrostatic unit.

The lack of electrostriction for aqueous solutions of sulfamic acid is somewhat surprising. As mentioned previously, sulfamic acid is dissociated completely into solvated protons and sulfamate ions (**3**). Owing to its large size and concomitant small charge density, the sulfamate ion probably is not solvated appreciably. The proton, however, is definitely solvated and, as reported by Weber (**14**), the process is accompanied by appreciable electrostriction. To account for the observed lack of electrostriction reported herein for aqueous solutions of sulfamic acid, it appears reasonable to postulate that the conversion of the dipolar ion into a proton and a sulfamate ion is accompanied by a volume increase which counterbalances the volume contraction resulting from the hydration of the proton.

Although most of the amino acids in water are associated with apparent molal volumes which increase with increasing solute concentration, Dalton and Schmidt (**15**) have reported data for several amino acids for which Φ (V_2) remains constant over the experimental concentration range. This latter behavior is analogous to our results for the apparent molal volume of sulfamic acid in each of the solvents.

For aqueous solutions, the average electostriction of dipolar amino acids is about 13.5 cc./mole (**5**) which is greater than that of —3.4 to 10.9 cc./mole for the alkali halides (**16**). In reverse nature, for DMA solutions, the electrostriction volumes for potassium thiocyanate (**17**) and sulfamic acid are 14 and 7 ml./mole respectively.

Aminoiminomethanesulfinic acid which exhibits a dielectric increment twice as great as that of sulfamic acid in DMSO at 25°C. (**1**) was not considered to be sufficiently soluble to make worthwhile a study of its apparent molal volume using the present experimental technique.

For an ideal study involving electrostriction phenomena associated with solutions of dipolar ions, it is desirable to use also an isomeric uncharged molecular species for comparison purposes, such as the combination of glycine ($H_3{}^+N—CH_2—COO^-$) and glycolamide ($HO—CH_2—CONH_2$) which have been used in aqueous solutions. However, no uncharged molecular isomer of sulfamic acid is known.

In conclusion, the phenomenon of electrostriction is very complex and is a function of several factors such as the nature of the crystalline lattice structure of the solute, the nature of the liquid structure of the solvent, and mechanisms and extents of solute-solvent interactions in solution. From the pattern of the results which have been obtained for sulfamic acid in the nonaqueous solvents, there exists an inverse relationship as expected between the dielectric increment and the apparent molal volume; however, no quantitative correlations between electrostriction and dipolar ionic structure are evident.

SUMMARY

1. The densities of solutions of sulfamic acid in DMA, DMP, DMB, DMSO and water have been determined at 25°C.

2. Equations have been deduced reproducing the density data for each system for concentrations up to unit molality.
3. The density of solid sulfamic acid at 25°C. has been determined using toluene and carbon tetrachloride as inert displacement solvents.
4. The apparent molal volume of sulfamic acid in each of the five solvents at 25°C. has been calculated from appropriate density data.
5. Within the estimated experimental error, the apparent molal volume of sulfamic acid in a given solvent does not vary with concentration.
6. For sulfamic acid in the nonaqueous solvents, there exists an inverse relationship as expected between the dielectric increment and the apparent molal volume; however, no quantitative correlations between electrostriction and dipolar ionic structure are evident.
7. Although sulfamic acid is dissociated completely into ions in aqueous solution, there is no evidence of electrostriction. A postulation has been made to account for this behavior.

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A Study of the Conductimetric Behavior of Electrolytes in an Equimolar Mixture of N-Methylacetamide and Tertiary Butyl Alcohol at 40° C.*

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THE CONDUCTIMETRIC BEHAVIOR of electrolytes in N-methylacetamide (NMA) has been discussed in earlier papers from this laboratory (**1**, **2**, **3**). NMA is characterized by a dielectric constant of 165 and a viscosity of 0.0302 poise at 40°. As expected for a system with an extremely high dielectric constant, the behavior of electrolytes in dilute NMA solutions corresponds very closely to that predicted theoretically by the Debye-Hückel-Onsager equation (**4**). Nevertheless, phoreograms (**5**) for uni-univalent electrolytes in NMA are slightly catabatic at concentrations above 0.005N. This may be the result of either ionic association or increasing viscosity of the solution with increasing concentration of the electrolyte, and the existing knowledge concerning the behavior of electrolytes in NMA is not sufficiently differential to adequately substantiate a preferential choice between the two factors. It is believed that studies involving binary solvent mixtures using NMA as one component will assist in better understanding or elucidating the role of NMA as an electrolytic solvent.

Solvent mixtures of tertiary butyl alcohol (TBA) and NMA, which contain at least 50 mole per cent of NMA, are characterized by dielectric constants which range from 45 to 165 and by viscosities which are essentially constant and equal to that of NMA (**6**). These mixtures represent very useful media for theoretical conductimetric studies in which the dielectric constant may be varied while maintaining an almost constant viscosity. TBA as the other component of a binary mixture with NMA is advantageous because its gram formula weight, and consequently the number of molecules of it per unit volume, does not differ appreciably from that of NMA. The principal

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objectives of the present study which is restricted to using the equimolar mixture of NMA and TBA as a solvent are as follows: (1) to obtain basic information concerning the behavior of electrolytes in a new and interesting medium, (2) to compare the results with those which have been obtained previously for NMA solutions, and (3) to obtain information concerning apparent relative or preferential solvation effects. Inasmuch as the previous NMA studies were made at 40°, the same temperature was selected for the present research.

EXPERIMENTAL

1. *Purification of NMA and TBA.*—The preparation and purification of NMA has been described in previous papers (**1**, **2**). Eastman Grade tertiary butyl alcohol was rendered anhydrous by ternary azeotropic distillation using benzene as the third component. The batches of NMA and TBA which were used in the preparation of the equimolar mixture were characterized with physical properties which were in excellent agreement with corresponding data which have been reported in the literature. The equimolar mixture had the following properties at 40°: conductivity, 3×10^{-7} ohm $^{-1}$ cm.$^{-1}$; density, 0.8463 g./ml.; viscosity, 0.0292 poise; and dielectric constant, 45.4.

2. *Purification of Salts.*—The three tetraalkylammonium bromides were synthesized from the proper tertiary amines and alkyl bromides. The crude products were recrystallized at least twice from suitable media. The melting points of the final samples of the salts were in excellent agreement with those which had been reported by other investigators. The purification of the other eleven electrolytes which were obtained commercially has been described previously (**1**, **2**, **3**).

3. *Apparatus and Procedure.*—These also have been discussed adequately in a recent paper (**7**).

RESULTS AND DISCUSSION

The conductance of each electrolyte in the equimolar mixture of NMA and TBA was measured for at least five concentrations in the range 1-123 x 10^{-4}N. Corresponding values of the equivalent conductance, Λ, and the concentration in gram-equivalents per liter, C, for each of the fourteen salts are presented in Table I.

Illustrative plots have been omitted inasmuch as the phoreogram for each salt is linear over the entire experimental concentration range. Data pertinent to the intercepts and the experimental and thoretical slopes are contained in Table II. The results indicate that all of the electrolytes are completely dissociated in the NMA-TBA equimolar mixture. Only in the cases of the tetraalkylammonium iodides is the experimental slope of the phoreogram numerically greater than that predicted theoretically by the Debye-Hückel-Onsager

TABLE I. EQUIVALENT CONDUCTANCES OF SALTS IN AN EQUIMOLAR MIXTURE OF NMA AND TBA AT 40°C

$C \times 10^4$	Λ	$C \times 10^4$	Λ	$C \times 10^4$	Λ
(a) KCl		(b) KBr		(c) KI	
1.095	17.03	2.414	17.82	3.583	19.07
3.124	16.82	6.983	17.50	10.42	18.68
6.140	16.62	14.73	17.16	19.30	18.35
11.16	16.36	25.75	16.81	33.01	17.98
16.87	16.13	41.03	16.44	46.20	17.67
21.17	16.00	62.50	16.05	71.20	17.27
(d) NaCl		(e) NaBr		(f) NaI	
2.864	16.11	4.451	16.90	3.073	18.28
7.855	15.79	12.25	16.51	8.051	17.96
16.88	15.43	23.65	16.13	17.68	17.59
29.20	15.08	40.22	15.72	28.86	17.27
47.88	14.65	63.11	15.32	49.08	16.82
67.27	14.29	84.84	15.00	60.14	16.63
(g) $\mathrm{Me_3PhNO_3SPh}$		(h) $\mathrm{Me_3PhNBr}$		(i) $\mathrm{Me_3PhNI}$	
1.904	18.01	2.743	19.40	2.819	20.63
6.200	17.68	9.728	18.94	24.43	19.52
14.07	17.25	22.40	18.43	52.24	18.71
23.54	16.89	39.36	17.94	81.66	18.11
45.81	16.31	66.62	17.38	122.8	17.44
		115.8	16.64		
(j) $\mathrm{NaO_3SPh}$		(k) $\mathrm{Pr_4NBr}$		(l) $\mathrm{Pr_4NI}$	
2.345	15.63	4.183	18.51	0.8870	20.00
5.814	15.36	11.65	18.09	3.452	19.67
10.23	15.12	24.82	17.60	6.900	19.40
20.24	14.72	50.80	16.95	14.88	18.93
32.44	14.36	81.07	16.38	24.24	18.53
49.72	13.97	122.7	15.79	40.72	17.98
(m) $\mathrm{Bu_4NBr}$		(n) $\mathrm{Bu_4NI}$			
1.168	17.40	1.863	18.53		
3.839	17.12	6.272	18.16		
8.835	16.80	12.57	17.75		
20.24	16.34	26.31	17.18		
52.02	15.52	41.52	16.69		
		55.67	16.34		

Bu-butyl
Pr-propyl
Me-methyl
Ph-phenyl

equation which may be written in general form for uni-univalent electrolytes as

$$1. \qquad \Lambda = \Lambda_0 - \left[\frac{8.204 \times 10^5}{(DT)^{3/2}}\ \Lambda_0 + \frac{82.42}{(DT)^{1/2}\eta}\right]\sqrt{C}$$

or preferably in a more simplified form for the equimolar NMA-TBA mixture at 40° as

TABLE II. TEST OF THE APPLICABILITY OF THE ONSAGER EQUATION TO THE CONDUCTANCES OF ELECTROLYTES IN AN EQUIMOLAR MIXTURE OF NMA AND TBA AT 40°C

Salt	Λ_0	Theoretical Slope (S_T)	Experimental Slope (S_E)	$\frac{(S_E - S_T)100}{S_T}$
KCl	17.33	−32.1	−28.7	−15
KBr	18.23	−32.5	−27.6	−15
KI	19.58	−33.2	−27.5	−17
NaCl	16.57	−31.7	−27.8	−12
NaBr	17.45	−32.1	−26.7	−17
NaI	18.76	−32.8	−27.8	−15
Me_3PhNO_3SPh	18.46	−32.6	−31.8	− 2
Me_3PhNBr	19.93	−33.1	−31.2	− 6
Me_3PhNI	21.20	−33.9	−34.8	+ 2
NaO_3SPh	16.07	−31.5	−30.0	− 5
Pr_4NBr	19.12	−33.0	−30.5	− 7
Pr_4NI	20.34	−33.5	−37.0	+10
Bu_4NBr	17.74	−32.3	−31.0	− 4
Bu_4NI	19.07	−32.9	−36.6	+11

$$2. \qquad \Lambda = \Lambda_0 - [0.483\ \Lambda_0 + 23.7]\ \sqrt{C}$$

Although this behavior may be considered to arise from a slight extent of ionic association, the continued linearity of the phoreograms for the iodides over a rather broad concentration range indicates that other factors may be operative such as the mutual cancellation of effects proportional to "C" and "C log C" which have theoretical basis but which are neglected in the limiting expression of the Debye-Hückel-Onsager equation (**8**).

The Shedlovsky rearrangement of the Debye-Hückel-Onsager equation (**9**) was utilized to evaluate Λ_0 directly from the individual values of Λ as shown below:

$$3. \qquad \Lambda'_0 \equiv \frac{\Lambda + 23.7\sqrt{C}}{1 - 0.483\sqrt{C}} = \Lambda_0 + BC$$

Inasmuch as the plot of Λ'_0 *versus* C for each salt was linear over the entire experimental concentration range, corresponding values of Λ'_0

TABLE III. DATA PERTINENT TO PLOTS OF EQUATION (3) FOR SOME ELECTROLYTES IN AN EQUIMOLAR MIXTURE OF NMA AND TBA AT 40°C

Salt	Λ_o	B	$B' = B/\Lambda_o$
KCl	17.36	33	1.9
KBr	18.35	50	2.7
KI	19.69	56	2.9
NaCl	16.64	28	1.7
NaBr	17.56	39	2.2
NaI	18.83	47	2.5
Me_3PhNO_3SPh	18.46	10	0.5
Me_3PhNBr	19.95	25	1.3
Me_3PhNI	21.19	0	0.0
NaO_3SPh	16.11	5	0.9
Pr_4NBr	19.19	21	1.1
Pr_4NI	20.31	−54	−2.6
Bu_4NBr	17.74	21	1.2
Bu_4NI	18.98	−40	−2.1

and C were analyzed by the method of least squares in order to evaluate Λ_0 and B. The resulting data from the plots of Λ'_0 *versus* C are summarized in Table III. The values of Λ_0 in this table differ approximately 0.1 unit from corresponding values in Table II; however, this is not uncommon and the values based upon equation No. 3 usually are preferred.

The constant differences in the limiting equivalent conductances of pairs of salts having anions or cations in common provide numerous confirmations of the Kohlrausch law of independent limiting ionic equivalent conductances. The differences also indicate, however, that the data for KI and Pr_4NBr may be slightly in error (about 0.4% high).

In comparing values for B in equation No. 3 for different salts in a given medium, it is best to normalize the B values by dividing them by the corresponding Λ_0 values. The resulting B′ values which are presented in Table III tend to exhibit certain regularities. For the alkali halides, the B′ values are $Cl^- < Br^- < I^-$; for the tetraalkylammonium halides, the B′ values are $Br^- > I^-$. The latter parallels the corresponding behavior which has been reported for aqueous solutions although the series for the alkali halides is reversed in comparison to aqueous solutions.

Using the trimethylphenylammonium benzenesulfonate approximation method (3, 10), the limiting ionic equivalent conductances in the equimolar NMA-TBA mixture have been calculated and the result-

TABLE IV. LIMITING EQUIVALENT CONDUCTANCES OF IONS IN AN EQUIMOLAR MIXTURE OF NMA AND TBA AT 40°C. BASED UPON THE TRIMETHYLPHENYLAMMONIUM BENZENESULFONATE APPROXIMATION METHOD

Cation	Λ_o^+	Anion	Λ_o^-
Me_3PhN^+	9.2	I^-	12.0
Pr_4N^+	8.4	Br^-	10.7
K^+	7.6	Cl^-	9.8
Bu_4N^+	7.0	$PhSO_3^-$	9.2
Na^+	6.9		

ing data are presented in Table IV. The relative series of anionic and cationic conductances also are discernible readily from the arrangement of the data within the table. The only change relative to comparable data for NMA solutions is that the limiting ionic conductances of the sodium and tetra-n-butylammonium ions are reversed.

At 40°C., the ratio of viscosity of NMA to that of the equimolar NMA-TBA mixture is 1.04. Consequently it would be predicted on the basis that the limiting equivalent conductance of a salt is inversely proportional to the viscosity of the medium that the ratio of the limiting equivalent conductance of a salt in NMA to that of it in the equimolar mixture would be 1/1.04 or 0.96. Quite unexpectedly, however, the ratio of the conductance of a salt in NMA to that of it in the mixture ranged from 1.11 to 1.21, or approximately 15-25% greater than would be predicted. This provides evidence that the effective sizes of the ions are greater in the mixed solvent probably as a result of selective solvent-electrolyte interaction involving electrostatic attractions between the ions and the dipoles of the alcohol or of depolymerized NMA. Additional evidence supporting increased sizes of the solvodynamic units in the mixed solvent arises from the slopes of the phoreograms for salts in the mixed solvent being numerically less than the theoretical slopes, whereas the reverse is true for NMA solutions. The sheath of orientated solvent dipoles about the ions in the mixed solvent apparently reduces interionic attractions thereby making an electorlyte more conducting at finite concentrations than calculated theoretically. The Onsager equation is a function of the physical properties (dielectric constant and viscosity) of the solvent and predicts on the basis of electrostatic and hydrodynamic considerations the decrease of Λ from Λ_0 at small but finite concentrations. It fails to take into account, however, the chemical nature of the solvent or phenomena which may arise from solvent-electrolyte interaction. On the basis of the relative conductance behavior of electrolytes in the equimolar NMA-TBA mixture and in pure NMA, the dissociating power of a solvent appears to be complex and involves other properties of the solvent in addition to the dielectric constant.

SUMMARY

1. The equivalent conductance of each of fourteen electrolytes in an equimolar mixture of NMA and TBA has been measured at several concentrations in the range of 1-123 x 10^{-4} N.
2. The phoreograms for the electrolytes are linear over the entire experimental concentration range and are anabatic with the exceptions of the three for the tetraalkylammonium iodides which are very slightly catabatic.
3. The data for all electrolytes were analyzed using Shedlovsky plots of Λ'_0 *versus* C.
4. The results indicate that the electrolytes are completely dissociated in the equimolar mixture.
5. Numerous confirmations of the additivity of limiting ionic equivalent conductances are discernible from the data for electrolytes having anions or cations in common.
6. In comparison to the results which have been obtained previously for NMA solutions, the electrolytes in the equimolar mixture are 15-25% less conducting than would be predicted on the basis of an inverse viscosity ratio.
7. Limiting ionic equivalent conductances have been approximated using trimethylphenylammonium benzene-sulfonate as the reference electrolyte.
8. The results also indicate that the ionic species in the equimolar mixture are more solvated than in NMA and that less interionic attraction occurs than predicted theoretically by the limiting expression of the Debye-Hückel-Onsager equation.

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ACADEMY AFFAIRS

News and Notes

The 1957 Spring Meeting of the Kentucky Academy of Science was held at Western State College and Mammoth Cave National Park on April 26 and 27. Members and guests registered at the Paul L. Garrett Student Center on the Western State campus on Friday, the 26th.

The program opened Friday afternoon with a talk on the birds of the Mammoth Cave area by Dr. Gordon Wilson. This was followed by motion pictures and a talk presented by Dr. William M. Clay.

Following dinner at the Manhattan Towers, Bowling Green, Mr. Preston McGrain, Assistant State Geologist spoke on "Geology of the Mammoth Cave Area." Mr. McGrain's speech was followed by a social hour.

Saturday morning was devoted to field trips in the Mammoth Cave National Park. Dr. Gordon Wilson led an early morning bird walk starting at 6:30. At 8 A.M. Mr. McGrain conducted a geology trip through the park, and Dr. Roger Barbour led a group interested in herpetology and mammalogy.

The planned portion of the meeting was concluded with luncheon at the Mammoth Cave Hotel, although many members remained in the park until Sunday.

Dr. Louis A. Krumholz has been appointed Assistant Professor of Biology at the University of Louisville, and field investigator for a study of the Ohio River. The University of Louisville has received a $27,000.00 contract with the Ohio River Valley Water Sanitation Commission, Cincinnati, to investigate the fish and other biotic resources of the river. Dr. Krumholz's duties will be concerned largely with this project, and he will work in cooperation with the states bordering the Ohio River.

On May 9, Dr. Willis King spoke at the University of Louisville. His lecture was entitled, "Looking Ahead in Fish Conservation," and was the concluding program in the 1956-57 series of Conservation Lectures sponsored by the Biology Dept. and the Tom Wallace Foundation of Conservation. Other lectures in the series were as follows:

October 26, 1956—Justin W. Leonard: "Conservation in an Expanding Economy."

January 24, 1957—Ira N. Gabrielson: "Modern Trends in Conservation."

February 21, 1957—Stanley A. Cain: "Resource Use and Conservation in Brazil."

March 28, 1957—Clarence W. Watson: "Fish and Game Management in the South."

The forty-third annual meeting of the Kentucky Academy of Science will be held at Berea College on October 25th and 26th. The general theme of the Friday program is Research in Kentucky. The banquet on Friday evening will be followed by a talk by Dr. Donald M. Bennett, Chairman of the Physics Department, University of Louisville. Dr. Bennett will discuss the Geophysical Year.

Saturday morning will be devoted to the presentation of papers in the various section meetings of the Academy.

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Line drawings will appear as *text-figures,* while half-tones or other processes requiring paper inserts will appear as *plates.* Text-figures should be jet-black on white; shading may be indicated by stippling or lines but not by half-tone washes. Drafting should be carefully done (hand lettering generally is not satisfactory). Photographs for plates should have good contrast and be printed on glossy paper. Plates and text-figures are to be numbered consecutively and independently; on the back of each its number and the author's name should be written lightly in pencil. Each plate and text-figure must be referred to specifically in the text and must be provided also with a legend, the latter to be supplied as typed copy separate from the figures. Figures should be arranged into groups whenever possible and the legend for each group written as a separate paragraph. The amount of reduction desired should be indicated and should be consistent with the page dimensions of this journal. Indications of magnification should apply to the reduced figure.

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Official Organ
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Nutritional Studies with *Clostridium parabotulinum*

JEROME BULMASH AND R. H. WEAVER
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ALTHOUGH ROESSLER and Brewer (1946) and Mager, Kindler, and Brewer (1954) had published the results of recent studies of the nutritional requirements of *Clostridium botulinum,* a need for biochemical mutants necessitated a study of the requirements of strains that were available to us, in view of the common observation that strains of a bacterial species may differ in some of their requirements. During the course of our study, or subsequent to its completion, the results of similar studies were published (Kindler, Mager and Grossowicz, 1956; Campbell and Frank, 1956). Differences in findings indicate strain variation as well as variations in requirements with different growth conditions.

The strains of *Clostridium parabotulinum* used in our study were: t-3, a type A strain isolated by Hampson from a fatal case of botulism following a wound infection; 300, a type B strain from McClung's collection; 455, a type B strain from the Schoenholz and Meyer collection; and 510, a non-toxigenic strain obtained from Gunnison.

To test the adequacy of media to support growth of the various strains, tubes were inoculated with 3-mm. loopfuls of suspensions of cells in saline with turbidities equivalent to a number 3 McFarland nephelometer tube. The cell suspensions were prepared by centrifuging 24-hour brain heart infusion broth cultures, resuspending in saline, washing twice with saline, and diluting to the desired turbidity. Duplicate sets of tubes were incubated in a hydrogen jar and in a hydrogen jar in which 10 per cent of the hydrogen was replaced with carbon dioxide.

Good growth of all the strains was obtained in a medium contain-

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ing, per 100 ml. of distilled water: L-arginine, 300 mg.; L-glutamic acid, 100 mg.; DL-methionine, 70 mg.; DL-leucine, 150 mg.; DL-lysine, 120 mg.; DL-aspartic acid, 100 mg.; DL-threonine, 100 mg.; DL-serine, 100 mg.; L-tyrosine, 30 mg.; glycine, 50 mg.; L-tryptophan, 30 mg.; L-histidine, 20 mg.; DL-valine, 200 mg.; DL-alanine, 50 mg.; L-cystine, 10 mg.; L-proline, 50 mg.; DL-isoleucine, 50 mg.; DL-phenylalanine, 200 mg.; hydroxy-L-proline, 10 mg.; adenine, 1 mg.; uracil, 1.7 mg.; pyridoxamine, 50 µg.; riboflavin, 50 µg.; Ca-d-pantothenate, 100 µg.; thiamine, 40 µg.; biotin, 1 µg.; folic acid, 1µg.; pyridoxine, 50 µg.; nicotinic acid, 100 µg.; choline, 250 µg.; inositol, 4 mg.; p-aminobezoic acid, 1 µg.; glucose, 1 g.; mineral supplement, 1 ml.; and buffer, 4 ml. The buffer and the mineral supplement, except for the addition of 125 mg. of $CuSO_4 \cdot 5H_2O$ and 100 mg. of $ZnSO_4 \cdot 7H_2O$ per 250 ml. of supplement, were made by the directions of Boyd, Logan and Tytell (1948). The medium had a pH of 7.4.

An attempt to determine which of the ingredients in the medium were essential or stimulatory for growth was made by omitting single ingredients from the medium. The results are shown in Table I.

TABLE I. Single Omission Studies

Growth in synthetic medium Minus:	Strain of *Clostridium parabotulinum*			
	t-3	300	455	510
——————	4*	4	4	4
Lysine	1	3	4	1
Threonine	(3)†	(2)	(4)	(3)
Methionine	2	1	1	2
Leucine	1	1	1	1
Tyrosine	1	1	1	1
Glucose	1	1	1	1

*Growth of each strain in complete synthetic medium denoted as 4.
†Growth only in the presence of CO_2.

Omission of phenylalanine, valine, trytophan, arginine, isoleucine, cystine, biotin or thiamin resulted in no growth of any strain. Omission of glutamic acid, hydroxy-proline, serine, alanine, glycine, histidine, proline, aspartic acid, folic acid, pyridoxine, nicotinic acid, choline, inositol, p-aminobenzoic acid, pyridoxamine, riboflavin, Ca-pantothenate, adenine or uracil resulted in no reduction of growth of any strain.

As a check on the validity of this procedure for determining essential and stimulatory substances, a medium containing only the ingredients that had been found to be essential or stimulatory was tested. This medium did not support growth of any of the strains. The addition of pyridoxine allowed some growth, and the further addition of p-aminobenzoic acid increased the amount. Even this amount of growth was short of that obtained with the complete medium. Carbon dioxide would not replace threonine in this less complex medium. The finding that a medium made of substances that are shown to be stimulating or essential by single-omission studies will not support growth as well as a more complex medium is in accord with the results of previous investigators (*e.g.,* Mager, Kindler and Grossowicz, 1954; Kindler, Mager and Grossowicz, 1956).

The results of this study are compared with those of other workers in Table II. It appears that certain substances such as biotin, isoleucine, and phenylalanine may be essential for the growth of *C. parabotulinum* under all conditions. Arginine was shown to be essential in all the experiments reported in Table II. However,

TABLE II. Growth factors for *Clostridium parabotulinum*

Substances	This study	Roessler and Brewer (1946)		Mager, Kindler and Grossowicz (1954), Kindler, Mager and Grossowicz (1956)		Campbell and Frank (1956)	
		Type A	Type B	Type A	Type B	Type A	Type B
Biotin	N	N	N	N	N	N	N
Thiamin	N	U	U	N*	S*	N	U
P-aminobenzoic acid	U or S§	S	S	N*	N	N	U
Riboflavin	U	S	S	U	..	..	..
Niacin or Niacinamide	U	S	S	N*	U	U	U
Pyridoxine, pyridoxal or pyridoxamine	U or S§	U	U	N*	N*	U	U
Leucine	S	N	N	N	N	N	U
Isoleucine	N	N	N	N	N	N	N
Valine	N	N	N	N	..	N	N
Arginine	N	N	N	N	N	N	N
Phenylalanine	N	N	N	N	N	N	N
Tryptophan	N	N	N	N	N	N	U
Tyrosine	S	N	N	N	N	N	N
Methionine	S	N	N	N	N	N	U
Threonine	N	N	U	N	N	N	U
Lysine	S*	..	..	U	S†	U	U
Histidine	U	U	N	U	S‡	U	U

N—Necessary
U—Unnecessary
S—Stimulatory
†—Reduces requirement for arginine

..not mentioned, presumably unnecessary
*some strains
‡Glycine or serine plus histidine replaced threonine
§Dependent upon basal medium

Kindler, Mager and Grossowicz (1956) found that ornithine and lysine could partially replace arginine and that citrulline could replace it completely. Apparently other substances are essential for some strains but not for others. In addition, some substances may be essential or stimulatory in a comparatively simple medium although they may be without effect in a more complex medium, as shown in our experiments, and some substances may substitute for others. For example, it has been shown (Kindler, Mager and Grossowicz, 1956) that glycine or serine can substitute for threonine.

Results with cystine have not been included in Table II. Although we found it to be essential, it is possible that some of it was transformed into cysteine which may have acted as a reducing agent rather than as an essential amino acid. Burrows (1933) found cystine to be essential and Fildes (1935), and Elberg and Meyer (1939) used cystine in their media. All the more recent workers whose results are included in Table II used cysteine in their media, presumably as a reducing agent.

SUMMARY

The nutritional requirements of four strains of *Clostridium parabotulinum* have been studied. A complex synthetic medium has been found to yield good growth. Studies of the essentiality of substances in this medium and a comparison of the results with those of previous workers indicate that a few substances such as biotin, isoleucine and phenylalanine may be essential for the growth of *C. parabotulinum* under all conditions. Other substances may be essential or stimulatory for some strains but not for others, or in some basic media but not in others. Some substances may substitute partially or completely for others.

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Species of Plant-parasitic Nematodes in Kentucky*

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DURING RECENT YEARS the investigation of nematodes as causal agents of plant diseases has become quite intensive. Now it is known that several nematodes are the primary causal agents of diseases of various kinds of plants. Information concerning the occurrence of known and suspected plant-pathogenic nematodes in Kentucky has been accumulated by identifying the nematodes in soil and plant samples received at the Plant Pathological Laboratories in the Kentucky Agricultural Experiment Station and by occasional surveys of certain crop plants. This report is a summary of the information to date on the occurrence of species of these nematodes in Kentucky.

All the species listed are considered to be parasites of flowering plants. They may or may not have been proved to be the cause of economic loss in the situations in which they were found. Some of them are known to be pathogenic under certain conditions; others have not been evaluated so far as their capabilities as pathogens are concerned.

Identifications have been confined to the nematodes associated with cultivated plants and the sampling of kinds of plants and geographic areas has been limited. Specific identifications have not been made in several genera that have been encountered. For these reasons this is only a partial list of the plant parasitic species that occur in Kentucky.

PHYLUM NEMATODA: PHASMIDIA

FAMILY TYLENCHIDAE

Genus *Pratylenchus:* Root-lesion or meadow nematodes.

P. penetrans (Cobb) Sher and Allen has been found in the roots of strawberry (*Fragaria chiloensis* Duchesne.), corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), various vegetable and ornamental plants, red clover (*Trifolium pratense* L.), alfalfa (***Medicago sativa***

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* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

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L.), lespedeza (*Lespedeza* spp.), bluegrass (*Poa pratensis* L.), tall Fescue (*Festuca arundinacea* Schreb.), orchard grass (*Dactylis glomerata* L.), and other forage legumes and grasses, and small grains throughout Kentucky.

P. scribneri Steiner has been found in the roots of corn, strawberry and forage legumes and grasses in Grant, Henderson, Johnson, Pulaski, Russell and Wayne Counties.

P. brachyurus Godfrey has been found in strawberry roots in Grant County and *P. zeae* Graham was found in the roots of the same plant in Pulaski County.

Genus *Tylenchorhynchus:* Stylet nematodes.

T. claytoni Steiner has been found in association with the roots of strawberry, tobacco, corn, and forage legumes and grasses in Adair, Bell, Carter, Johnson, Knott, Laurel, Lawrence, Letcher, Martin, Pulaski, and Rowan Counties.

T. dubius (Bütschli) Filipjev has been found in association with the roots of strawberry and forage legumes and grasses in Grant, Hickman, Pulaski, Simpson and Woodford Counties.

T. maximus Allen has been found associated with the roots of tobacco and forage grasses in Fayette and Mercer Counties.

T. parvus Allen has been found in association with the roots of sorghum (*Holcus* sp.) and various ornamentals in Carroll and Jefferson Counties.

T. acutus Allen and *T. capitatus* Allen have been found associated with the roots of sorghum in Carroll County.

Genus *Hoplolaimus:* Lance nematodes.

H. coronatus Cobb has been found associated with the roots of many plants and is widespread throughout the state. It has been found most frequently in association with corn, small grains and forage legumes and grasses.

Genus *Helicotylenchus:* True spiral nematodes.

H. nannus Steiner has been found in association with the roots of corn, small grains, strawberry, tobacco, forage legumes and grasses and vegetables and ornamentals throughout the state.

Genus *Rotylenchus:* Spiral nematodes.

R. brachyurus Steiner has been found in association with the roots of *Clivea mineata* Lindl. in a greenhouse in Fayette County.

R. buxophilus Golden was found in association with the roots of barberry (*Berberis thunbergii* DC) in Fayette County.

FAMILY APHELENCHIDAE

Genus *Aphelenchoides:* Foliar nematodes.

A. ritzema-bosi (Schwartz) Steiner, the bud and leaf nematode, has been found in the leaves of chrysanthemum (*Chrysanthemum* sp.) from Fayette County.

FAMILY HETERODERIDAE

Genus *Heterodera:* Cyst nematodes.

H. trifolii Goffart, the clover cyst nematode, has been found in several instances in the roots of red clover, white clover (*T. repens* L.) and lespedeza (*Lespedeza stipulacea* Maxim.) in Fayette, Garrard and Hardin Counties.

H. glycines Ichinoe, the soybean cyst nematode, has been found in soil from a soybean field in Fulton County.

Genus *Meloidogyne:* Root-knot nematodes.

M. incognita (Kofoid) Chitwood, the southern root-knot nematode, has been found in the roots of vegetable and ornamental plants in home gardens throughout the state. It also occurs frequently in greenhouses. It has not been found frequently in farm land except in fields of vegetable crops. It has been recorded from ten fields of tobacco in Clay, Jessamine, Laurel, Madison, Magoffin and Wolfe Counties.

M. hapla, Chitwood, the northern root-knot nematode, has been found in the roots of strawberry throughout the state. It has also been found in the roots of vegetable and ornamental plants from home gardens in Fayette, Jefferson and Pike Counties and in the roots of red clover and alfalfa from Oldham, Shelby and Woodford Counties.

PHYLUM NEMATODA: APHASMIDIA

FAMILY DORYLAIMIDAE

Genus *Xiphinema:* Dagger nematodes.

X. americana Cobb has been found associated with the roots of tobacco, strawberry, vegetable and ornamental plants in home gardens and forage legumes and grasses throughout Kentucky.

DISCUSSION

The association of certain nematodes with certain plants does not necessarily mean the nematodes are strongly pathogenic. In many cases where such an association is recorded above, it is yet to be proved that disease situations occur, or, for that matter, that host-parasite relationships are involved. Only rarely are soil samples obtained from areas containing a single species of plant and it is quite possible that some of the nematodes found are remnants of a population that developed on a host raised previously in a crop rotation system.

Where nematodes, especially in larval stages, are found within the roots of plants there is a much greater probability that a host-parasite relationship exists and that the nematodes are pathogens.

Inferences about the distribution of these nematodes that are not now known to be widespread in Kentucky are highly suspect. For those that are widespread certain conclusions seem to be justified.

Meloidogyne incognita, the southern root-knot nematode, is widespread in home gardens throughout the state. However, it has been found very infrequently in farm fields except where vegetables have been raised. Roots of thousands of tobacco plants and hundreds of red clover and alfalfa plants from thousands of fields throughout the state have been examined over a period of several years for signs of various diseases and this nematode has been found only as listed above. These plants are all susceptible and the signs of the presence of this nematode are fairly obvious and would not often be overlooked. Therefore it seems reasonable to assume that this nematode is not present in large areas in the state. There is a possibility that it may be indigenous in some areas with light, sandy soils.

If it is not indigenous, it must have been introduced, and introduction readily occurs in the roots of vegetable transplants and other plants imported from the coastal southeastern states where the nematode is endemic. This introduction explains its widespread occurrence in home gardens, greenhouses and fields of vegetable crops, and probably also explains its occurrence in tobacco in several of the recorded cases, because, in these cases, the tobacco was raised in close association with vegetable gardens.

The experience of home gardeners shows that once the nematode has been introduced it can maintain itself as an economically important pest in the presence of a succession of susceptible plants as occurs in a vegetable garden. Because of the current increase in vegetable production with its increased importation of vegetable transplants, the same situation can develop rapidly in farm fields. Systems of crop rotation that contain economically valuable crops will provide a succession of susceptible plants in which the nematode can maintain itself.

Meloidogyne hapla, the northern root-knot nematode, will be found wherever strawberries are raised and it is readily disseminated in the roots of transplants. The signs of the presence of this nematode are frequently inconspicuous and can be easily overlooked. It has not been found in the specimens of tobacco mentioned above. Many legumes are susceptible to this nematode and it may prove to be more widespread in them than the known instances indicate.

Pratylenchus penetrans appears to be indigenous in large areas in the state as do *Helicotylenchus nannus* and *Hoplolaimus coronatus.*

SUMMARY

1. An annotated list of the known and suspected plant-pathogenic nematodes found in plant and soil samples and during surveys of certain crop plants in Kentucky is presented.
2. The nematodes are referable to three families, eight genera, and 17 species of the Phasmidia, and to one species of the Aphasmidia.
3. *Pratylenchus penetrans, Helicotylenchus nannus,* and *Hoplolaimus coronatus* are widespread throughout the state. Several other species appear to be much more restricted in distribution.

Accepted for publication 17 October 1957.

A New State and Size Record for the Spiny-tailed Fairy Shrimp, *Streptocephalus seali* Ryder

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THERE ARE NO published accounts of the occurrence of the spiny-tailed fairy shrimp, *Streptocephalus seali* Ryder, in Kentucky. Dexter (1953) listed records of this species from 15 states, Mexico, and Canada. Later he brought the known distribution up to date with the addition of six more states to the list (Dexter, 1956). The purpose of the present paper is to record, for the first time, the occurrence of *S. seali* in Kentucky, and to review our knowledge of Kentucky anostracans.

Dexter (1953) summarized the geographic distribution of the North American anostracans, but failed to make note of published records of two species from Kentucky. Garman (1920) described a new species, *Eubranchipus neglectus,* from specimens collected in "Bluegrass, Kentucky." Chambers (1874) was cited by Ward (1940) as having collected *E. vernalis* (Verrill) near Covington, Kentucky. In 1956 Dexter corrected those earlier omissions and, in addition, recorded *E. vernalis* from eight stations in Jefferson Co., Ky., on the basis of specimens sent to him by Dr. Gerald A. Cole of the University of Louisville. *E. neglectus* had not been reported since Garman's original description, but Dexter (1956) stated that specimens taken from Ohio, and now in the U. S. National Museum, have been determined as members of this species.

Streptocephalus seali was collected by the present author in a temporary pond two miles south-west of Rich Pond in Warren Co., Ky. This region of Kentucky is located in the Pennyroyal Plateau and exhibits a typical karst topography. Some of the numerous sinkholes of the region have subterranean connections, and contain waters which are mainly the result of overflowing subterranean channels. The depth of water in such sinkholes fluctuates with the subterranean water level. During the late autumn and early winter, the waters rise and overflow into surface sinks and valleys. The

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collection site was located in a valley of approximately 500 to 700 acres which filled with water of subterranean origin. In late spring and early summer this large expanse of water gradually receded to the underground channels. Much of the valley at that time of year was dotted by small, temporary ponds in the process of recession and evaporation. *S. seali* was collected in such a pond on June 16, 1957. The water temperature in that pond was 17° C. The pond was about eight feet across and three feet deep, and had a bottom composed of rocks and silt. All of the water had disappeared from this sinkhole by June 29, 1957.

Several other species typical of both subterranean and temporary-pond habitats were collected with the fairy shrimps. Various developmental stages of the leopard frog, *Rana pipiens* Schreber, and of the crayfishes, *Cambarus* spp. were collected along with several widely distributed temporary-pond plankters. Agassiz's cave-fish, *Chologaster agassizi* Putnam, uses both the subterranean and epigean habitats at this site, and was taken in the samples from the pond.

Moore (1951) published a new size record for *Streptocephalus seali,* a specimen measuring 42 mm. from the top of the head to the tip of the cercopods. The present author made similar measurements of seven of the specimens from Warren Co., Ky., with the following results:

Length of males in mm.	*Length of ovigerous females in mm.*
45.3	42.0*
44.6	40.5
43.0	38.5
41.0	

Therefore, a new size record for *Streptocephalus seali* is a male, 45.3 mm. in length. This specimen is on deposit with the other six in the Biology Department of the University of Louisville.

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* The egg sac of this female measured 8.5 mm. x 2.0 mm. and contained approximately 1,000 ova.

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A Bird's Inborn Navigational Device

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This paper presents an hypothesis to account, on a sensory basis, for the ability of birds to find their way, through unfamiliar territory, to their desired destination.

THE ARGUMENT

JUST AS ONE'S hair, while being brushed, becomes electrified, so also a flying pigeon's feathers, swept by the wind, acquire electrical charges (electrons). However, the two cases differ in one important respect: the charges on the hair remain essentially unmoved, but those on the feathers rush through space. Under this circumstance, a speeding electron on a feather may be regarded as an electric current. But, every electric current is always surrounded by its own magnetic field. Hence, all the magnetic fields accompanying the multitude of electric currents which arise from the translation of the electrons adsorbed on the feathers are in position, under certain conditions, to react with the earth's magnetic field. Such reactions tend to move the feather tips; and these movements, when transmitted to the quills, may be detected by the bird through the sense of feeling, and used as directional signals for navigational purposes.

EXPERIMENTS AND DISCUSSION

When a stream of air impinges upon an experimental pigeon in the laboratory, the feathers acquire a charge. The sign of the charge was determined by standard procedure: a gold leaf electroscope was constructed and partially charged from a vulcanite rod which had been rubbed with a woolen cloth; when the electrified pigeon then approached the negatively charged electroscope, the leaves diverged still farther, indicating a negative charge on the feathers. (Parenthetically, in this connection, it is suggested that free nitrogen of the air may have been the source of the electrons which charged the feathers, nitrogen molecules simultaneously becoming temporarily oxidized; this proposal is somewhat supported by the observation that

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a jet of commercially "pure" nitrogen from a steel cylinder appeared to equal, and possibly excel, air in charging an unattached pigeon feather; alternatively, the source may have been "free" electrons in the air.)

In order to reverse the conditions of the experiment just described, and learn if the feathers would become charged when the bird was rushed through still air—somewhat as in natural flying, many experiments involving simulated flight were undertaken. Most of these trials either failed to charge the feathers—especially those which operated from the side, top or front of a moving, steel-topped automobile, or were only mildly successful in this respect. Some of the causes of failure are believed to be: leakage of captured electrons directly or indirectly to ground, due partly to the humidity of the atmosphere; confinement of the experimental bird, during observation, in a cage or net, the restrainer itself appearing to acquire the charge; thus the possibility of accumulating electrons on the feathers became zero, because a charge cannot exist within a conducting shell (the humid atmosphere rendering the wood and silk better conductors).

However, by eliminating, as nearly as was thought practical, the interfering elements, entirely satisfactory results were finally achieved: after fastening the bird's feet together with a rubber band, the feet, tail feathers and wing tips were assembled and wrapped with several layers of dry silk cloth; the handler, then, with his neoprene-gloved hand, grasped the collected items just enumerated, and whirled the bird, at arm's length, about his head, the bird's estimated speed being approximately 10 mph. After swinging the pigeon for a minute or so, the treatment was stopped, and the electroscope, applied immediately, indicated a relatively strong charge over the whole area of exposed feathers. Had the bird moved at normal flying speed, say 40 mph, a much more intense charge could have been expected.

In homing trials, pigeon speeds up to 45 mph have been recorded (1). This figure (great circle distance divided by total time of flight) does not take into account the probability that the pigeon flew at times much faster (perhaps 60 mph), and at other times more slowly. The actual momentary velocities are important to this discussion, not only because the magnitude of the charge acquired by the feathers is doubtless a function of the bird's speed through the air (and charges acquired at high speed are retained somewhat at lower speeds), but also because a rate, such as the one just suggested, approaches the maximum velocity of travel (about 68 mph) which Rowland (2), in 1876, impressed upon his electric charge when he arrived at the memorable proof that an electric charge, during translation, constitutes essentially an electric current. With this in mind, one may say that each and every one of the multitude of electric charges (electrons) adsorbed on the feathers of a bird in flight is a true creator, delineator and bearer of an electric current through space.

The question arises, How may a bird in flight detect the reaction between the earth's field and those of the electric currents generated by the electrons on his feathers? The author is inclined to believe that this should be possible through the sense of feeling. Loosely speaking, the proximal part, or quill, of a feather is more or less fixed in position, with reference to the bird, but the distal part, which bears the barbs, is more free to move. One may reasonably assume that the electrons, which account for the charge acquired by the bird in flight, reside mostly on the barbs of the exposed parts of the feathers. Hence, because the feathers are epidermal outgrowths, and the quills are contiguous to the sensitive derma, any force, or torque, applied to the barbs will be transmitted through the shafts to the quills, and be noted by the bird.

One may now recall that the direction of the field (the earth's magnetic field in this instance), that of the current (the forward direction of flight) and the direction in which motion may occur in an electric motor may be symbolized, in three dimensions, with the forefinger, the second finger and the thumb respectively of the right hand. Then, when the reaction of the earth's magnetic field with the currents' magnetic fields tends to turn the distal part of the feathers through even an inconceivably small angle, the bird will note the "feel" at his surface nerve terminals over almost his entire body.

The following suggestions and remarks may help to clarify the problem under consideration. On a sheet of paper draw two straight lines at right angles to each other, and intersecting at their midpoints; label the extremities of the lines appropriately north, east, south and west respectively; also draw many arrows pointing south to indicate the direction (north to south) of the earth's magnetic force field.

We may now consider the question: What will be the effect, of a west heading, on a bird flying somewhere between the Tropic of Cancer and the Tropic of Capricorn—a region in which the earth's magnetic lines are more nearly horizontal than elsewhere? The Fleming's rule, as used above, will give the answer quickly. Over the prepared chart point the forefinger (field) south, and the second finger (current) west; then the thumb will point down, indicating that there will be a tendency for all charged feathers to be pressed down toward the earth. In a similar fashion, an east heading engenders a tendency for all feathers to rise up and away from the earth. We now have two signals for the bird to observe and correlate with direction. On a heading due North, there will be no lowering or elevating effects; the same is true when the course is due South. However, on a course which is not precisely North or South, for example, a SW course, i.e., one slightly West of South, the downward pressure, already referred to, begins to be effective; likewise, when turning SE, the lift will be noticed. Continuance of this line of reasoning for the other six headings to the right and left of the remaining three cardinal points would show how forces, up or down, fade out or come into being as the bird changes his course slightly. The author is inclined to suspect that migrating birds, while in flight, make it a practice to continually turn slightly off course, first to the right, then to the left, in order to detect the changing signals, and thus to assure themselves of their heading. Indeed, a correct but unchanging signal surely would become ineffectual in time due to its tiring action on the receiving nerve system. Perhaps the comments just made account for the winding paths of some migrating birds through the sky. Even automatic steering devices, whether controlling sea or air-borne craft, execute similar gyrations.

We are now in a position to remove the geographic limitations which were imposed earlier, and to consider bird migration into, or out of, the higher latitudes where the earth's magnetic lines may dip steeply. The solution of the navigational problem, arising when the route extends North and away from the regions of horizontal lines, will be met when the bird occasionally dips or dives momentarily with the sloping lines, and notes the signals; conversely, when the bird is in the North Latitudes, and is migrating South, it will be necessary, now and then, for him to rise sharply with the lines, and then resume his accustomed horizontal stance. However, the transition from one zone to another is so smooth and gradual that little difficulty need be anticipated.

To recapitulate, then, the fundamental principle of this type of bird navigation is conceived to be this: after the bird has decided on the direction to take, he starts—flying naturally (horizontally), and notes the strongest signal of that course; thereafter, his task is to so direct his heading that the character and strength of the signal will be maintained constantly; if he loses the signal, or needs to verify it, he may do so by turning momentarily to the right and left, or by dipping and rising briefly.

When studying this procedure, many may choose to employ, along with the Fleming's rule, the left-hand screw aid. This device can be made by marking the stopper of a plastic pill bottle "forward," and by inserting in the bottle a slip of paper bearing arrows which point in the direction a left-hand advancing screw would turn; the stopper, then, indicates the direction in which the current (the advancing bird) moves, and the arrows show the direction of the current's magnetic force field. Thus, for example, let a bird, in a northern region, where the earth's magnetic dip is considerable, fly West. The direction of the earth's field is up against the bird's lower right side; hence, the direction of the earth's lines will be contrary to those of the current's field on his lower right side (lowering the force); and the two sets of lines will be in unison on his upper left side (increasing the force at that point). Therefore, the net effect observed by the bird, will be a pressure down against his upper left side, tending to push the feathers down and somewhat to the north. This signal the bird must maintain.

Just as no simple compass can inform a mariner of the direction in which his home lies (unless he resides at one of the magnetic poles), so also it must be stressed that the usefulness of the bird's navigational device (a name conveniently shortened to "aviguide") begins after the animal has decided on his heading. Wild birds, summering in the far north, may know, from observations made on extensive flights about the countryside, which direction looks towards a warmer climate and longer days. But the domesticated homing pigeon, when released for making the homeward journey, must have other means for determining the starting direction. The author suggests that these birds depend upon the kinesthetic impressions of the outward journey. When this matter of heading has been settled—and he often miraculously appears to settle it in seconds—has aviguide will lead him home. (This view differs from the tenets of the well known hypothesis (3) which requires these impressions not only to tell the bird which way to start home, but to guide him on the way.) A point, favorable to the concept of kinesthesia, as here proposed, is the circumstance that birds have only two legs: hence, they should be supersensitive to changes occurring in the direction of travel when they are outbound from the home loft.

The present paper confines its attention quite exclusively to its own problem and, to avoid duplication of effort, makes no pretension of reviewing the many excellent papers that have already been published on the subjects of homing and migration; yet, there is on record one unique study which may be mentioned, not because of any close relation with the present paper, but due to its employment of the concept of magnetic field. Reference is made to Yeagley's (4) well-known work in which it was conceived that there exists some mechanism "even though man does not yet comprehend its full import," relating bird navigation to a gridwork of coriolis effect and the vertical component of the earth's magnetic field. Because of the interdependence of these two factors, the hypothesis could be strengthened by testing favorably one of the forces. The magnetic field was chosen for experimentation. Little magnets were ingenuously attached to the wings of one group of pigeons; others received nonmagnetic weights. Theory held that the magnets would interfere with the homing ability of the one group, while birds carrying no magnets would find their way as usual. Both groups of birds seemed to home equally well in the tests.

Many years ago, when work on bird navigation began at the University of Kentucky, the initial effort was devoted to an investigation of the possibility that the enigmatic pecten in a bird's eye was the seat of the "homing instinct." Special interest was attached to (a) the relatively large qualities of blood flowing anteriorly through the pectens, and to (b) the properties of the attendant streaming electric potentials which might be expected to develop as a result of the intermittent action of the heart. Naturally, considerable instrumentation, for detecting magnetic fields near the eye, characterized the management of this early work. Although this preliminary study was unsuccessful in its mission, it did lead eventually to the new outlook which was then developed, and is now reported in the present paper.

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SUMMARY

This paper, without invoking a new "sensory window" (1), presents what is believed to be a new approach to the "homing instinct" problem. It is based on the circumstance that a bird's feathers become charged negatively in flight; and the electromagnetic consequences of this observation suggest that a bird has an inborn navigational instrument, the supposed structure of which is given.

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Studies on Fresh-Water Bryozoa, XVIII
Lophopodella carteri in Kentucky

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THE PURPOSE of the present study is to report the occurrence of *Lophopodella carteri* (Hyatt) 1866 in Kentucky and to bring its distribution records up to date.

The present specimens were collected by William B. Echols, Biology student at the University of Louisville, and given to Dr. Gerald A. Cole who identified them as *L. carteri* and sent them on to the author for verification. To quote from information furnished by Dr. Cole, the specimens were collected about Sept. 20, 1957, "from an intermittent brook in George Rogers Clark Park, Louisville, Jefferson County, Kentucky. This brook is hardly more than a grassy ditch. The bryozoans were in an isolated remnant of the dried up stream. This was a small pool about 4 square feet and 6 inches deep. The colonies continued thriving in a finger bowl for several weeks in the lab." The sample consisted of three small but very handsome and well nourished colonies containing about 12 zoids and 10 nearly mature statoblasts.

The shallow, Kentucky habitat from which the samples were collected is reminiscent of the similarly shallow, vegetation-rich East Harbor, Lake Erie, Ohio site where specimens were exceedingly abundant in 1933 (Rogick, 1934a, 1934b).

Lophopodella carteri is very easy to identify by its spined statoblasts. According to Toriumi (1956: 42), who has made the most recent and critical taxonomic study of this species, *L. carteri* "may consist of many different groups . . .These groups may be small ones such as strains and may not be varieties." He then lists a number of synonyms, a few of which are listed below:

1859. *Lophopus.* Carter
1907. *Pectinatella davenporti.* Oka
1907. *Lophopus lendenfeldi* var. *himalayanus.* Annandale
1919. *Lophopodella carteri* forma *typica.* Annandale

On the basis of the above list of synonyms the distribution of *L. carteri* can be said to extend to three continents: Asia, Africa and

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North America. Carter found it at Bombay, India; Oka near Tokyo, Japan; Annandale, in various papers, reported it from eastern Persia, thru India, into China; Vorstman (1928) from Java; Rao (1929) from Burma; Lee (1936) from China; and Toriumi, in several papers, from a number of localities in Japan, Formosa and Korea. Meissner (1895) reported the species from East Africa. North American records for the species are few:—from New Jersey by Dahlgren (1934); and from various parts of Ohio by Rogick (1934a, 1934b) and Masters (1940). In addition, the writer has seen specimens from Pennsylvania and now from Kentucky.

Two samples of the Pennsylvania specimens, both from Hankins Pond, Pleasant Mount, Wayne County, Pa., had been sent to the writer for identification,—one in 1947 by the Smithsonian Institution (collector not indicated) and the other, in 1948, by Dr. H. Radclyffe Roberts, of the Academy of Natural Sciences of Philadelphia.

Dr. Roberts' letter of Oct. 7, 1948, stated that "The Pennsylvania Fish Commission has found in some of their fish ponds very abnormal growths of these Bryozoa. It appears as though this condition was induced by the fertilizer added to the ponds. The serious aspect of this condition is that when these Bryozoa are crushed in the vicinity of the fish, it has a very quick, lethal action. This is a very serious condition in cases where they rear the small fish in wire cages and when they are netting the fish and disturb the bottom or other surfaces."

Dr. Shujitsu Oda, of Tokyo Bunrika University, in Japan, upon learning that *L. carteri* is toxic or lethal to fish if crushed or damaged, experimented with a related bryozoan species, *Pectinatella gelatinosa,* and found that it too possessed similar toxic or lethal properties when crushed near the common Japanese fish *Oryzias latipes* (from Dr. Oda's letter of June 9, 1951).

In view of Roberts' and Oda's observations it would seem that *L. carteri's* toxic properties should be investigated further by someone with an interest in either biochemistry or in fisheries problems.

The writer wishes to express her most grateful thanks to the collector, Mr. Echols, to Dr. Gerald Cole who suggested that this article be undertaken and who furnished all the essential Kentucky collection data and specimens, and to the National Science Foundation which so generously supported the author's bryozoan research for the past several years.

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Studies on a Kentucky Knobs Lake. III. Some Qualitative Aspects of the Net Plankton*

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INTRODUCTION

PLANKTON SAMPLES were collected at irregular intervals from Tom Wallace Lake in Jefferson County, Kentucky, between September 1950 and December 1956. Tow nets of No. 20 silk bolting cloth were used for all collections. Some samples were secured by casting from the shore, whereas others were taken from a boat and involved vertical as well as horizontal tows. The collections represent every month of the year, although comparatively long intervals separated some sampling dates, and midsummer data are relatively scarce. Altogether, 55 samples, of which 40 were preserved and 15 were examined fresh and not saved, constitute the basis for this report.

Some physical, chemical, and biological characteristics of Tom Wallace Lake have been presented by Distler (1952), Cole (1954), and Neff (1955). The lake, which has an area of 2.34 hectares and a mean depth of 4.1 meters, is an artificial impoundment, eutrophic in nature, with an anaerobic summertime hypolimnion and pronounced thermal stratification. The dam was built during the summer of 1946 and the basin filled the following winter so that 1947 is considered the lake's first year. The only natural inlet is a small intermittent brook from a steep wooded ravine at the west end of the lake. This stream is insignificant as a source of plankton organisms.

Some changes in the water chemistry have occurred since the lake was first impounded and these may very well have influenced plankton populations. First, the annual application of two tons of 6-8-6 commercial fertilizer was discontinued after the first five years. Second, there has been an increase in the total alkalinity of the lake since the first data were recorded; in late September 1950, the total alkalinity of surface waters was 22 p.p.m., whereas during the same

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period in 1955 it was 44 p.p.m., and in 1956 after the rainiest summer since 1951 it was 38 p.p.m.

Collections made at different seasons revealed both oligothermal and polythermal species. In this study, those species described as cold-water forms were usually collected while epilimnion temperatures were below 15° C., and the warm-water species were those taken usually at temperatures of 17° and higher.

Annotated List of Plankters

During the six years of this study, the following plankton organisms were collected and identified. The sequence in which the taxonomic groups of species are listed is in no way an indication of their relative abundance. The arrangement has involved a grouping of the chromatophore-bearing flagellates as Protista, and these are treated along with the ameboid and ciliate protozoans as 'Protozoa and Protista.'

CYANOPHYTA

Anabaena planktonica Brunnthaler
Anabaena spiroides Lemmermann
Aphanizomenon flos-aquae (Linn.)
Aphanocapsa sp.
Chroococcus sp.
Coelosphaerium naegelianum Unger
Merismopedia sp.
Microcystis aeruginosa Kützing
Oscillatoria sp.
Trichodesmium lacustre Klebahn

These blue-green algae were largely warm-water plankters. All but three collections which contained members of this group were taken when epilimnion waters were warmer than 15° C. Several blooms were noted early in the study; these will be discussed later.

CHRYSOPHYTA

Asterionella formosa Hassall
Fragilaria spp.
Homeocladia sigmoides (Nitz.)
Navicula spp.
Surirella robusta Ehrenberg
Synedra spp.
Other diatoms

The diatoms were rarely abundant in the collections, and, when compared to other phytoplankters and the pigmented flagellates, they never attained bloom proportions. *Surirella robusta* and *Synedra* exhibited mild autumnal and vernal pulses. *Asterionella* *formosa* and *Fragilaria* showed minor autumnal and vernal peaks, respectively. The relative unimportance of diatoms in the net plankton and the fact that there was never evidence of any winter maxima in Tom Wallace Lake are similar to the findings reported by Moore (1950) for Lake Providence, Louisiana.

CHLOROPHYTA

Botryococcus sp.
Closterium aciculare T. West
C. acerosum (Schrank)
C. moniliferum (Bory)
Closterium sp.
Coelastrum microporum Nägeli
Cosmarium botrytis (Bory)
Crucigenia tetrapedia (Kirchner)
C. quadrata Morren
Euastrum sp.
Golenkinia sp.
Hyalotheca sp.
Micrasterias sp.
Mougeotia sp.
Netrium digitus (Ehrenberg)
Pediastrum duplex Meyen
Penium margaritaceum (Ehrenberg)
Planktosphaeria sp.
Pleurotaenium ehrenbergii (De Brébisson)
Scenedesmus armatus (Chodat)
S. bijuga (Turpin)
Sphaerocystis sp.
Spirogyra spp.
Staurastrum spp.

Green algae large enough to be taken by the plankton net were not abundant in Tom Wallace Lake and no single species was present consistently. Furthermore, striking blooms, comparable to those exhibited by the blue-green algae in the early years, were never noted.

The commonest desmids were *Staurastrum* spp., *Closterium* spp., and *Pleurotaenium ehrenbergii.* Several green algae, other than desmids, appeared from time to time in the samples, but only three were observed on several occasions. One of these, *Coelastrum microporum,* occurred in samples from each season of the year. At least two species of *Spirogyra* were especially conspicuous in early spring collections and to a lesser extent in late fall and winter. The limnetic filaments of these algae probably came from littoral benthic mats which were present at those times. The third common alga was *Sphaerocystis,* frequently noted, but never in bloom proportions.

PROTOZOA AND PROTISTA

Actinophrys sp.
Arcella vulgaris Ehrenberg
Ceratium hirundinella (O.F.M.)
Codonella cratera (Leidy)

Difflugia lobostoma Leidy
Dinobryon bavaricum Imhof
D. sertularia Ehrenberg
Eudorina elegans Ehrenberg
Mallomonas caudata Iwanhoff
Peridinium cinctum (Müller)
Phacus longicauda (Ehrenberg)
P. pleuronectes (O.F.M.)
Synura uvella Ehrenberg
Trachelomonas volvocina Ehr.
Uroglenopsis americana (Calkins)
Vorticella sp.

With the exception of the flagellates, the Protozoa were limited to three important species, *Codonella cratera, Vorticella* sp., and *Difflugia lobostoma.* The last-mentioned form was almost entirely limited to warm-water periods. Thriving populations of *Codonella* and *Vorticella* were springtime phenomena, although representatives of these species occurred in collections during all seasons.

Five species of flagellated organisms were important as members of the plankton community of the lake. The first species, *Dinobryon,* will be discussed later. The second, *Ceratium hirundinella,* was the most frequently occurring flagellate, although found only once during the winter. It was present in only eight collections taken when temperatures of epilimnion waters were below 15°. It attained bloom proportions during spring, summer, and fall. The most striking bloom of *Ceratium* occurred during the spring of 1952 when it was the predominant plankter; that was the only spring when *Dinobryon sertularia* was absent from all collections.*

Eudorina elegans was found many times, although it seemed to be essentially a warm-water form. It was the predominant plankter during early September 1951, and colored the upper waters of the lake, but became scarce by the end of the month.

Mallomonas caudata appeared each autumn and usually could be found during the winter and spring. It was never collected during midsummer.

Peridinium cinctum, characterized by summertime blooms, was never present when epilimnion temperatures were below 15°.

ROTATORIA

Asplanchna sp.
Brachionus angularis Gosse
B. quadridentata Hermann
Conochiloides sp.
Conochilus sp.
Filinia sp.
Kellicottia bostoniensis (Rousselet)

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* Cole (1954: 39) erroneously stated that *Dinobryon* was present that spring. The statement actually referred to 1953.

Keratella cochlearis (Gosse)
K. gracilienta Ahlstrom
K. valga (Ehrenberg)
Lecane sp.
Monostyla sp.
Pedalia sp.
Polyarthra spp.
Ptygura libera Myers
Synchaeta sp.
Trichocerca spp.
Unidentified saccular-type rotifers

Rotifers occurred in more collections and were represented by more species than any other group of zooplankton. The number of species in individual collections ranged from one to nine; the mean number was 4.4 and the mode was 4. Among those taken, *Keratella cochlearis* was the most consistently collected and *Polyarthra* ranked second. Although numbers of those two species fluctuated, large populations were present every season. In addition, some unidentified, small, somewhat opaque, saccular rotifers were present during all seasons of the year, but were not consistently collected. Some of the largest populations of these were present during cold-water periods. Members of the cosmopolitan genus *Asplanchna* were found very rarely and never in large numbers.

Synchaeta was typical of cold-water periods, although one pulse occurred during spring when the epilimnion had warmed to 17°. It was never found during summer or when the water was warmer than this. *Kellicottia bostoniensis* was also considered an oligothermal rotifer.

Polythermal species, never collected in winter, were *Keratella gracilienta, Filinia, Conochilus, Pedalia, Trichocerca, Ptyqura libera,* and *Conochiloides.* Rare occurrences of the following were noted also during warm-water periods: *Monostyla, Lecane, Brachionus quadridentata,* and *B. angularis.*

Mixed populations of the very common *Keratella cochlearis* and the much less common *K. gracilienta* were found a few times, but one collection (July 25, 1952) contained *K. gracilienta* alone and in great abundance. At this time the two species of *Brachionus* were common also.

A single record for *Keratella valga* was from subsurface samples (3 m.) in May 1952. On the same day none were present in the samples from the surface waters which contained *K. cochlearis.* The subsurface samples were taken with a Kemmerer water sampler, capacity 1,500 ml. Because many collections on other occasions consisted only of surface tows, *K. valga,* which is common in the Louisville area, may have been missed.

CLADOCERA

Alona guttata Sars
Alona rectangula Sars
Alona sp.
Bosmina longirostris (O.F.M.)
Ceriodaphnia lacustris Birge
Chydorus sphaericus (O.F.M.)
Daphnia longispina (O.F.M.)
Daphnia pulex (de Geer)
Diaphanosoma brachyurum (Liéven)
Ilyocryptus sordidus (Liéven)
Leydigia quadrangularis (Leydig)
Macrothrix laticornis (Jurine)
Pleuroxus denticulatus Birge
Scapholeberis mucronata (O.F.M.)
Simocephalus serrulatus (Koch)
S. vetulus (O.F.M.)

The cladocerans which occurred most frequently in the collections were *Bosmina longirostris, Daphnia longispina, Chydorus sphaericus, Ceriodaphnia lacustris, Daphnia pulex,* and *Diaphanosoma brachyurum. Chydorus* was found during all seasons and in many samples, but, in contrast to the others, was never especially abundant. Other species occurred sporadically or at rare intervals.

The number of cladoceran species in individual collections ranged from one to six. The average number of species collected in single samples was 3.2, and the mode was 3.

The two species of *Daphnia* were found together in several samples. With the exception of these instances and those already mentioned among the rotifers, no two species of zooplankton of the same genus were ever observed together. Furthermore, vertical plankton hauls cannot show the stratification that exists in plankton populations, and the occurrence of closely related species in such samples does not necessarily mean that they occupy the same stratum. This was discussed earlier in the case of the relationship between *Keratella cochlearis* and *K. valga.*

COPEPODA

Cyclops vernalis Fischer
Diaptomus pallidus Herrick
Eucyclops agilis (Koch)
Harpacticoid copepods
Macrocyclops albidus (Jurine)
Mesocyclops edax (Forbes)
Naupliar larvae
Tropocyclops prasinus (Fischer)

Diaptomus pallidus and *Tropocyclops prasinus* were the only abundant copepods in the plankton of Tom Wallace Lake during the period of this study. *Diaptomus* was not collected until May 1951, but after that it was always the more numerous of the two. Both exhibited pulses at all seasons and were present in almost every collection after May 1951. There were rare occurrences of littoral and semi-benthic copepods which will be discussed later. *Mesocyclops edax,* although rarely found, was the only other truly limnetic species, and will be discussed later. No more than three species of the Copepoda occurred in single samples, the mean number of species being 1.88, and the mode two species, the calanoid *Diaptomus pallidus* and the cyclopoid *Tropocyclops prasinus.*

OSTRACODA

Cypridopsis vidua (O.F.M.) *Physocypria pustulosa* Sharpe

These were the only ostracods taken in the collections, and will be mentioned again as adventitious plankters.

Adventitious Plankters

Examinations of bottom samples from littoral and sublittoral depths revealed that there were several microscopic arthropods in Tom Wallace Lake associated with the sediments. These were either true microbenthic forms or species that swim about close to the bottom in shallow areas. Their occurrences in plankton samples from the upper waters of the lake were rare and did not indicate their actual abundance in the lake. Typical of these species are: (1) the cladocerans, *Simocephalus serrulatus, S. vetulus, Ilyocryptus sordidus, Leydigia quadrangularis,* and *Macrothrix laticornis;* (2) the copepods, *Eucyclops agilis, Macrocyclops albidus* and some harpacticoids; (3) the ostracods, *Cypridopsis vidua* and *Physocypria pustulosa;* and (4) various species of water mites.

Larvae of *Chaoborus punctipennis* (Say) are very common in the lake and during the day occur as benthic and deep-water planktonic organisms. These dipterous predators were occasionally taken in plankton hauls.

Scapholeberis mucronata was collected in plankton tows during warm-water periods, but, because it is primarily a neuston form, its consideration as a plankter is probably unwarranted.

Of the rotifers, at least *Lecane* and *Monostyla* are not typical limnetic species, and belong to the microbenthos or the periphyton rather than to the plankton.

SUCCESSION AND CHANGES IN SPECIES COMPOSITION

Certain trends and fluctuations occurred in the species composition of the plankton population of Tom Wallace Lake during its first 10 years of existence. However, any reasons for these changes must be regarded as largely speculative.

In spite of the fact that no plankton collections were made before September 1950, it was possible to learn something about limnetic populations that were present during the three previous years by studying microfossils in the sediments. Profundal deposits are varved, and the annual layers sedimented since the lake was first impounded are readily identified (Cole, 1954). By January 1956, about 6.5 cm. of sediment had accumulated over the original basin in the profundal region and the collecting of vertical core samples had become relatively easy.

The 1947 stratum is characterized by the presence of many filaments of *Melosira*. This is a small species with finely punctate valves, five or six times longer than wide. The next two strata, which represent the 1948 and 1949 deposits, contain occasional filaments of this diatom, but they are not abundant and *Melosira* has never been collected since the net sampling was initiated in 1950.

The 1948 and 1949 strata are rich in well-preserved colonies of the blue-green algae, *Microcystis* and *Coelosphaerium*. *Synedra* frustules are common in the three layers from 1949 through 1951.

The phytoplankton relics in Tom Wallace Lake suggest a history comparable in some ways to the much longer one of Linsley Pond, Connecticut, as revealed by the diatom remains in the sediments there (Patrick, 1943). There is no evidence of a stage comparable to the initial post-glacial period in Linsley Pond when *Cyclotella compta* made up an important segment of the plankton. Tom Wallace Lake began with a *Melosira* flora, perhaps not unlike the eutrophic second stage in Linsley Pond, and which was replaced within a year or two by a *Synedra* and blue-green population. In Linsley Pond a later decline of the *Melosira* community probably reflects cultural influences. In Tom Wallace Lake the replacement may have been hastened by rapid accumulation of nutrient materials from the applied commercial fertilizer.

Additional evidence for the relationship between the decline of *Melosira* populations with increasing pollution is shown by studies in Crystal Lake, Minnesota. The superficial sediments of this lake include 10 or 12 cm. of sapropel which overlie gyttja (Cole, 1955). There are no *Melosira* frustules in the upper 4 or 5 cm. of sapropel, but they do exist below that level. Swinyard (1933) found *Melosira* abundant in plankton taken from Crystal Lake in 1931 and 1932, but repeated plankton sampling there by the present writer from 1947 through 1949 revealed no limnetic *Melosira* at any time.

The blue-green algae were more abundant during the years fertilizer was applied to Tom Wallace Lake than afterward, and the relics in the sedimentary strata of 1948 and 1949 suggest that heavy blooms occurred during the years before collecting was initiated. During 1950 and 1951, there were large populations of *Microcystis aeruginosa, Anabaena spiroides, Trichodesmium lacustre*, and *Chroococcus. Trichodesmium* exhibited a minor pulse in the fall of 1952, following a conspicuous bloom of *Aphanizomenon flos-aquae* that summer. Since that time there has been no evidence of blooms of blue-green algae, but the scarcity of midsummer data precludes any definite statement that they have not occurred.

Although the desmids were represented by several species, and occurred in many samples, they were usually collected during the early spring and were never abundant. Furthermore, more species were found during 1951 and 1952 than in later years. Each spring the total alkalinity of the lake water is at its annual lowest, and there seems to have been an increase in bicarbonates from year to year (Cole, 1954). Perhaps the increasing hardness of the water played a part in the apparent decrease in the desmid population. In South Park Pond, two and one-half miles away, where total alkalinity is much lower than in Tom Wallace Lake, there is a rich and varied desmid population.

The colonial flagellate, *Dinobryon sertularia,* present in a great many collections, exhibited some puzzling fluctuations in abundance that are not readily explained. It first appeared in late March 1951, bloomed luxuriantly during April, and disappeared by late May. It did not reappear until mid-November of 1952, but then persisted throughout the winter, and was present during the spring and summer of 1953 until at least September, when further collecting was prohibited by forest fires in the Jefferson County Forest. It was again abundant in January 1954, but disappeared completely, except for a tremendous number of cysts, by March. There was a reappearance and bloom in April 1954, but by June it was gone, and did not occur again until late March of 1955. That spring population waned and died out in May, and did not recur until October of that year. In the meantime a large, summertime population of *D. bavaricum* appeared and persisted until late October when *D. sertularia* began to occur in the samples, and, apparently, replaced *D. bavaricum* during November. In January 1956, *Dinobryon* was absent once more, but it was abundant later in the summer samples and it persisted in large numbers through December. No collections were made during the spring of 1956.

Two other species were also characterized by long periods of absence followed by reappearance. The first, *Kellicottia bostoniensis,* was considered an oligothermal rotifer, its prominent pulses occurring in winter. In general it was monocyclic, appearing in autumn and disappearing as the water became warmer in the spring. For some reason, this species was not collected between April 17, 1951, and the fall of 1954. The second was the cladoceran, *Ceriodaphnia lacustris,* which was absent in all collections from December 6, 1952, until April 26, 1955. It was very common during the warm-water periods of 1950, 1951, 1952, and 1955, but was not present in samples taken in 1956. Recently Pennak (1955) described similar enigmatic disappearances of limnetic entomostracans from a Colorado mountain lake.

Possible late comers to the lake include two rotifers, two cladocerans, and a copepod. *Ptygura libera* and *Conochiloides* sp. were not encountered until June 1954. A few individuals of *Diaphanosoma brachyurum* were collected in May 1951, but none were seen again until late September of 1952. Since that time it has been fairly common during late spring, summer, and early fall, but has not occurred in any winter or cold-water collections. It has been a common plankter in nearby South Park Pond for many years. *Daphnia pulex* was not collected until February 1952, but was found during all seasons after that, although less frequently than *D. longispina.*

In South Park Pond, *Mesocyclops edax* has been a conspicuous member of the plankton community for several years. Although searched for in TomWallace Lake since the collecting started, it was not found until the summer of 1954. Later, it was present in the spring and fall samples of 1955, but there were none in the collections of 1956. Prior to 1954, this species had been collected at only five places in the Louisville area (Cole, 1953): three artificial ponds, a limestone quarry pit, and a stream. These bodies of water differed greatly in size, total alkalinity, and pH, but each had been in existence for many years. It is not impossible that *Mesocyclops edax* is a form with poor means of dispersal and/or inferior colonizing ability. It may be a species that eventually can succeed in becoming established in suitable habitats, perhaps at the expense of earlier arrivals which may be fugitive species in the sense of Hutchinson (1951). Although such a statement may be pure speculation, there is little information available to the contrary. Yeatman (1956) reported *Mesocyclops edax* from a new reservoir in Tennessee, but it had been present in an older pond which was engulfed by the impoundment, and hence gained immediate access to the new habitat.

DISCUSSION

From examination of the collections it would appear that the larger pigmented flagellates are the important primary producers in Tom Wallace Lake. In the early years, when inorganic commercial fertilizer was applied, the blue-green algae may have been more important, but they certainly have not been since 1952. The intestines of the common filter-feeding cladocerans, *Daphnia, Bosmina, Ceriodaphnia,* and *Diaphanosoma* were filled with green material in some instances when the only chromatophore-containing forms taken were the colonial *Dinobryon* and the large, armored *Ceratium,* and at other times when pigmented species were almost entirely lacking in the samples. In addition to the cladocerans mentioned above, the list of zooplankters is composed largely of small-particle feeders such as ciliates, and the rotifers *Keratella, Filinia, Conochilus,* and *Kellicottia.* Probably the calanoid copepod, *Diaptomus pallidus,* is a filter-feeder and belongs in this group. The presence of such large populations of filter-feeding and small-particle-feeding zooplankters, coupled with the relative scarcity of small autotrophic net plankters, suggests the presence of a significant nannoplankton population not taken by the net. On the other hand, it is possible that planktogenic detritus was an important trophic factor where these zooplankters were concerned.

With respect to the secondary consumers there also seems to be inadequate representation. Only one limnetic cyclopoid, *Tropocyclops prasinus,* was common. Because some cyclopoids are predaceous, this species also might well be carnivorous and capable of feeding on animals and algae denied to the filter feeders. The predaceous rotifer, *Asplanchna,* was extremely rare, and except possibly for *Synchaeta, Polyarthra,* and *Trichocerca,* the common planktonic rotifers collected probably were mostly small-particle-detritus and nannoplankton feeders. It seems reasonable to assume that the secondary consumer niche was filled by *Chaoborus* larvae making nocturnal forays on the zooplankton. At times *C. punctipennis* has been represented in the deeper areas of the lake by as many as 55,000 individuals per square meter (Neff, 1955).

The difficulties and complexities encountered in a study of the autecology of any limnetic species are underlined by consideration of the vagaries of *Dinobryon* populations. Hutchinson (1944) pointed out that periods of *Dinobryon* maxima are not characterized by uniform chemical and temperature conditions, and that complex biotic factors may control the incidences of blooms. The lack of quantitative data assembled at regular and close intervals precludes definite conclusions concerning the rise and fall of the Tom Wallace populations. However, it should be mentioned that members of the genus *Dinobryon* were found only rarely and never in large numbers during blooms of blue-green algae, or when *Ceratium* or *Eudorina* were predominant. They were, however, abundant on several occasions during *Peridinium* and *Mallomonas* blooms. Hutchinson (*loc. cit.*) stated that in Linsley Pond, Connecticut, *Dinobryon* appeared after catastrophic falls in the standing crop of phytoplankton. The collections from Tom Wallace Lake do not reveal anything clearly consistent with this, but it is possible that quantitative data concerning the rather scanty net phytoplankton, if gathered at close intervals, might show a similar correlation.

The factors that operated against the appearance of *Dinobryon* during the spring of 1952 are obscure. Perhaps the tremendous success of *Ceratium* that year was related in some way, but Yeatman (1956) described *D. sertularia* as abundant during May in Woods Reservoir, Tennessee, while *Ceratium hirundinella* was blooming. Therefore, in Tom Wallace Lake the consistent scarcity of *Dinobryon* during *Ceratium* maxima may not necessarily be ascribed to the latter *per se.*

The reason why *D. bavaricum* replaced *D. sertularia* in the summer and early fall of 1955 (the only time the former was collected) is also obscure. Furthermore, the reason for the appearance of members of this genus during only three summers out of the six under observation is not known. If cool weather favors the growth of *Dinobryon,* an assumption not necessarily warranted, it could be pointed out that the three summers it did occur were the coolest of the six except for 1951. Its absence during 1951 might be explained on the basis of competitive action of *Microcystis* and other blue-green algae which were abundant that summer. The September and October collections of 1950 represent at least the end of an additional summer when there was a luxuriant *Microcystis* bloom and *Dinobryon* was absent.

ACKNOWLEDGMENTS

I am grateful to Dr. Arland T. Hotchkiss for his aid in the taxonomic determinations of the phytoplankters, and to Dr. Frank F. Hooper and Dr. Louis A. Krumholz (to whom I am especially indebted) for their valuable suggestions concerning the manuscript.

SUMMARY

1. Plankton populations present during the first 10 years of the existence of Tom Wallace Lake, Jefferson County, Ky., were studied through examination of profundal sediments and net collections.
2. An early *Melosira* community disappeared after three years, possibly in response to the accumulation of nutrients from applied commercial fertilizers.
3. *Microcystis aeruginosa* bloomed luxuriantly during the years fertilizer was added to the lake, but with cessation of the fertilizing program this species was never again conspicuous. *Aphanizomenon* and *Trichodesmium* bloomed the first year no fertilizer was applied, but since then blue-green algae have been unimportant.
4. Desmids were more common in the spring months and during the early years of the lake's existence than at other times. The decline in the desmid population may have been due to the increase in total alkalinity of the lake.
5. Puzzling fluctuation in the populations of some species, and the late arrival of such zooplankters as *Conochiloides, Ptygura, Diaphanosoma, Daphnia pulex,* and possibly *Mesocyclops edax* are discussed. Where possible, polythermal and oligothermal species are pointed out.

6. With respect to the number of species present, the zooplankton community on most occasions consisted of four rotifers, three cladocerans, one cyclopoid copepod, and one calanoid copepod.
7. The holophytic net plankters were largely flagellates, and the majority of the zooplankters were filter feeders or small-particle feeders. Secondary consumers were scarce, although the common *Tropocyclops prasinus* may have belonged to this trophic level. *Chaoborus* larvae, which were very abundant in profundal sediments, probably filled this niche, but were not common in daytime plankton samples.

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ACADEMY AFFAIRS

THE 1957 FALL MEETING OF THE KENTUCKY ACADEMY OF SCIENCE

The forty-third annual meeting of the Kentucky Academy of Science was held at Berea College, October 25 and 26, 1957. Members and guests commenced registering in Hall Science Building Friday afternoon, the 25th, and the meeting began with an official welcome to the Berea campus from Dean Kenneth Thompson. A program of invited papers on ''Research in Kentucky'' followed. Participants were: Phil M. Miles, speaking on ''Industry and Research''; Merl Baker, whose subject was ''The Scope of the Kentucky Research Foundation''; and Dr. Edmund K. Hall, who presented an illustrated discussion of ''Research in Clinical Fields, and Contributions of Pure Science to Medicine.''

The Academy's annual banquet was held at the Boone Tavern Hotel on Friday evening. Following the dinner, Dr. D. M. Bennett, Chairman of the Department of Physics, University of Louisville, addressed the gathering with a talk entitled, ''The Geophysical Year.''

At the business session on Saturday morning the following officers were elected for 1957-58:

President: William M. Clay, University of Louisville.
President-elect: Phil M. Miles, Lexington, Ky.
Vice President: William B. Owsley, Morehead State College.
Secretary: Gerrit Levey, Berea College.

Directors elected to serve on the Board until 1961 were L. Y. Lancaster, Western State College, and H. P. Riley of the University of Kentucky.

Robert M. Boyer introduced Miss Susie Caudill, who spoke to the members on ''The Human Brain.'' This was her subject in the Junior Academy's Science Discussion Contest, in which she was a winner.

The announcement was made of the first two members in the newly established Business and Industrial Membership category: Jack M. Hamilton and Spencer T. Jones, Philip Morris and Co., Louisville.

The various sections of the Academy met, following the business meeting, to elect officers and to hear the programs of research papers as follow:

SECTION MEETINGS

Bacteriology and Medical Technology Section

Genevieve Clark, Chairman; William Cantrell, Secretary

(1) The growth effect of several inorganic sources of nitrogen on *Nocardia*. Margaret Simms and Margaret Hotchkiss, University of Kentucky. Presented by Miss Simms.)

(2) Fluorescence microscopy applied to exfoliated cytology. H. Davis Chipps, Central Baptist Hospital, Lexington.

(3) The effects of various carbohydrates in rations upon the intestinal microflora of chickens. Ralph F. Wiseman, University of Kentucky.

(4) The in vitro sensitivity of leukocytes from allergic and non-allergic subjects to products of growth of pathogenic cocci. H. E. Hall and M. Scherago, University of Kentucky.

(5) Methods for demonstrating bacterial flagella. Charles R. Bennett, Jr., and O. F. Edwards, University of Kentucky. (Presented by Edwards.)

(6) The recognition of primary colonies of Clostridia by neutral red fluorescence. Leo Kaufman and R. H. Weaver, University of Kentucky.

(7) The influence of hormones of the adrenal cortex upon hematopoesis in the rat. James W. Fisher, University of Louisville.

(8) Some observations on infectious diseases and other health problems in Costa Rica. Emil Kotcher, University of Louisville.

(9) The effect of X-irradiation on the blood volume. K. C. Huang, University of Louisville.

(10) Antigenic variation and mutation rate in *Trypanosoma equiperdum*. William Cantrell, University of Louisville.

BIOLOGY SECTION

L. Y. Lancaster, Chairman; Dwight M. Lindsay, Serecretary

(1) The occurrences of *Aegilops cylindrica* Host in Kentucky. Charles R. Gunn, Ross Seed Company, Louisville.

(2) Two common Hydras of Kentucky. Robert R. Bryden, Union College.

(3) Polyploidy in *Gasteria*. Herbert Parkes Riley, Department of Botany, University of Kentucky.

(4) A four-year study of the breeding birds on the Kleber Song Bird Sanctuary. H. B. Lovell and A. L. Stamm, Department of Biology, University of Louisville. (Presented by H. B. Lovell.)

(5) A new North American leafhopper previously confused with *Typhlocyba andromache* McAtee. Paul J. Christian, Department of Biology, University of Louisville.

(6) Biogeochemical prospecting—a new field for biologists. Hansford T. Shacklette, Department of Biology, Georgetown College.

(7) Notes on the present distribution of the Mammoth Cave fish, *Amblyopsis spelaea* De Kay, in Kentucky. William M. Clay, Department of Biology, University of Louisville.

(8) Chromosomes in South African plants. Victor Hoff and Herbert Parkes Riley, Department of Botany, University of Kentucky. (Presented by Victor Hoff.)

(9) Studies on the wild yeasts ingested by *Drosophila* Populations. J. K. Komatsu, Department of Zoology, University of Kentucky.

(10) Correlation of phases of the testicular cycle with behavior in the gray squirrels, *Sciurus carolinensis*. Alfred Brauer and Albert Dusing, Department of Zoology, University of Kentucky. (Presented by Alfred Brauer).

(11) A comparative study of seasonal fluctuations in *Drosophila* species. E. R. Newport and J. M. Carpenter, Department of Zoology, University of Kentucky. (Presented by E. R. Newport).

(12) On our knowledge of Kentucky crustaceans. Gerald A. Cole, Department of Biology, University of Louisville.

(13) The effect of ecological factors on *Drosophila* populations. J. F. Jones, Department of Zoology, University of Kentucky.

(14) Observations on the oviposition and feeding of *Amblyomma dissimile* (Koch, 1944). Elden G. Burcham, Department of Zoology, University of Kentucky.

(15) Studies in alpine lake ecology. I. Heat Budgets. Vera and R. C. Dugdale, Department of Zoology, University of Kentucky. (Presented by Vera Dugdale.)

(16) Some chromosome numbers of Kentucky Characeae. A. T. Hotchkiss, Department of Biology, University of Louisville.

(17) Preliminary studies on the herpetology of Mason County. J. T. Wallace, Department of Zoology, University of Kentucky.

(18) Spawning and early embryology of the frog fish *Phrynelox scaber*. Louis A. Krumholz, Department of Biology, University of Louisville.

(19) Problems of nutrient supply in a group of sub-Arctic Lakes. Vera and R. C. Dugdale, Department of Zoology, University of Kentucky. (Presented by R. C. Dugdale).

CHEMISTRY SECTION

Paul G. Sears, Chairman; Sister M. Concette Waller, Secretary

(1) Polarization of the N-methylacetamide—water system and five N-methlyacetamide—n-alcohol systems at 30° C. W. K. Plucknett, P. G. Sears, and R. A. Hovermale, University of Kentucky. (Presented by Hovermale).

(2) A bicyclic hemiketal from benzal-bis-acetylacetone. Walter T. Smith, Jr. and John W. Ryan, University of Kentucky. (Presented by Smith).

(3) The dehydrogenation of pyrrolidine. John M. Patterson and Peter Drenchko. University of Kentucky. (Presented by Drenchko).

(4) A study of the *in vitro* reaction of catalase and hemin with organic mercury compounds. Sister M. Angelice Seibert, O.S.U., Ursuline College, Louisville, Ky. (Presented by Sister Angelice).

(5) Chloroform carrying chelates. J. F. Steinbach and J. H. Burns, University of Kentucky. (Presented by Steinbach).

(6) The reaction of isophorone with cupric bromide. Arthur W. Fort, University of Kentucky.

(7) Absorption behavior of Th234. Kinziro Aizawa and F. J. Johnston, University of Louisville. (Presented by Aizawa).

(8) Spectrophotometric studies of chelates in non-aqueous media. William N. McNerney, University of Kentucky.

ENGINEERING SECTION

W. M. Carter, Chairman

(1) On the testing of aerial camera shutters. Fred C. Curtis.

(2) The parachute research program at the University of Kentucky. James F. Lafferty.

(3) Work activity analysis of non-repetitive workers. O. W. Gard.

(4) Anthropometric contourometry. A. L. Wittwer.

PSYCHOLOGY SECTION

Kenneth Purcell, Secretary

(1) A test of Newcomb's theory of communication in a small group setting. Albert Lott, University of Kentucky.

(2) The stages in creative thought. Clara Cooper, Berea College.

(3) An experimental approach to the problem of empathy. Hans Hahn, Transylvania College.

(4) Some aspects of clinical judgment. John Donahoe, University of Kentucky.

(5) Some aspects of inter-personal perception. Roy Yamahiro, University of Kentucky.

(6) Film on progress in several researches. Hans Hahn, Transylvania College.

KENTUCKY ASSOCIATION OF PHYSICS TEACHERS

JUNIOR ACADEMY OF SCIENCE

TREASURER'S REPORT

Balance, October 1, 1956	$1029.38
Receipts, October 1, 1956 to October 1, 1957	
Membership dues	930.00
Subscriptions to Transactions	12.00
Contributions	10.00
Meeting fees	16.00
AAAS Research Grant	50.00
University of Louisville for 300 copies of Transactions	300.00
	$1318.00
Disbursements, October 1, 1956 to October 1, 1957	
Secretary's expenses	$ 97.32
Treasurer's expenses	19.50
Junior Academy expenses	256.03
AAAS Research Grant	50.00
Publication of Transactions	958.07
Tax on bank account	.79
	$1381.71
Balance, October 1, 1957	$ 965.67

RICHARD A. CHAPMAN, *Treasurer.*

News and Notes

The Spring Meeting of the Kentucky Academy of Science will be held at Natural Bridge State Park, May 2-3, 1958.

Tentative plans for the forty-fourth annual meeting of the Kentucky Academy of Science call for a two-day session at the University of Kentucky late in October, 1958.

Dr. Ralph H. Weaver was recently announced as the Distinguished Professor of the Year in the University of Kentucky's Arts and Sciences College. Dr. Weaver is the 14th recipient of this award, an honor which recognizes academic and scholarly achievement. A native of Pennsylvania, Professor Weaver joined the faculty of the University of Kentucky in 1926. He is the author of many papers on various phases of bacteriology.

Dr. and Mrs. Richard C. Dugdale, Department of Zoology, University of Kentucky, have received a National Science Foundation grant in the amount of $8,100.00. This award will permit them to carry on their limnological work in Alaska. The problems of lacustrine fertility, and nitrogen fixation in aquatic communities will be investigated on Afognak Island during the summer months of 1958.

The Biology Department of the University of Louisville has announced the appointment of Dr. Paul B. Sears as Visiting Professor in the Tom Wallace Chair of Conservation. Dr. Sears, who was president for 1957 of the AAAS, will present a course entitled "The Importance and Care of Natural Resources." In addition, he will direct the faculty and graduate Biology seminar for the spring semester.

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INSTRUCTIONS FOR CONTRIBUTORS

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Titles must be clear and concise, and provide for precise cataloging. Textual material should be in clear, brief, condensed form. Footnotes should be avoided. Tables and illustrations are expensive and should be included only to give effective presentation of the data. Articles with an excessive number of tables or illustrations, or with poorly executed tables or illustrations, may be returned to the author for modification.

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TRANSACTIONS OF THE
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Volume 19 **May, 1958** **Numbers 1-2**

Synthesis of Mannich Derivatives of Some Symmetrical Bisphenols*

GEORGE O'BRIEN AND JACOB R. MEADOW
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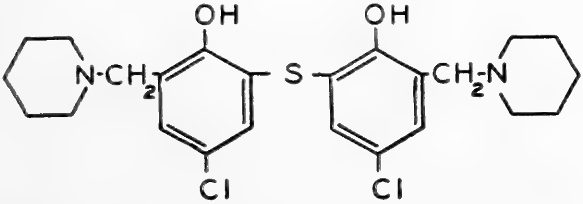
Lexington, Ky.

INTRODUCTION

THE introduction of Mannich groups into a large number of aromatic phenolic compounds has been reported by Meadow and Reid (1), Burckhalter and his coworkers (2,3), and others. Because of continued interest in the physiological activity (4) shown by certain types of nitrogen-containing bisphenols, the preparation of several additional amino bisphenols has been carried out and reported here.

We prefer to select symmetrical bisphenolic compounds which have a blocking group either in the ortho or para position of each ring, thus leaving open only one position in that ring for the introduction of a Mannich group. This is illustrated in the following formula:



The bisphenols used as starting materials were as follows: 2,2′-thio-*bis*-(4-chorophenol), 4,4′thio-*bis*-(2-*tert.*-butyl-5-methylphenol), 4,4′-sulfonyl-*bis*-(2-aminophenol), 2,2′-methylene-*bis*-(4-chlorophenol),

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* Supported in part by a research grant from the Geschickter Fund for Medical Research, Inc. Acknowledgment is made for permission on the part of the Geschickter Fund to publish these results.

Transactions of the Kentucky Academy of Science, **19** (1-2): 1-5. May, 1958.

TABLE I. DI-MANNICH DERIVATIVES OF SYMMETRICAL BISPHENOLS WITH SULFUR LINKAGES[a]

Mannich group in each ring[b]	M.p., ° C.	Formula	Nitrogen, %		Neutralization Equiv.	
			Calcd.	Found	Calcd.	Found
From 2, 2′-Thio-*bis*-(4-chlorophenol)[c]						
Dimethylaminomethyl[d]	141-2.5[e]	$C_{18}H_{22}Cl_2N_2O_2S$[f]	6.98	6.90	201	203
Pyrrolidinomethyl	156-7[g]	$C_{22}H_{26}Cl_2N_2O_2S$	6.18	6.26	227	227
Piperidinomethyl	165.5-7[h]	$C_{24}H_{30}Cl_2N_2O_2S$[i]	5.82	5.79	241	242
Morpholinomethyl	132-3[e]	$C_{22}H_{26}Cl_2N_2O_4S$	5.77	5.55	243	246
From 4, 4′-Thio-*bis*-(2-*tert.*-butyl-5-methylphenol)[j]						
Dimethylaminomethyl	143.5-5[h]	$C_{28}H_{44}N_2O_2S$	5.93	5.74	236	245
Diethylaminomethyl	133.5-5[h]	$C_{32}H_{52}N_2O_2S$	5.30	5.39	264	273
Pyrrolidinomethyl	174-5[h]	$C_{32}H_{48}N_2O_2S$	5.34	5.62	263	266
Piperidinomethyl	183-4[k]	$C_{34}H_{52}N_2O_2S$	5.07	5.10	276	276
Morpholinomethyl	199-200[k]	$C_{32}H_{48}N_2O_4S$	5.03	4.76	278	281
From 4, 4′-Sulfonyl-*bis*-(2-acetamidophenol)[l]						
Dimethylaminomethyl[h]	193 dec.[m]	$C_{24}H_{36}N_4O_6S$[n]	10.69	10.58	262	261
Pyrrolidinomethyl[o]	190-5 dec.[m]	$C_{26}H_{34}N_4O_6S$	10.56	10.30	265	263
Moropholinomethyl[p]	250-60 dec.[m]	$C_{26}H_{34}N_4O_8S$	9.96	9.90	281	280
N-Methylbenzylaminomethyl[q]	195-200 dec.[m]	$C_{34}H_{38}N_4O_6S$	8.89	8.89	315	323

[a] All derivatives prepared as free bases. [b] Mannich group is ortho to OH in each case. [c] Prepared according to method of Dunning (5). [d] Full name of compound; 2, 2′-thio-*bis*-(4-chloro-6-dimethylaminomethylphenol). [e] From methanol. [f] Calc'd for $C_{18}H_{22}Cl_2N_2O_2S$; C, 53.91; H, 5.53. Found; C, 54.09; H, 5.84. [g] From benzene-ethanol. [h] From ethanol. [i] Calc'd for $C_{24}H_{30}Cl_2N_2O_2S$; C, 59.86; H, 6.28. Found; C, 60.14; H, 6.46. [j] Obtained from Monsanto Chem. Co., M.p. 161-2°. [k] From benzene-methanol. [l] Prepared by acetylation of 4, 4′-sulfonyl-*bis*-(2-aminophenol), M.p. 226-8°, (courtesy Monsanto Chem. Co.). [m] Melting point indefinite because major dec. product fails to melt below 275°. [n] Solvated. Calc'd. for $C_{22}H_{30}N_4O_6S$ plus one mole ethanol; S, 6.12; found 6.11. [o] Purified by extr. with acetone. [p] Crystals from dimethylformamide. [q] From benzene-naphtha.

TABLE II. DI-MANNICH COMPOUNDS FROM BISPHENOLS WITH ALKYLIDENE LINKAGES

Mannich group in each ring^{a}	M.p., ° C.	Formula	NITROGEN, %		NEUTRALIZATION EQUIV.	
			Calcd.	Found	Calcd.	Found
From 2, 2′-Methylene-*bis*-(4-chlorophenol)^{d}						
Dimethylaminomethyl^{c}	142-3^{d}	$C_{19}H_{24}Cl_2N_2O_2$	7.32	7.13	192	202
Diethylaminomethyl	101-2^{e}	$C_{23}H_{32}Cl_2N_2O_2$	6.38	6.23	220	223
Pyrrolidinomethyl	192-3^{d}	$C_{23}H_{28}Cl_2N_2O_2$	6.43	6.28	218	224
Piperidinomethyl	152-3^{d}	$C_{25}H_{32}Cl_2N_2O_2$	6.05	6.00	232	237
Morpholinomethyl	180-1^{d}	$C_{23}H_{28}Cl_2N_2O_2$	6.00	5.67	234	236
From 2, 2′-Methylene-*bis*-(4-methylphenol)^{f}						
Dimethylaminomethyl	110.5-1.5^{g}	$C_{21}H_{30}N_2O_2$	8.18	7.96	171	173
Diethylaminomethyl	59-60^{g}	$C_{25}H_{38}N_2O_2$	7.03	7.02	199	200
Pyrrolikinomethyl	130-2^{e}	$C_{25}H_{34}N_2O_2$	7.11	6.98	197	205
Piperidinomethyl	117-8^{e}	$C_{27}H_{38}N_2O_2$	6.63	6.35	211	214
Morpholinomethyl	147-8^{e}	$C_{25}H_{34}N_2O_2$	6.57	6.29	213	217
From 4, 4′-*iso*-propylidene-*bis*-(2-methylphenol)^{h}						
Dimethylaminomethyl	96.5-7.5$^{e,\ i}$	$C_{23}H_{34}N_2O_2$	7.56	7.49	185	185
Diethylaminomethyl	96-7$^{e,\ i}$	$C_{27}H_{42}N_2O_2$	6.57	6.56	213	213
Pyrrolidinomethyl	96-7$^{e,\ i}$	$C_{27}H_{38}N_2O_2$	6.67	7.03	211	212
Piperidinomethyl	137.5-9^{e}	$C_{29}H_{42}N_2O_2$	6.22	6.20	225	225
Morpholinomethyl	177-9^{d}	$C_{27}H_{38}N_2O_4$	6.16	6.14	227	229
From 4, 4′-*iso*-propylidene-*bis*-(2-*iso*-propylphenol)^{j}						
Dimethylaminomethyl	105.5-6.5$^{e,\ k}$	$C_{27}H_{42}N_2O_2$	5.93	5.74	236	245
Diethylaminomethyl	49-9.5^{e}	$C_{31}H_{50}N_2O_2$	5.30	5.39	264	273
Pyrrolikinomethyl	108-9$^{e,\ k}$	$C_{31}H_{46}N_2O_2$	5.34	5.62	263	266
Piperidinomethyl	107.5-9$^{l,\ k}$	$C_{33}H_{50}N_2O_2$	5.07	5.10	276	276
Morpholinomethyl	126.5-7^{l}	$C_{31}H_{46}N_2O_2$	5.03	4.76	278	281

[a] Reported as the free base with Mannich group ortho to OH in each case. [b] Courtesy Dow Chem. Co., M.p. 175-6°. [c] Full name of compound; 2, 2′-methylene-*bis*-(4-chloro-6-dimethylaminomethylphenol). [d] From benzene-ethanol. [e] From ethanol. [f] Courtesy Sindar Corp., N. Y., M.p. 121-3°. [g] From methanol. [h] Obtained from Dow Chem. Co., M.p. 140-1°. [i] M.p. depressed by each of other two compounds in this group having m.p. at approx. 96°. [j] Also obtained from Dow Chem. Co., M.p. 95-7°. [k] M.p. depressed by each of other two derivatives having similar m.p. [l] From benzene-methanol.

2,2′-methylene-*bis*-(4-methylphenol), 4,4′-*iso*-propylidene-*bis*-(2-methylphenol), and 4,4′-*iso*-propylidene-*bis*-(2-*iso*-propylphenol). By using a variety of secondary amines, each of these bisphenols was converted into symmetrical di-Mannich derivatives by the method described by Meadow and Reid (**1**). These compounds are listed as free bases in Tables I and II. The yields in most cases ranged from 70 to 85%.

EXPERIMENTAL

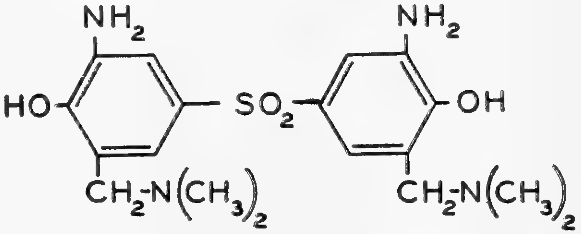
All melting points were taken with a Fisher-Johns apparatus, and are uncorrected. The analytical results were furnished by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

1. *2,2′-Thio bis-(4-chlorophenol)* was prepared by the method of Dunning (**5**) using a cold SCl_2 solution in CS_2 and *p*-chlorophenol dissolved in CCl_4 After recrystallization from benzene, crystals melting at 174-175.5° (lit. 173°) were obtained. The other bisphenols were obtained from commercial sources and were purified by recrystallization from the appropriate solvents.

2. *Preparation of Mannich Compounds.*—A representative example of the di-Mannich derivatives from the bisphenols is 4,4′-thio-*bis*-(2-*tert.*-butyl-5-methyl-6-piperidinomethylphenol). A mixture of 7.2 g. (0.2 mole) of the phenol (See Table I), 7.7 g. (0.09) of piperidine, and 30 ml. of absolute alcohol was cooled and 5.7 g. (0.07 mole) of aqueous HCHO (37%) was added slowly over a 20-minute period with intermittent shaking and cooling. After standing 45 minutes at room temperature the mixture was refluxed for 4 hours. An oil separated during the first hour of heating; this later solidified on cooling or chilling for several hours. A pale pink solid, 11.2 g., was removed by suction filtration, washed with methanol, and then recrystallized by dissolving in a minimum amount of benzene, filtering, and adding 75 to 100 ml. of hot methanol; 9.2 g. (83%) of small white crystals melting at 183-184° were obtained. (See Table I for analyses.)

Other Mannich derivatives were made in a similar way, but each compound usually required a slightly different solvent technique in order to recover it and purify it; some of these details are mentioned briefly in Tables I and II. Each of the free bases was readily solubilized in water by salting it with a strong acid.

3. *Isolation of 4.4′-Sulfonyl-bis-(2-amino-6-dimethylaminomethylphenol).*—By acetylating the two amino groups in 4,4′-sulfonyl-*bis*-(2-aminophenol) it was possible to introduce a Mannich group in each of the other two ortho positions (See Table I). The acetamido groups could then be removed by hydrolysis and a free base with the following structure obtained:



150 ml. of 20% aqueous HCl was added cautiously to 28.7 g. (0.06 mole) of 4,4′-sulfonyl-*bis*-(2-acetamido-6-dimethylaminomethylphenol) and the mixture refluxed for 3 hours. The solvent was then removed by reduced pressure distillation, after which 50 ml. of benzene and 50 ml. of absolute ethanol were added and distilled from the reaction mixture. The crude tetra-hydrochloride so obtained was dried overnight in a vacuum desiccator; after two recrystallizations from methanol 29.8 g. (92%) of a tan crystalline powder, M.p. 176-7.5° dec., was recovered.

An aqueous solution of 1.0 g. of the tetra-hydrochloride of 4,4′-sulfonyl-*bis*-(2-amino-6-dimethylaminomethylphenol) in 40 ml. of water was made slightly basic with ammonia and extracted with four 40 ml. portions of $CHCl_3$. The solvent was evaporated from the combined extracts by a stream of air and the residue recrystallized from anhydrous methanol to give 0.35 g. (45%) of tan crystals which decomposed without melting when heated above 180°. *Anal.* Calcd. for $C_{18}H_{26}N_4O_4S$: N, 14.21. Found: N, 14.18.

SUMMARY

Several bisphenols containing central linkage groups such as -S-, $-SO_2-$, $-CH_2-$, $-C(CH_3)_2-$, have been converted into various symmetrical di-amino and tetra-amino derivatives by use of the Mannich reaction. Thirty-three such compounds are listed in Tables I and II. Many of these new derivatives have shown some interesting physiological properties.

ACKNOWLEDGMENTS

The authors wish to thank Dr. C. F. Geschickter, Georgetown University Medical School, and Dr. E. Emmet Reid, Baltimore, Md., for their helpful suggestions in connection with this work.

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Accepted for publication 16 January 1958.

Effect of Certain Variables on the Determination of Iodine Values of Waxes by the Hanus Method

SISTER MARY CONCETTA WALLER, O. S. U. AND JANE F. FUSSENEGGER
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INTRODUCTION

WITH the increasing attention given to the properties and uses of plastic materials there has been a renewal of interest in the chemistry and technology of the natural waxes. A series of studies of the physical constants of the natural waxes and wax mixtures has been made in recent years (1, 2, 3). In this paper of the series the iodine number or ''iodine absorption value'' of the natural waxes and wax mixtures will be discussed.

Iodine number values given in the literature range over a number of units. Values determined by the same method and reported by different investigators are often quite dissimilar. This is true because of the impurities sometimes present in the samples under study. Highly purified samples should show better agreement of results. The method itself was questioned. Can the Hanus method, as it is usually followed, be expected to give accurate results when determinations are made on natural waxes and natural wax mixtures?

Iodine values were studied using the following methods of determination: (1) Hanus method (4) modified by shaking the mixture for 30 minutes in the dark using a Burrell-Wrist-Action Shaker; (2) Ubaldini-Capizzi-Maitan method (5) modified by shaking the mixture for 30 minutes; (3) Ubaldini-Capizzi-Maitan method modified by using chloroform in place of carbon tetrachloride for the solvent.

RESULTS AND DISCUSSION

Any conclusions from iodine determinations must be drawn cautiously. Many factors influence these results and reproducible iodine values can be obtained only under controlled conditions. Table I shows that such conditions are possible.

These values were obtained using different preparations of Hanus solution and sodium thiosulfate titrating solution. The effect of time using the Hanus method was studied over a week's period.

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TABLE I. IODINE NUMBERS. PRECISION OF RESULTS

Wax Sample	Conditions	I	II	III	Average
Refined Beeswax..	Sample in contact with Hanus Solution, 30 minutes, 25° C	7.4	7.7	7.5	7.5
Refined Beeswax..	Sample in contact with Hanus Solution, one week, 25° C	12.9	13.2	12.6	12.9
Candelilla Wax...	Sample in contact with Hanus Solution, 30 minutes, 25° C	29.2	29.2	29.3	29.2
Candelilla Wax...	Sample in contact with Hanus Solution, one week, 25° C	44.9	44.5	44.2	44.5

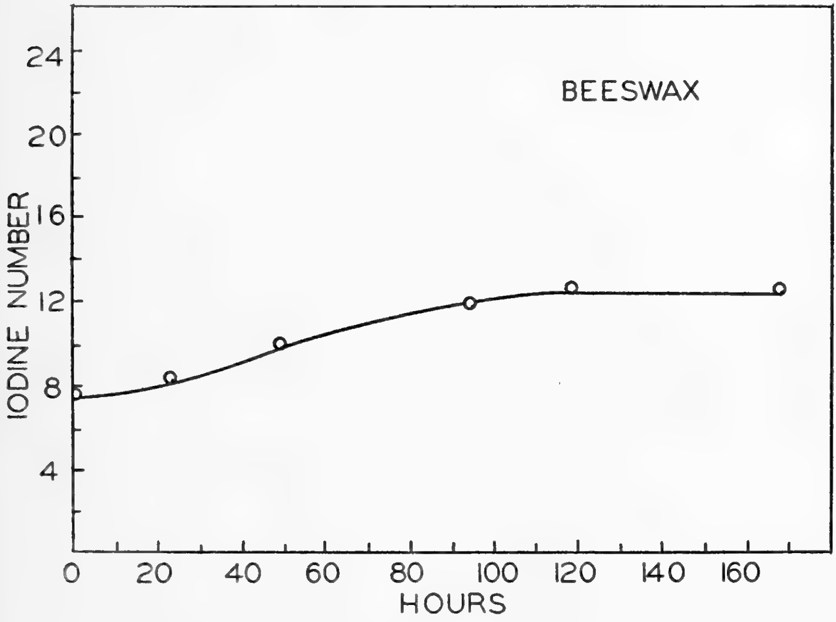

FIGURE 1. Iodine Numbers for Refined Beeswax

Figure 1 shows that the maximum iodine number for refined beeswax was reached after 5 days. The maximum value for carnauba wax was reached after about 50 hours with the greatest increase in the values occurring during the first five hours. Figure 2 shows that the maximum iodine number for candelilla wax was not reached until the end of a week's period. In each of these cases the reaction is more rapid in the beginning and gradually levels off to some constant value. A closer inspection of Figures 1 and 2 and a study of the values in Table I will convince the reader that unless the time element is allowed for, variations in the iodine number values will result.

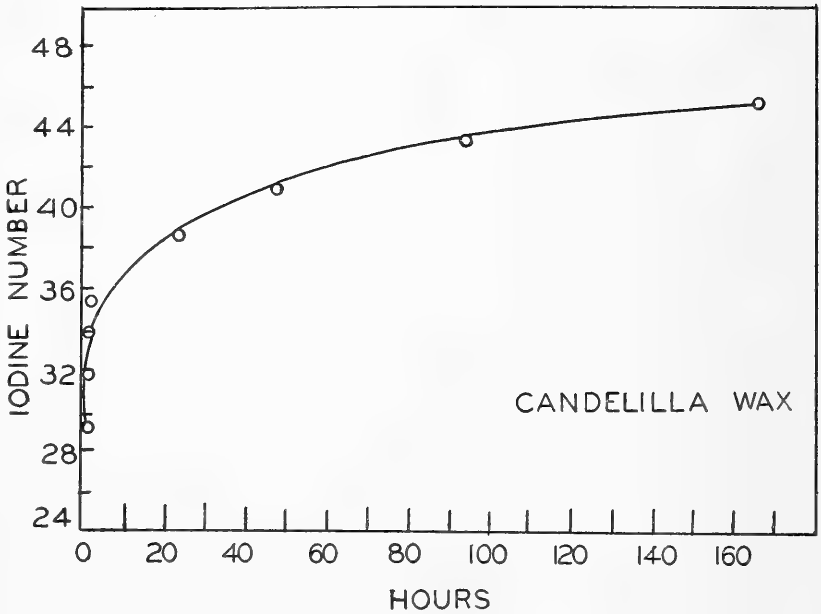

FIGURE 2. Iodine Numbers for Candelilla Wax

Possible side reactions during an iodine determination can be considerable, so that it is dangerous to assume that the highest value is the correct one. In waxes where there is a variety of unsaturated bonds it can be quite difficult to arrive at the absolutely correct iodine number. Table II gives the iodine numbers as determined by the three methods used in this study. A comparison of these values indicated the importance of the solvent in these determinations. Good checks were obtained when chloroform was used as the solvent. (Compare columns I and II of Table II.) Higher values were obtained when carbon tetrachloride together with mercuric acetate were employed. The waxes are more soluble in carbon tetrachloride than in chloroform.

TABLE II. IODINE NUMBERS

Wax Sample	Hanus Method	I_2 in $CHCl_3$	I_2 in CCl_4
Insect Waxes			
Refined Beeswax, Sample 1	7.5	7.5	7.9
Refined Beeswax, Sample 2	12.4	12.1	13.0
Chinese Insect Wax	4.0	4.4	7.0
Vegetable Waxes			
Candelilla Wax, Sample 1	29.2	29.3	31.1
Candelilla Wax, Sample 2	33.0	33.0	34.5
Carnauba Wax, Sample 1	19.4	19.1	37.8
Carnauba Wax, Sample 2	19.7	19.4	40.4
Japan Wax, Sample 1	13.9	14.4	15.2
Japan Wax, Sample 2	21.3	21.8	22.2
Mineral Wax			
Montan Wax	42.6	42.9	45.9
Ozocerite Wax	2.2	2.9	1.7
Paraffin Wax	0.3	0.4	1.0

Figure 3 is an example of how combined conditions effect results. (1) Japan wax is more soluble in chloroform than is candelilla wax. (2) Shaking the samples will hasten the solubility of the waxes in the reaction mixture. (3) Candelilla wax is a more unsaturated wax than Japan wax and therefore has a higher iodine number than Japan wax. These results indicate that complete solution of the sample may be the second variable responsible for the disagreement of Hanus iodine number values in the literature. A comparison of Figure 3 with Figure 2 shows that these may not be the final values for the mixtures because the curve has not reached the maximum value for candelilla wax.

The effect of allowing the sample to remain in contact with the chloroform for a week or two weeks before adding the Hanus solution was studied. The iodine number values agreed within eight parts per thousand with those obtained when the Hanus solution had been added after dissolving the sample. This indicates very little solvent interaction between wax sample and chloroform when the latter is the solvent. Spectra in the visible and ultraviolet regions of wax samples dissolved in carbon tetrachloride were studied with the Beckman DU spectrophotometer over a period of eight days. The spectra remained the same over this period of time again indicating no interaction between wax and solvent.

After these studies had been completed the iodine numbers of

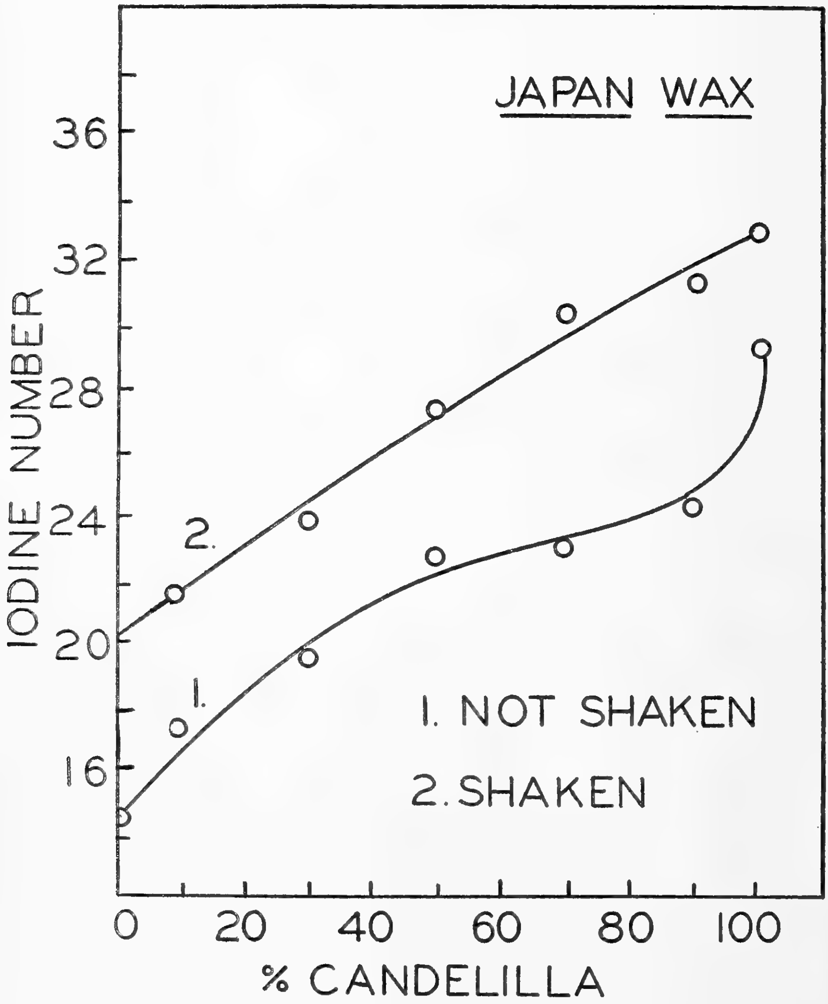

FIGURE 3. Iodine Numbers for Japan Wax—Candelilla Wax Mixtures

wax mixtures according to the Hanus method were experimentally determined. The mixtures were shaken during the time they were kept in contact with the Hanus solution to hasten the solution of the wax samples in the reaction mixture, and to provide uniform treatment of the components in each of the determinations. Figure 4 gives the iodine number values for carnauba wax mixtures. These curves present some of the greatest deviations from calculated values

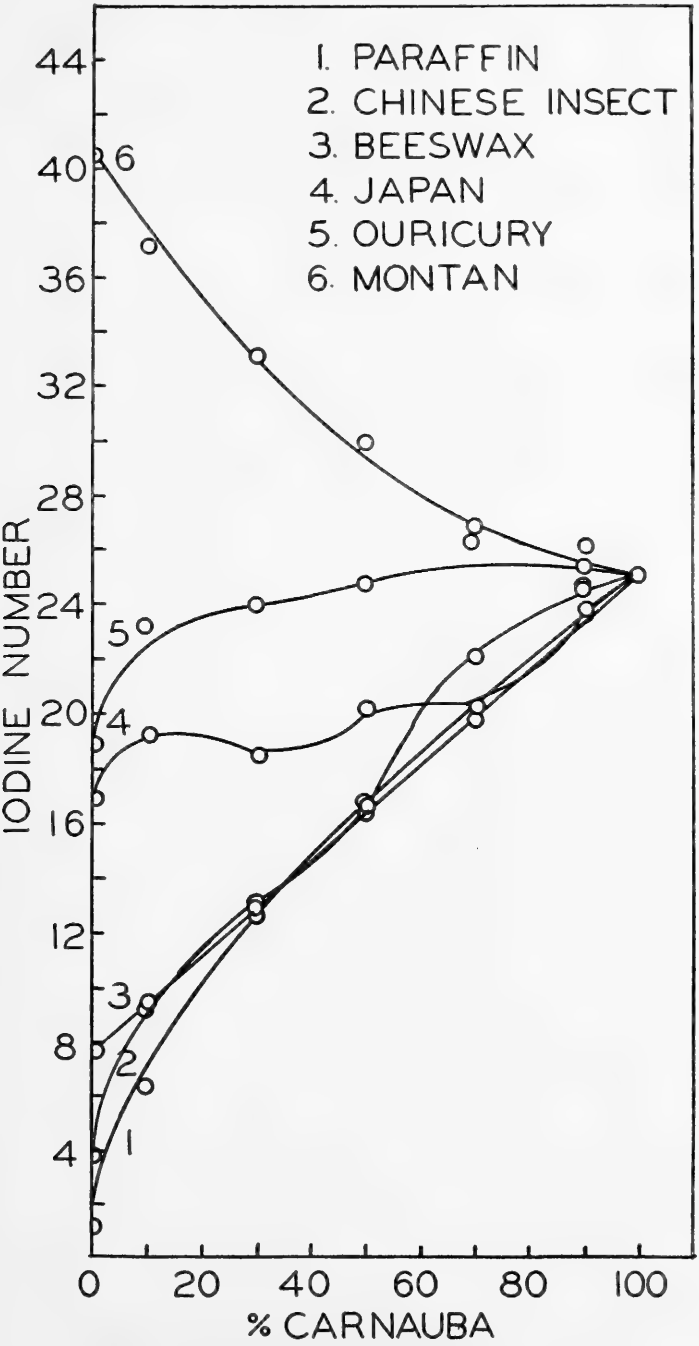

FIGURE 4. Iodine Numbers of Carnauba Wax Mixtures

that were observed in the study. Ouricury-carnauba wax mixtures gave much higher than anticipated values, whereas the montan-carnauba wax mixtures gave values lower than the calculated values.

Several other interesting combinations were noted. The candelilla wax-montan wax mixtures gave a sigmoid curve. The eutectic mixture for paraffin wax-beeswax gave a minimum iodine number in that series. Montan wax (the so-called Czechoslovakian Pure Montan Wax) a mineral wax with the highest iodine number of any of the waxes studied did not show straight line relationships in any series of its mixtures. The greatest deviations from calculated straight line values were obtained with waxes that have a high degree of unsaturation. Most of the paraffin wax and beeswax blends gave values that deviated by less than 5% from the calculated values.

SUMMARY

1. Procedures used during the determination of iodine number values according to the Hanus method may not be suitable for waxes and may be responsible for some of the deviations to be noted in reported data.
2. The iodine numbers of natural waxes and wax mixtures are greatly influenced by light, temperature, the solvent, and the length of time the sample remains in contact with the iodine solution.
3. Shaking the mixtures while they are in contact with the iodine solutions provides uniform treatment of the components in each of the determinations and gives greater precision in the final calculations.
4. A higher iodine number value than that calculated may be due to side reactions such as substitution reactions. At other times the functional groups present in the waxes may retard addition reactions.
5. A suggestion for further work would be the determination of hydrogen numbers of the waxes and the comparison of these values with the iodine number values. This would give a clearer idea of how much of the iodine number value is measuring the unsaturation of the wax, and to what extent substitution reactions and side reactions are involved.

ACKNOWLEDGMENTS

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Some Chromosome Numbers in Kentucky Characeae

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Introduction

Two genera of charophytes, *Chara* and *Nitella,* are known to occur in Kentucky, and a third genus, *Tolypella,* may possibly be found in the state. From the general lack of references concerning the Kentucky Characeae, it is apparent that very little collecting has been done in this state. The specimens collected for the present study were taken from a limited area comprising Jefferson, Meade, Nelson, and Daviess counties as shown in Table I. They have been deposited with Mrs. F. K. Daily, Butler University, Dr. R. D. Wood, University of Rhode Island, and in the Herbarium of the University of Louisville.

Table I. Chromosome Numbers in Some Species of Characeae from Kentucky

Species Name	Chromosome Number	Location
Chara contraria A. Br.......	28	1. Muldraugh Hill, Fort Knox, Meade Co. 2. Knobs area, Deatsville, Nelson Co.
Chara globularis Thuill......	28	1-3. Small ponds, Knobs area, Deatsville, Nelson Co. 4. Carpenter's Lake, Daviess Co.
Chara sejuncta A. Br.......	14	1. Sinkhole pond, limestone area, Meade Co.
Nitella acuminata var. *subglomerata* A. Br......	18	1. Sinkhole pond, limestone area, Meade Co. 2. South Park Pond, Knobs area, Jefferson Co.

Following the squash method and aceto-orcein staining, chromosome counts from antheridial mitoses were made on each of the four species shown in Table I. Camera lucida drawings of these species are shown in Figs. 1-5. Slides of *Chara contraria* A. Br. consistently

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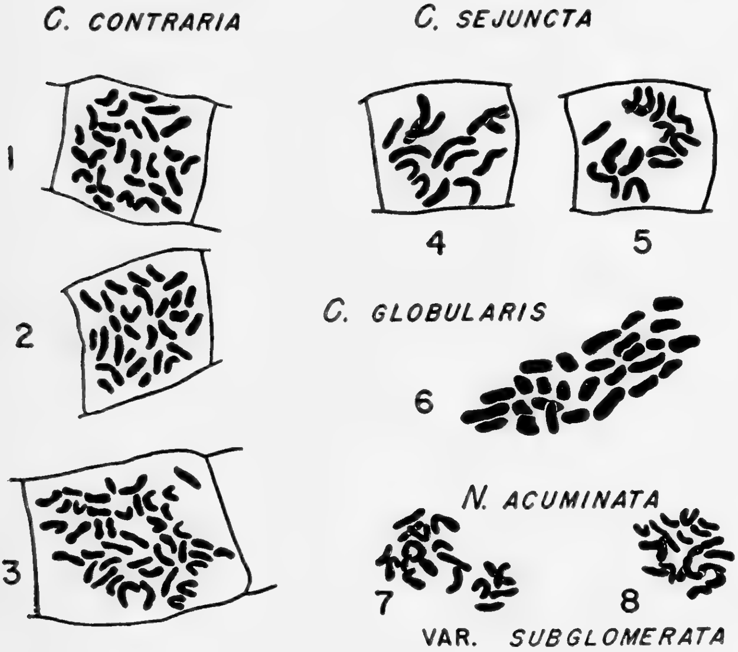
showed 28 chromosomes with the exception of one plant found to have 42 (Fig. 3).



FIGURES 1-3: *Chara contraria,* metaphase of mitoses in antheridial filaments. Figures 1-2 showing the usual chromosome complement, n = 28. Figure 3 showing the aberrant complement, n = 42. All ×900.

FIGURES 4-5: *Chara sejuncta,* metaphase of mitoses in antheridial filaments, n = 14. ×900.

FIGURE 6: *Chara globularis,* metaphase of mitosis in antheridial filament, n = 28. ×1200.

FIGURES 7-8: *Nitella acuminata* var. *subglomerata,* metaphase of mitoses in antheridial filaments, n = 18. ×900.

DISCUSSION

Various workers have reported chromosome numbers for the Characeae. In 1897 Debski, who was searching for the reduction division in *Chara fragilis* Desv. (= *C. globularis* Thuill.), found 23, 24, and 25 chromosomes in mitotic divisions of the antheridial filaments. He interpreted the 23 as "hidden," the 25 as a probable knife dissection, and arrived at 24 as the chromosome number for this species. The difficulties encountered in working with sections are summarized in Debski's conclusion that: "Im Mittel ergeben sich aber 24, und ich habe nie weniger als 20 und nie mehr als 28 gefunden.'' Oehlkers (1916) by microtome sectioning at 30 μ found 24 chromosomes in *Chara fragilis.* Lindenbein (1927) and Telézýnski (1929) have made lists of chromosome numbers for the Characeae. The information and sources they accumulated, together with some more recent data are included in Table II.

TABLE II. CORRELATION OF PLOIDY AND SEX IN THE GENUS *Chara.*

Species Names (as published)	Chromosome Number	Authority		Dioecious, Monoecious or Disjunct.
Chara aspera	12	Ernst	1918	Dioecious
	12	Lindenbein	1927	
Chara crinita	12	Ernst	1918	Dioecious
	24	Lindenbein	1927	
Chara galioides	12	Ernst	1918	Dioecious
Chara australis	14	Macdonald & Hotchkiss	1955	Dioecious
* *Tolypellopsis stelligera*	14	Telézýnski	1929	Dioecious
Chara ceratophylla	14	Telézýnski	1929	Dioecious
Chara coronata	14	Karling	1928	Dioecious
Chara sejuncta	14	A. & D. Hotchkiss		Disjunct
Chara fragilis	16	Riker	1921	Monoecious
	18	Strasburger	1908	
	24	Debski	1897	
	24	Oehlkers	1916	
	24	Lindenbein	1927	
	24	Telézýnski	1929	
	28	A. & D. Hotchkiss		
Chara contraria	*ca.* 30	Lindenbein	1927	Monoecious
	ca. 24	Schmucker	1927	
	28	Telézýnski	1929	
	28	A. & D. Hotchkiss		
Chara delicatula	24	Lindenbein	1927	Monoecious
	more than 40	Karling	1928	

*Included here for comparison.

Because of the difference of opinion among the older workers, it is apparent that verification of chromosome numbers in some species by use of newer methods may be necessary. Material of many additional species, and material from wide-ranging sources for each species must be studied before a complete list of chromosome numbers in the Characeae can be given. Since our number, 28, for *Chara globularis* is different from several previously published reports for this species (*C. fragilis* Desv.), we have carefully rechecked our count. Variations in chromosome number may exist and this factor is another reason for making confirmatory studies. The rather surprising number 42 appearing in a single plant of *C. contraria* (Fig. 3) was found in antheridial filaments of a larger diameter than those with 28 chromosomes (Fig. 1, 2). In this small group of species the chromosomes of *C. sejuncta* A. Br. appear distinctive because of their somewhat larger size.

Telézýnski (1929 suggested that *Chara fragilis* with a chromosome number of 24 was an autopolyploid. The arrangement of selected species of *Chara* in Table II suggests a polyploid series with 12 and 24, and another series with 14 and 28 chromosomes.

It has been observed (Macdonald and Hotchkiss, 1955) that a dioecious species has a chromosome number of 14. When *Chara* species with the lower chromosome numbers are tabulated as in Table II, it is seen that, in general, they are dioecious or disjunct in the distribution of sex organs. Furthermore the species with higher chromosome numbers are monoecious with the antheridia and gametangia together at the same node. From these facts comes the suggestion that there may be a relationship between ploidy and sex-determination in *Chara*. At this time, however, it is impossible to make a general statement which will fit all cases or which will describe the mechanism of sex-determination. Any such hypothesis will be on firmer ground when the chromosome numbers of all or most of the species have been determined.

ACKNOWLEDGMENTS

I am greatly indebted to Mrs. F. K. Daily, Butler University, and to Dr. R. D. Wood, University of Rhode Island, for the identification of this material. Thanks are due to the Department of Botany, Indiana University, for the use of a microscope, and to the Cornell University Library for the use of its facilities. Finally, I must acknowledge with thanks the aid and assistance of my wife in counting the chromosomes and preparing the illustrations.

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Influence of Polyvinyl-Pyrrolidone Compounds on the Post-Irradiation Syndrome

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Introduction

Blood volume expanders and plasma substitutes, of limited use in peacetime, may become essential for use in the event of certain civilian and military emergencies. In some situations a high percentage of victims will probably suffer from exposure to ionizing radiations in addition to blast, burns, crash and shock injury. The possibility of detrimental effects of administered expanders and substitutes in such cases has been pointed out at several occasions, leading to detailed reinvestigations of the combined effects of these compounds and irradiation injury. While in the case of the glucose polymer, dextran, a recent investigation by Blondal (1) establishes definitely the beneficial effect of this compound in modifying the acute post-irradiation syndrome, the role of the polyvinyl-pyrrolidone compounds (PVP) is not yet clear. Rugh, Suess and Scudder (2) found no protective action of PVP-macrose and PVP-dextrose (average molecular weight about 40,000) on the post-irradiation syndrome in mice. Burger, Grabinger and Lehmann (3), using the low molecular weight compound Periston "N" (average molecular weight 12,600)* in post-irradiation treatment of total body X-irradiated rats, reported beneficial effects. However, their results could not be confirmed by other investigators (4, 5). In addition, a critical review of some of the later studies indicates a tendency of reverse effects caused by PVP plasma expanders in a total-body X-irradiated organism, justifying a detailed study of the different factors entering the picture. Thus the effects of post-irradiation-administered PVP compounds on total body X-irradiated mice were systematically studied as to the dependence of mole-

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* Periston "N" is produced by Farbenfabriken Bayer, Leverkusen, Germany and is recommended for "serum and tissue-lavage." The Periston "N" used in these experiments was generously supplied by Farbenfabriken Bayer, Leverkusen, Germany.

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cular weight, administered amounts, radiation quality and radiation quantity.

EXPERIMENTAL

875 female CF-1 mice, weighing 21 ± 2 grams, were used in the study. They were irradiated, in groups of ten, in a well ventilated lucite cage with soft (100 kvp) and hard (250 kvp) X-rays from a General Electric Deep-Therapy Unit. The softer radiation was delivered at 100 kvp, 30 ma, 50 cm TSD, 4.75 mm inherent Be filtration, no added filter, HVL 0.83 mm Al, dose rate of 170 r/min in air. The harder radiation was given at 250 kvp, 30 ma, 1 mm Al plus 0.5 mm Cu added filtration, HVL 1.1 mm Cu and a dose rate of 115 r/min in air, all other conditions remaining unchanged.

The PVP compounds were injected intraperitoneally at time intervals of 1, 24, 48 and 72 hours after irradiation. Control animals were injected with saline and treated in a like manner. All animals were fed Purina Chow pellets and given water *ad libitum.* They were observed for 30 days post irradiation, the number of deaths being recorded every 24 hours.

RESULTS AND DISCUSSION

In order to test the toxicity of the compounds, Periston "N", PVP K-21, K-30, K-60, and K-90 were injected in amounts of 0.25, 0.5, 1.0 and 2.0 ml per injection per mouse, four times at 24 hour intervals. Survival of the animals was taken as toxicity criterion. For this purpose the animals were kept under observation for a thirty-day period, during which no ill effects were noted.

The influence of molecular weight on survival after 700 r X-irradiation with a relatively soft X-radiation and with a harder quality radiation is presented in Figure 1. While in the case of the harder radiation (250 kvp) neither beneficial nor damaging effects of the different PVP compounds are afforded, the experiment with the softer radiation (100 kvp) shows the tendency of a detrimental effect for the higher molecular weight compounds, especially for PVP K-90 with an average molecular weight of approximately 150,000. In addition, the results do not verify the findings by Burger *et al* (**3**) who speculate that low molecular weight PVP compounds, able to easily pass the kidney barrier, also should excrete to a high degree possible radiation-produced "toxic" substances (**6**). The curves for the 250 kvp radiation show practically no beneficial effects of this kind, thus confirming recent findings by Becker and Kirchberg (**4**) and others.

Also changing the amounts of the administered expanders does not contribute significantly to counteract the radiation damage. Figure 2 shows the influence of different amounts of Periston "N" on animals

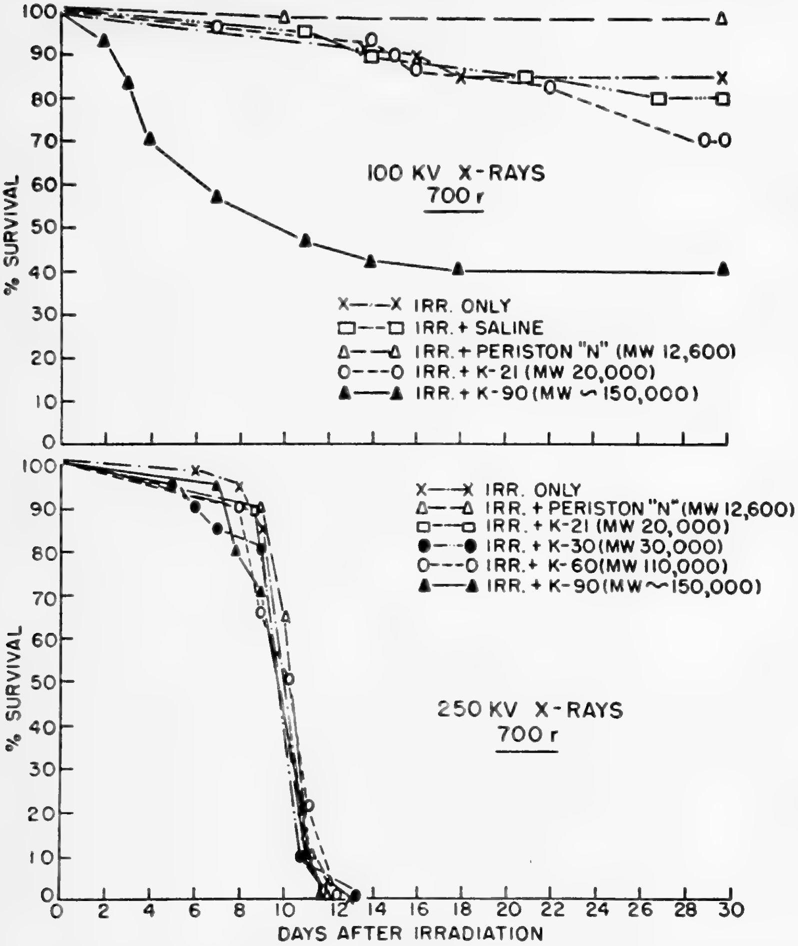

FIG. 1 INFLUENCE OF PVP MOLECULAR WEIGHT ON SURVIVAL OF X-IRRADIATED MICE, FOUR 0.5cc POST-IRRADIATION INJECTIONS 24 HOURS APART.

irradiated with 700 r, 900 r and 1100 r, respectively, of a 100 kvp X-radiation. While in the 700 group neither beneficial nor detrimental effects exist, in the 900 r-group higher amounts of the PVP-compound produce reverse effects, a tendency which becomes even more evident in the 1100 r X-irradiated group.

It must be concluded, therefore, from the reported study that the PVP-compounds K-21, K-30, K-60 and K-90 with molecular weights

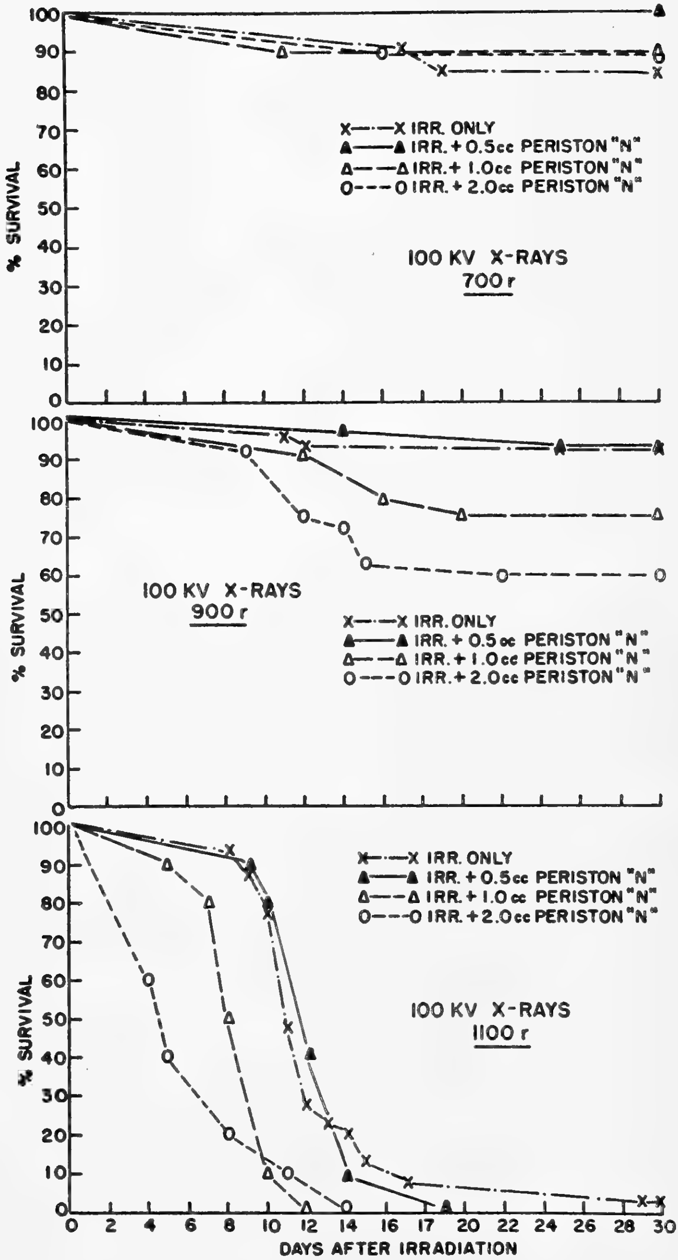

FIG. 2 INFLUENCE OF PERISTON "N" AMOUNTS ON SURVIVAL OF X-IRRADIATED MICE, FOUR POST-IRRADIATION INJECTIONS 24 HOURS APART.

between 20,000 and 150,000 do not afford any post-irradiation protection. On the contrary, depending on radiation dose and radiation quality, they may produce reverse effects by enhancing lethality in critical dose ranges.

Also, the low molecular weight compound Periston "N" (average molecular weight 12,600) does not prove of value in counteracting the post-irradiation syndrome. There may be the tendency to protect in the lower radiation-dose-ranges, when administered in small amounts; amounts higher than 0.5 ml per 20 gram mouse detrimentally affect animals irradiated with 900 r and higher doses.

These facts should be kept in mind during emergency situations, when the victim to be treated with expanders may also have been exposed to ionizing radiations. In the case of dextran, as recently shown, the danger of reverse effects may be less and it is thoroughly possible to find in a systematic research substances which will allow replacement of blood losses and at the same time afford beneficial effects in the post-irradiation syndrome. However, as long as such substances have not been studied in detail as to possible synergistic effects, caution should be taken in applying them to cases where the victim may have suffered not only from blast, crash, shock and burns, but also from exposure to high doses of ionizing radiations.

SUMMARY

The influence of post-irradiation administered polyvinyl-pyrollidone compounds, including the low-molecular weight Periston "N", on the post-irradiation syndrome in mice was studied in detail. No beneficial effects were observed for the high molecular weight PVP-compounds. For certain radiation doses and radiation qualities, the high molecular weight PVP compounds even produced detrimental effects by enhancing lethality. Low molecular weight Periston "N" in large amounts caused reverse effects for the higher dose levels. These results should be kept in mind when in emergency cases victims, who may have been exposed to ionizing radiations, require treatment with plasma substitutes.

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The Rate of Development of *Sigmodon hispidus* as Compared with Some Other Rodents

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THIS paper is based on a comparative study of the developmental rates of some rodents with particular emphasis on *Sigmodon hispidus,* the cotton rat. Data for this publication were taken from the papers of the various authors listed in the bibliography which follows, and are presented graphically in Figure 1.

It is particularly significant that developmental rates in *Cavia, Mus, Rattus, Cricetus,* and *Sigmodon* almost coincide at 84 hours of development, the early blastocyst stage (Figure 1). The cleaving egg, prior to implantation, seems to have approximately equal developmental potentialities in all these rodents.

In spite of the general similarities in developmental rates, there are some significant differences. Through the first 38 hours of development *Sigmodon* has the most rapid rate of development of the rodents compared (Figure 1). In the 38 to 84 hour interval *Mus* has the fastest rate. At 84 hours *Sigmodon* reassumes the most rapid rate of development, and this rate is not exceeded again by any other animal compared until 170 hours, when *Cricetus* and *Mus* exceed *Sigmodon* in rapidity of development. After implantation the developmental rates begin to diverge widely, and seem to correspond closely to the gestation period for each rodent (Figure 1).

Upon first inspection, the rapid rate of development of *Sigmodon* is surprising, because this animal has a gestation period as long as 27 to 28 days. However, this developmental rate is readily understood when one is cognizant of the advanced state of the young at birth and shortly thereafter. At parturition *Sigmodon* has a furry coat; a few hours after paturition the young will run over the bottom of the cage, if alarmed; approximately two days following birth the eyes of the young rats are open; at the age of eight days the young feed on adult rations, and they can be weaned by 15 days.

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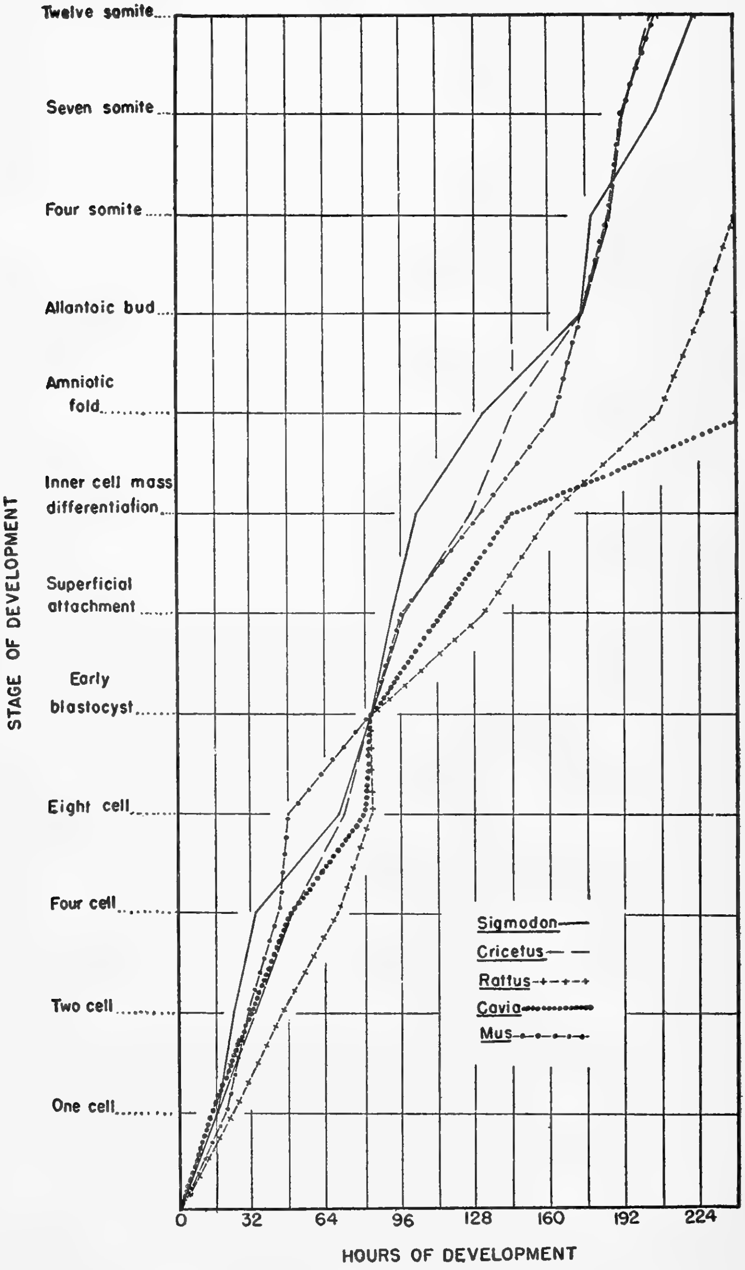
Figure I. Graph Showing Comparative Developmental Rates Among Some Rodents



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The Conductance Behavior of Some Lithium, Strontium and Barium Salts in N,N-Dimethylacetamide at 25°C.*

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THE conductance behavior of twenty potassium, sodium and tetraalkylammonium salts in N,N-dimethylacetamide (DMA) has been discussed in a recent paper from this Laboratory (**1**). With exceptions for a few bromides, nitrates and benzenesulfonates, the behavior of uni-univalent electrolytes in DMA was found to conform very closely with that predicted theoretically by the Onsager equation (**2**). Probably the most interesting result of the previous study, however, was finding the first medium in which the limiting equivalent conductance of the sodium ion is greater than that of the potassium ion. Subsequent studies from this Laboratory (**3**, **4**) have revealed that the unusual reversal of the ionic conductances occurs also in N,N-dimethylpropionamide and N,N-dimethylbutyramide solutions.

The objectives of further research on solutions of electrolytes in DMA, which is discussed herein, were to investigate the behavior of some lithium salts and to compare the results with those for corresponding potassium and sodium salts, to investigate the behavior of some multivalent electrolytes, to compare the behavior of corresponding hydrated and anhydrous salts, and to extend generally the existing knowledge concerning the potentialities of DMA as an electrolytic solvent.

EXPERIMENTAL

1. *Preparation and Purification of Solvent.*—These have been explained in detail in the previous paper related to DMA solutions (**1**).

2. *Salts.*—Reagent grade salts which were purchased from the Mallinckrodt Chemical Company and the G. Frederick Smith Chemical Company were used without further purification. The non-hydrated salts were dried to constant weight in a vacuum oven at an appropriate temperature. All of the salts were analyzed for water content by the Karl Fischer method (**5**) using Fisher stabi-

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lized single-solution Karl Fischer reagent which was standardized against sodium acetate trihydrate (**6**). The molecular weights of the hydrated salts were adjusted in necessary cases to conform with the analytical data. For convenience, each hydrate is listed in the following tables as the one containing the nearest integral number of associated water molecules.

3. *Procedure and Apparatus.*—The electrical equipment, cells, temperature bath and control, and procedures involved in the determination of the physical properties of the solvent, the preparation of solutions and the measurement of resistances have been described previously (**1**, **3**, **7**).

Discussion and Results

Corresponding values of the equivalent conductance, Λ, and the concentration in gram-equivalents per liter, C, for each of the twelve solutes are presented in Table I.

Table I. Equivalent Conductances of Salts in DMA at 25°C.

$C \times 10^4$	Λ	$C \times 10^4$	Λ	$C \times 10^4$	Λ
(a) $LiNO_3$		(b) $LiClO_4$		(c) $LiClO_4.3H_2O$	
1.489	66.7	1.010	63.6	2.469	62.9
4.344	65.6	2.726	63.0	6.889	61.7
14.70	63.4	11.85	60.9	17.04	60.1
33.54	61.0	23.84	59.4	29.19	58.6
64.72	58.4	39.95	58.0	43.51	57.4
103.8	56.0	60.12	56.7	59.10	56.2
(d) $NaO_3SC_6H_4NHC_6H_5$		(e) $Ba(O_3SC_6H_4NHC_6H_5)_2$		(f) $BaBr_2$	
0.9435	44.7	0.6114	49.9	3.059	71.1
5.386	42.2	3.110	42.6	8.192	66.6
11.77	40.1	8.011	35.9	17.03	62.0
21.94	37.8	15.44	30.9	29.75	57.7
33.81	35.7	26.78	26.8	44.51	54.3
(g) $Ba(ClO_4)_2$		(h) $Ba(ClO_4)_2.3H_2O$		(i) $Sr(ClO_4)_2$	
4.999	71.9	4.670	72.0	4.733	71.5
12.35	68.8	15.69	67.8	13.43	68.0
28.56	65.0	48.69	62.2	26.06	65.1
49.43	62.1	89.56	58.6	43.49	62.7
72.77	59.8	127.0	56.4	66.74	60.1
96.82	58.1	174.0	54.4	86.33	58.6
(j) $Sr(ClO_4)_2.3H_2O$		(k) $SrBr_2.H_2O$		(l) $SrBr_2.6H_2O$	
7.982	69.9	0.6467	74.6	0.5894	74.5
20.11	66.2	1.741	72.7	1.595	72.7
42.62	62.4	6.859	67.3	5.517	68.3
74.26	59.2	13.81	63.2	14.53	62.7
109.5	56.8	22.13	59.8	26.35	58.3
152.4	54.8	33.99	56.6	42.54	54.4

The phoreograms (**8**) for all of the salts except sodium diphenylaminesulfonate are convex with respect to the concentration axis but exhibit differing slopes. In order to compare the observed results with those predicted theoretically, utilization is made of the Onsager equation which may be written conveniently in the simplified general form

1. $$\Lambda = \Lambda_0 - [\alpha\Lambda_0 + \beta]\sqrt{C}$$

in which C is the concentration in gram-equivalents per liter, Λ and Λ_0 are the equivalent conductances at concentration C and at infinite dilution respectively, and α and β are constants for which the values are fixed by the dielectic constant and viscosity of the solvent, the number of charges on each of the ions, and the absolute temperature. For DMA solutions at 25°C., the following expressions of equation (1) applicable to 1-1 salts and

2. $$\Lambda = \Lambda_0 - [0.686\,\Lambda_0 + 84.5]\sqrt{C}$$

2-1 salts respectively.

3. $$\Lambda = \Lambda_0 - \left[\frac{3.824\,\Lambda_0}{(1 + T_0^- + 0.816\sqrt{1 + T_0^-})} + 155.3\right]\sqrt{C}$$

In equation (3), T_0^- is the limiting value of the transference number of the anion. Existing values of the limiting equivalent conductances, λ_0^-, of the pertinent anions from the earlier study (**1**) make possible the convenient evaluation, $T_0^- = \frac{\lambda_0^-}{\Lambda_0}$. According to the Onsager equation, a phoreogram (or plot of Λ *versus* $\sqrt{C}$) should have the limiting slope, $-[\alpha\Lambda_0 + \beta]$. Data pertinent to comparisons of experimental and theoretical slopes are summarized in Table II. These results reveal that the phoreograms for lithium nitrate and all of the anhydrous and hydrated perchlorates are anabatic whereas those for the remaining five solutes are catabatic.

The conductance data for the completely dissociated lithium salts can be analyzed advantageously and effectively through the use of plots of Λ'_0 *versus* C which are based on the Shedlovsky modification of the Onsager equation (**9**):

4. $$\Lambda'_0 \equiv \frac{\Lambda + \alpha\sqrt{C}}{1 - \beta\sqrt{C}} = \Lambda_0 + B\,C$$

The values for the intercept, Λ_0, and the slope, B, for each plot are

TABLE II. TEST OF THE APPLICABILITY OF THE ONSAGER EQUATION TO THE CONDUCTANCES OF ELECTROLYTES IN DMA AT 25°C.

Salt	Limiting Experimental Slope (S_E)	Limiting Theoretical Slope (S_T)	$\frac{(S_E - S_T)\,100}{S_T}$
$LiNO_3$	−123	−132	− 7
$LiClO_4$	−107	−129	− 17
$LiClO_4.3H_2O$	−111	−129	− 14
$NaO_3SC_6H_4NHC_6H_5$	−185	−117	58
$Ba(O_3SC_6H_4NHC_6H_5)_2$	−682	−246	177
$BaBr_2$	−392	−271	45
$SrBr_2.6H_2O$	−382	−270	42
$SrBr_2.H_2O$	−374	−270	39
$Sr(ClO_4)_2$	−256	−270	− 5
$Sr(ClO_4)_2.3H_2O$	−256	−270	− 5
$Ba(ClO_4)_2$	−255	−271	− 6
$Ba(ClO_4)_2.3H_2O$	−255	−271	− 6

summarized in Table III. The results in Table III are valuable in enabling a comparison to be made between the behavior of lithium salts and that of corresponding sodium and potassium salts which was described in the previous paper concerning DMA solutions. Although the limiting equivalent conductance of a sodium salt in DMA is, uniquely, slightly greater than that for the similar potassium salt, the limiting equivalent conductance of a lithium salt is at least 3 Λ-units less than that of either of the corresponding potassium and sodium salts. Hence, one of the objectives of this research is clarified in finding the conductance behavior of lithium salts in DMA to be relatively normal with respect to that of other alkali metal salts.

TABLE III. DATA PERTINENT TO PLOTS OF EQUATION (4) FOR SOME LITHIUM SALTS IN DMA AT 25°C.

Salt	Λ_o	B
$LiNO_3$	68.3	112
$LiClO_4$	65.0	287
$LiClO_4.3H_2O$	64.9	229

Solutions of sodium diphenylaminesulfonate in DMA are characterized by considerable ionic association. This is not surprising, however, inasmuch as similar behavior was observed previously for sodium benezenesulfonate solutions. Treatment of the data for sodium diphenylaminesulfonate by the Fuoss-Shedlovsky method (10) yields values of 45.9 ohm^{-1} cm.^{2} equiv.$^{-1}$ and 2×10^{-2} for the limiting equivalent conductance and the dissociation constant respectively.

The barium and strontium salts may be divided appropriately into two classes—the completely dissociated perchlorates and the incompletely dissociated bromides and barium diphenylaminesulfonate. It is interesting that the data for each of the eight 2-1 salts may be fitted to an equation of the form

5. $\Lambda = \Lambda_* - A\sqrt{C} + B\,C$

in which Λ_* is an arbitrary constant the value of which will make possible a linear plot of $(\Lambda_* - \Lambda)/\sqrt{C}$ *versus* $\sqrt{C}$ having an ordinate-intercept of A and a slope of B respectively. The results are presented in Table IV. Onsager (2) first showed that values of A from plots of equation (5) for many 2-1 salts in water often agree with the theoretical slope, $-[\alpha\,\Lambda_o + \beta]$, within 10-15%. As discernible from comparable data in Tables II and IV, values of A for the 2-1 perchlorates agree within 4% with the corresponding theoretical slopes. It is observable also that large deviations exist for the bromides and especially for barium diphenlyaminesulfonate. When there is a large deviation between A and the value of the Onsager slope, Λ_* and Λ_o are not synonymous.

TABLE IV. DATA PERTINENT TO PLOTS OF EQUATION (5) FOR SOME BARIUM AND STRONTIUM SALTS IN DMA AT 25°C.

Salt	Λ_*	A	B
$Ba(ClO_4)_2$	77.6	277	795
$Ba(ClO_4)_2.3H_2O$	77.6	277	795
$Sr(ClO_4)_2$	77.1	278	865
$Sr(ClO_4)_2.3H_2O$	77.1	277	795
$BaBr_2$	79.0	477	1620
$Ba(O_3SC_6H_4NHC_6H_5)_2$	56.6	900	6180
$SrBr_2.6H_2O$	78.5	481	1720
$SrBr_2.H_2O$	78.4	464	1560

Lange (11) has demonstrated that an equation of the form

6. $\Lambda + [\alpha\,\Lambda_o + \beta]\sqrt{C} = \Lambda_o + k\,C$

accurately represents the data for 560 strong (completely dissociated) electrolytes in a variety of solvents. The data for the solutions of barium and strontium perchlorates in DMA also may be represented very well by the equation. Value of the ordinate-intercepts, Λ_o, and of the slopes, k, from plots of $\Lambda + [\alpha\Lambda_o + \beta]$ *versus* C are compiled in

Table V. It may be noted that corresponding values of Λ_* and Λ_o in Tables IV and V are in excellent agreement.

TABLE V. DATA PERTINENT TO PLOTS OF EQUATION (6) FOR SOME BARIUM AND STRONTHIUM SALTS IN DMA AT 25°C.

Salt	Λ_o	k
$Ba(ClO_4)_2$	77.5	750
$Ba(ClO_4)_2.3H_2O$	77.5	740
$Sr(ClO_4)_2$	77.0	780
$Sr(ClO_4)_2.3H_2O$	76.9	740

It appears worthwhile also to mention that plots of equation (4) for the 2-1 perchlorates are curved quite similarly to those for barium chloride in water (12) and that an accurate linear extrapolation to Λ_o which is based upon data covering an appreciable concentration range is not feasible.

From the Λ_o-values for the lithium salts, sodium diphenylaminesulfonate and the 2-1 perchlorates together with data pertinent to limiting ionic equivalent conductances from the previous study, the values of the limiting equivalent conductances of the lithium, barium, strontium and diphenylaminesulfonate ions in DMA have been evaluated as 21.9, 34.8, 34.3 and 20.1 ohm^{-1} $cm.^{2}$ $equiv.^{-1}$ respectively. From the addition of known limiting equivalent conductances, it can be shown that the values of Λ_* in Table IV for the bromides and barium diphenylaminesulfonate are 1 to 2 Λ-units greater than the Λ_o values.

The limiting equivalent conductances of the strontium and barium ions are intermediate between those of the sodium and potassium ions in aqueous solutions but are 30-40% greater than those of the lithium, sodium and potassium ions which are leveled in DMA solutions. Obviously relative extents of solvation for given ions differ appreciably in the two media. Owing to the larger size of the solvent molecules, steric factors probably strongly influence the extent of solvation of small ions in DMA solutions.

Another interesting result from this study is that the conductance behavior of corresponding anhydrous and hydrated salts in DMA is identical within experimental error. Apparently the water originally associated with a hydrate loses its identity as such upon dissolution of the solute in DMA and effectively becomes a part of the solvent medium. In a 0.01 N solution of an alkaline earth salt trihydrate, for example, the DMA/water mole ratio is greater than 700/1. As a result of this preponderance in concentration together with the greater dipole moment of DMA (13), the ions undoubtedly should be solvated preferentially with DMA and the properties of the resulting mixed solvent should differ negligibly from those of pure DMA. Recently it has been observed that anhydrous and hydrated salts behave similarly in N-methylacetamide solutions.

SUMMARY

1. The equivalent conductance of each of 12 alkali metal and alkaline earth salts in DMA has been measured at several concentrations in the range of 1-174 × 10^{-4} N.
2. The limiting equivalent conductances of the lithium salts have been determined using Shedlovsky plots of Λ'_0 *versus* C. Lithium salts in DMA are dissociated completely and exhibit normal conductance behavior in being less conducting than corresponding sodium and potassium salts.
3. The conductance behavior of DMA solutions of barium and strontium perchlorates (both anhydrous salts and trihydrates) agrees very closely with the theoretical behavior predicted by the Onsager equation; however, solutions of barium and strontium bromides and barium diphenylaminesulfonate are characterized by considerable ionic association.
4. The equivalent conductance data for solutions of each multivalent electrolyte in DMA are described very well within the experimental concentration range by an empirical equation of the form,

   $$\Lambda = \Lambda_* - A\sqrt{C} + B\,C.$$

   In the cases of the perchlorates, the values of A compare excellently in magnitude with the values of the corresponding Onsager slopes.
5. In contrast to the results for aqueous solutions, the limiting equivalent conductances of the barium and strontium ions are 30-40% greater than those of the lithium, sodium and potassium ions which are leveled in DMA.
6. The behavior of corresponding anhydrous and hydrated salts has been observed to be identical within experimental error.

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Preparation of Iron (III) Nitrilotriethoxide*

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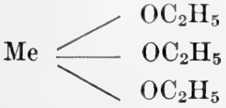
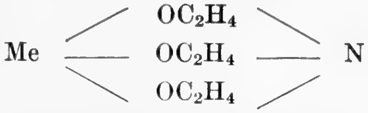
INTRODUCTION

TRIETHANOLAMINE (nitrilotriethanol) can act as a weak tribasic acid toward metals. The greater stability of the nitrilotriethoxides as compared to ordinary alkoxides can be attributed to chelation:





Accordingly, Hein and coworkers (**1,2**) prepared the boron and aluminum derivatives by replacing alkoxide groups. This method is limited since the starting materials are rather sensitive and not generally available.

An attempt to use a more general reaction was made by Starke (**3**) in the preparation of iron (III) nitrilotriethoxide:

1. $NH_4FeCl_4 + (HOC_2H_4)_3N.HCl + 4NH_3 \rightarrow Fe(OC_2H_4)_3N + 5NH_4Cl\downarrow$

The reaction appears applicable to other metal halides, but some improvements in the method seemed desirable. One problem was to replace the often hygroscopic anhydrous halides by the more convenient hydrated halides. Also it was preferable to precipitate the nitrilotriethoxide directly instead of the side-product. A solvent had to be found in which the nitrilotriethoxide is relatively insoluble while it readily dissolves the halide formed by the proton acceptor:

---

* Taken from a thesis submitted by Forrest C. Burns in partial fulfillment of the requirements for the degree of Master of Science.

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$$2.\ FeCl_3 + (HOC_2H_4)_3N.HCl + 4R_3N \rightarrow Fe(OC_2H_4)_3N \downarrow + 4R_3N.HCl$$

EXPERIMENTAL

1. *Materials.*—Nitrilotriethanol hydrochloride was prepared from technical grade triethanolamine by precipitation with hydrochloric acid (**3**). The 2,2-dimethoxypropane was of technical grade. All other reagents and solvents were of practical grade.

2. *Preparations.*—(a) Iron(III)nitrilotriethoxide monohydrate. Ferric chloride hexahydrate (5.40 g., 0.02 mole) was warmed with 30 ml. of 2,2-dimethoxypropane until it had completely dissolved. A suspension of 3.90 g. (0.02 mole) of nitrilotriethanol hydrochloride in 75 ml. of acetonitrile and 20 ml. of triethylamine was heated to boiling in a 250-ml. round-bottomed flask until the hydrochloride had dissolved. The hot solution of ferric chloride was now added slowly with constant stirring. A precipitate formed when about one-half of the ferric chloride had been added. After addition of all the ferric chloride the hot mixture was immediately filtered through a Buchner funnel, and the filtrate was allowed to cool to room temperature. Triethylammonium chloride crystallized out and was separated by suction.

The filtrate was returned to the round-bottomed flask with the filter paper containing the iron complex, and 5 ml. of triethylamine was added. The above procedure was repeated twice until no more triethylammonium chloride could be obtained after cooling the filtrate. All the iron(III)nitrilotriethoxide was then collected and washed with 10 ml. of acetonitrile. 3.81 g. of the crude product was obtained; the yield was 94.5%.

The iron(III)nitrilotriethoxide was dissolved in boiling absolute methanol, the solution concentrated to about 60 ml. and allowed to cool. On slow addition of 30 ml. of triethylamine a precipitate formed which was separated by suction and allowed to dry. 3.60 g. of pure iron(III)nitrilotriethoxide monohydrate was obtained; the yield was 81.8%. A small amount of the compound was hydrolyzed by warming it with water. The filtrate from the ferric hydroxide precipitate contained only a slight trace of chloride ion.

(b) Anhydrous iron(III)nitrilotriethoxide. The monohydrate (4.0 g.) was dehydrated on the waterbath in the presence of dioxane as described by Starke (**3**). 2.0 g. of anhydrous iron(III)nitrilotriethoxide was obtained; the yield was 50.0%.

3. *Analysis.*—The two compounds were dried in a vacuum desiccator under 25 mm.Hg pressure at room temperature overnight. Approximately 0.3 g. samples were heated slowly with a Fisher burner to decompose the organic matter. Then full heat was supplied until constant weight was reached, and the iron was determined gravimetrically as ferric oxide. The analysis for the nitrogen was done with approximately 0.1 g. samples by the Kjeldahl method.

The equivalent weight of iron(III)nitrilotriethoxide monohydrate was determined by titration in glacial acetic acid (**4**). Samples of 40-60 mg. were dissolved in 50 ml. of boiling glacial acetic acid. A 0.0997 N glacial acid solution of perchloric acid was used for titration. The end point was detected by finding the point of maximum deflection of the needle on a Beckman pH meter. A standard glass electrode was used against a saturated calomel cell. The equivalent weight was calculated from the relation:

$$3.\ \text{Equivalent weight} = \frac{\text{sample weight in milligrams}}{\text{normality of } HClO_4 \times \text{milliliters of } HClO_4}$$

TABLE I. IRON AND NITROGEN CONTENT AND EQUIVALENT WEIGHT OF IRON(III)NITRILOTRIETHOXIDE AND ITS MONOHYDRATE. VALUES ARE THE AVERAGE OF THREE DETERMINATIONS.

	$Fe(OC_2H_4)_3N$		$Fe(OC_2H_4)_3N.H_2O$	
	Found	Calculated	Found	Calculated
Iron	27.60	27.66	25.41	25.39
Nitrogen	6.95	6.94	6.32	6.37
Equivalent Weight	—	—	165.3	165.0

RESULTS AND DISCUSSION

The experimental data in Table I agree closely with the theoretical values. By comparison with the results obtained previously (3) the present method is an improvement with respect to purity of product as well as ease of preparation.

The dehydration of the starting material presumably proceeds according to:

$$4.\ Fe(H_2O)_6Cl_3 + 6(CH_3O)_2C(CH_3)_2 \rightarrow FeCl_3 + 12CH_3OH + 6CH_3COCH_3$$

The resulting organic solution is sufficiently free of water and hydroxyl ions which could cause side reactions in the basic reaction mixture. 2,2-Dimethoxypropane, now commercially available, should become a valuable reagent in all such inorganic preparations.

Unlike ammonia gas, liquid triethylamine can be added to the nitrilotriethoxide solution in all proportions and serve as a precipitating agent. However, triethylammonium chloride is not soluble in the amine, and too large an excess must be avoided. The acid constant of triethylamine ($pK = 10.65$) indicates that it is a more efficient proton acceptor than ammonia ($pK = 9.21$).

The fact that the solubility of triethylammonium chloride in acetonitrile increases with temperature makes it possible to extract it repeatedly with the mother liquor, keeping the loss of the main product at a minimum. Experiments with chromium (5) show that the extraction by acetonitrile is not complete if the chloride is bound preferentially to water in the fourth coordinative position of the metal ion. Only three of the four moles of triethylammonium chloride (equation 2.) are then soluble and easily extractable.

The determined equivalent weight of 165.3 agrees with the assumption that the trivalent metal has replaced all three hydrogens of the triethanolamine. At first glance one would expect the equivalent weight to be one quarter of the molecular weight of 220.03 according to the equations

5. $Fe(OC_2H_4)_3N + 3H^+ \rightarrow Fe^{+3} + (HOC_2H_4)_3N$

6. $(HOC_2H_4)_3N + H^+ \rightarrow (HOC_2H_4)_3NH^+$

However, according to Casey and Starke (**6**,**7**), the ferric ion is complexed so strongly in glacial acetic acid solution that only one ninth of an equivalent can be titrated:

7. $$[FE_3(OH)_2(OCOCH_3)_6]\ OCOCH_3 + HClO_4 \rightarrow [Fe_3(OH)_2(OCOCH_3)_6]\ ClO_4 + CH_3COOH$$

The calculated equivalent weight of the compound is therefore:

8. $$\text{Equivalent weight} = \frac{220.03}{3 \text{ x } \frac{1}{9} + 1} = 165.0$$

SUMMARY

1. A fast and efficient method of preparing nitrilotriethoxides has been worked out.
2. The experiments prove 2,2-dimethoxypropane to be an excellent dehydrating agent for some hydrated inorganic compounds.
3. The equivalent weight of iron (III) nitrilotriethoxide monohydrate has been determined. The value agrees with the structure of the compound and the abnormal behavior of the ferric ion in glacial acetic acid.

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Influence of Top-Dressing Alfalfa with Potash on Winterkilling*

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INTRODUCTION

WINTERKILLING OF ALFALFA, especially following the first or second season's crop, is a serious problem in most alfalfa growing areas. Since a ton of alfalfa hay removes 30 to 40 pounds of potassium from the soil, it is apparent that this crop exacts a heavy drain on the exchangeable potassium supply of the soil, and hence, yields of hay and vigor of the plants will go down rapidly unless the potassium-supplying power of the soil is high, or some means of adding potassium is provided. Progressive deterioration in the stand of alfalfa and increases in the proportion of grasses and weeds in alfalfa fields has often been attributed to winterkilling of the alfalfa. This results, of course, in lower yields and poorer quality of hay.

Nightingale (1943) pointed out that in plants, potassium seems to be essential for nitrate reduction and perhaps protein synthesis, and for CO_2 assimilation, and hence, carbohydrate synthesis. He stated that absorption of nitrates by plants is accelerated by the presence of rapidly absorbable cations like Ca and K, and that plants abounding in potassium nearly always have a high protein and carbohydrate content.

Grandfield (1943) found that during the winter hardening period of alfalfa, a rapid hydrolysis of starch to sugar occurred with a subsequent translocation of the sugar to the crown buds. The average nitrogen content of the crown buds found during this period was 4.19%, while that of the roots was 1.98%. He maintained that the high nitrogen content, and hence, protein content in the crown bud accounted for the decrease in free water and the high content of bound water found. This bound water and increased sugar concentra-

* This study was initiated at the University of Wisconsin while the author was a Research Fellow in the Soils Department. Later work done at the University of Kentucky has been published elsewhere. Special appreciation is expressed to Dr. O. J. Attoe & Prof. E. Truog, Soils Department, University of Wisconsin, for their helpful suggestions and criticisms during this study.

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tion lowered the freezing point of the crown bud. Thus, it appears that an abundant supply of potassium favors the formation and accumulation of sugar and proteins to an extent that may materially increase winter hardiness.

The present study was undertaken to investigate the influence of top-dressing established stands of alfalfa with potash on winterkilling, and hence, yield and quality of hay.

METHODS

In order to determine whether top-dressing with potash in October, 1948, prevented winterkilling, counts of alfalfa plants per unit area on plots receiving potash and on their respective check plots were made in September, 1949. A wire hoop 19 inches in diameter, thus enclosing an area of two square feet, was placed on the ground, and the number of alfalfa plant crowns inside the wire was counted. Five counts per plot were made, placing the hoop at various places in the plot.

TABLE I. THE EFFECT OF TOP-DRESSING FIRST YEAR ALFALFA ON ALMENA SILT LOAM WITH MURIATE OF POTASH (60% K_2O) IN OCTOBER, 1948 ON THE NUMBER OF ALFALFA PLANTS PRESENT PER TWO SQUARE FEET AREA IN SEPTEMBER, 1949.

Approximate lbs. per acre of available nutrients present in plots before topdressing		Lbs. per acre of KCl applied to sub-plots	Mean of five counts of no. of alfalfa plants per 2 ft.^{2}	Std. deviation of the no. of alfalfa plants per 2 ft.^{2}
P	K			
10	100	0 200	7.8 10.4	3.18 2.96
10*	100*	0 200	12.3 13.8	2.16 2.99
50	150	0 200	14.0 14.8	1.47 3.19
75	200	0 200	13.8 16.4**	1.00 2.19
125	300	0 200	9.6 10.2	3.20 2.86

*This plot received the common fertilizer application of 300 lbs. of 0-20-20 per acre on oats seeded down and 150 lbs. of 3-12-12 per acre in the hill for corn. All plots were in a corn, oats, alfalfa, alfalfa rotation.

**Difference from check significant (5% level).

Results and Discussion

The data in Table I do not indicate that top-dressing with 200 pounds of muriate of potash per acre on Almena silt loam in central Wisconsin had any effect on preventing winterkilling of alfalfa. In only one instance was the number of alfalfa plants significantly greater on a top-dressed plot. It is noteworthy that in all cases the mean number of plants per unit area was greater where potash was applied and that a trend of more plants wintering over was evident.

Tables II and III contain data for Spencer and Antigo silt loams in northern Wisconsin. On these soils, on all plots top-dressed with 200 pounds of muriate of potash per acre, the differences in alfalfa counts per unit area from that on the respective check plots were highly significant. The differences in the number of alfalfa plants per unit area between plots top-dressed with 100 pounds of muriate of potash per acre and the respective check plots were highly significant.

Table II. The Effect of Top-Dressing First Year Alfalfa on Antigo Silt Loam with Muriate of Potash (60% K_2O) in October, 1948 on the Number of Alfalfa Plants Present per Two Square Feet Area in September, 1949.

Approximate lbs. per acre of available nutrients present in plots before topdressing		Lbs. per acre of KCl applied to sub-plots	Mean of five counts of no. of alfalfa plants per 2 ft.^{2}	Std. deviation of the no. of alfalfa plants per 2 ft.^{2}
P	K			
10	100	0	13.2	2.58
		100	18.2*	1.30
		200	21.8*	1.64
50	100	0	11.6	1.81
		100	14.4**	1.67
		200	17.0*	2.00
50	150	0	14.6	1.81
		100	15.8	0.84
		200	19.6*	3.04
50	200	0	16.6	0.89
		100	19.6*	1.34
		200	22.8*	2.86
50	300	0	19.4	1.34
		100	22.2	3.34
		200	24.6*	1.51

*Difference from check highly significant (1% level).
**Difference from check significant (5% level).

TABLE III. THE EFFECT OF TOP-DRESSING FIRST YEAR ALFALFA ON SPENCER SILT LOAM WITH MURIATE OF POTASH (60% K_2O) IN OCTOBER, 1948 ON THE NUMBER OF ALFALFA PLANTS PRESENT PER TWO SQUARE FEET IN SEPTEMBER, 1949.

Approximate lbs. per acre of available nutrients present in plots before topdressing		Lbs. per acre of KCl applied to sub-plots	Mean of five counts of no. of alfalfa plants per 2 ft.^{2}	Std. deviation of the no. of alfalfa plants per 2 ft.^{2}
P	K			
10	100	0	11.4	1.14
		100	13.4*	1.14
		200	16.4*	2.70
50	100	0	9.4	2.70
		100	14.0*	2.32
		200	16.2*	1.64
50	150	0	15.4	2.88
		100	19.8**	2.48
		200	22.2	3.42
50	200	0	15.4	3.20
		100	20.4*	1.51
		200	21.2*	1.79
50	300	0	12.2	2.38
		100	13.6	2.50
		200	21.0*	3.16

*Difference from check highly significant (1% level).
**Difference from check significant (5% level).

The differences in the number of alfalfa plants per unit area between plots top-dressed with 100 pounds of muriate of potash per acre and the respective untreated plots was highly significant or significant with three exceptions. Two of these exceptions were plots which had been fertilized previously to a high level of exchangeable potassium. It is evident that top-dressing these soils with potash was important in preventing winterkilling of alfalfa.

In a study of alfalfa fertilization in Kentucky, Seay and Weeks (1955) found that potassium fertilization increased the K content of the crown. The crown tended to accumulate K during the autumn and winter. K rapidly decreased in the crown during the first early growth in the spring. Thus, insufficient soil or fertilizer K to provide for proper K storage in the crown which supplies K for rapid spring top growth, may be a factor in loss of alfalfa plants during winter.

Population counts were not made in that study but it was evident that plots which were not fertilized with K contained more grass and weeds and fewer alfalfa plants than plots which had been fertilized with muriate of potash.

SUMMARY

In September, 1949, plots on Antigo and Spencer silt loams in northern Wisconsin, not top-dressed with potash in 1948 contained significantly fewer alfalfa plants per unit area than top-dressed plots. The general trend was the same on Almena silt loam in central Wisconsin, but the differences in alfalfa population were not statistically significant. These differences in alfalfa population indicate that top-dressing with potash, even as late as October, was important in increasing survival rates of alfalfa during the winter and spring. A later study in Kentucky showed this same trend and provided some information as to reasons why K fertilization provided protection against winterkilling.

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Resistance to Oxophenarsine and Antimony Potassium Tartrate in *Trypanosoma equiperdum**

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Introduction

In rats infected with a strain of *Trypanosoma equiperdum* resistant to oxophenarsine we have encountered strains resistant to antimony potassium tartrate** on several different occasions. Since it is difficult to obtain strains resistant to oxophenarsine or to pararosaniline in rats (1) this experience with antimony tartrate was rather surprising. Therefore, it seemed worthwhile to publish an account of the circumstances under which antimony tartrate resistance appeared. Some observations on oxophenarsine resistance that were made at the same time will also be described in this report.

Materials and Methods

The rats were mostly males of the Wistar (Purdue) albino strain weighing between 170 and 230 grams. In the experiment with strain D one male and one female rat was used at each dosage level. Reading from left to right on the bottom row of the chart (Figure 1) the rats were female, male, female, male and male. The female rat at the 40 micromole dose died soon after treatment. The course of the infections was followed with the aid of a hemocytometer with 0.9% NaCl as the diluent. Blood was obtained from the tail. Injections were made with a 1-cc. tuberculin syringe and a 27-gauge needle into a vein on the inner aspect of the thigh, with ether anesthesia. The infections were initiated by intravenous inoculation of parasitized, defibrinated blood. The untreated infection is invariably fatal after a highly predictable course illustrated by the first four curves of the top row in Fig. 1. A moderate decelaration of the upward course of the parasatemia curve is seen in the untreated controls but never an actual decline in numbers. Any actual decrease in parasite count or failure

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** Antimony potassium tartrate (tartar emetic) will be referred to as antimony tartrate in what follows.

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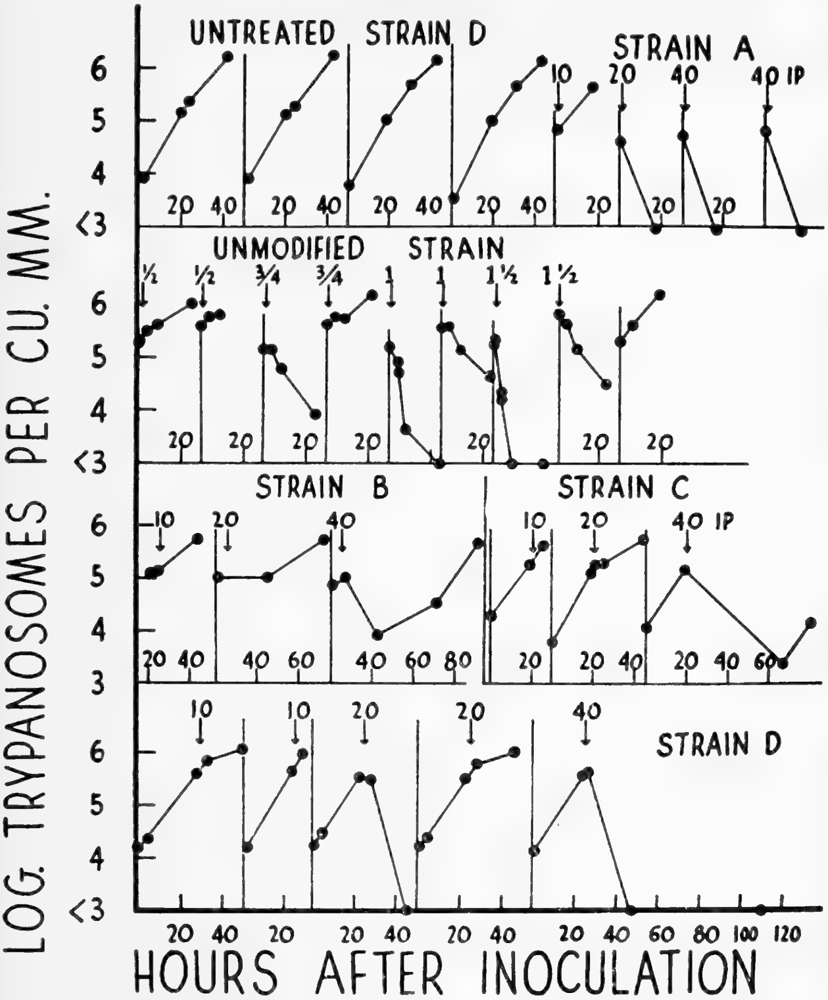
Figure 1.



The trypanosome counts are plotted as logarithms to the base 10. The legend *hours after inoculation* should be changed, in the instances of the rats infected with the unmodified strain, to read *hours after treatment.* The vertical arrows indicate the time of intravenous injection of the indicated doses of oxophenarsine (micromoles per kg.) except the two instances marked IP where the injection was by the intraperitoneal route.

All the rats eventually died of the infection. Reading from left to right and top to bottom the times of death were as follows: First (top) row: died 13 min. after last count (1,622,000); died 3 hrs. after last count (1,705,000); died 42 to increase following treatment can be attributed to the action of the drug.

The standard errors of the counts recorded on the chart vary from 4% to 8% of the values depending on the actual number counted. We refer to the error due to random distribution of the trypanosomes in the counting chamber which is a function of the square root of the number counted. Empirical evidence that the counting procedure is adequate for tracing the true course of these infections is seen in the good agreement between closely spaced counts and in the regular conformation of the curves. In several places in the chart counts made within a few minutes were so close together they could not be indicated at the scale used.

RESULTS

The strain that gave rise to the antimony tartrate resistant strains was resistant to oxophenarsine. It was obtained in mice by repeated subcurative doses of oxophenarsine (2) and had been kept in mice for about nine months without contact with the drug. It was transferred to rats and resistance to oxophenarsine was checked at the beginning of these experiments and found to be about 20-fold. Figure 1 illustrates the data upon which this estimate of resistance to oxophenarsine is based. In the figure the first oxophenarsine resistant strain is designated Strain A. The estimate of its resistance to oxophenarsine was made by comparison with the unmodified oxophenarsine sensitive strain (second row of figures). The doses that just fail to halt the upward course of the parasitemia curves are 10 micromoles per kg. for strain A and ½ micromole per kg. for the unmodified strain.

Seventeen rats infected with strain A were treated with antimony tartrate given intravenously at doses of 40, 20, 10 or 5 mg. per kg.

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min. after last count (1,450,000); died 4 hours after the last count (1,360,000); died within 24 hours of last count; dead 48 hours after last count; died 13 days after last count; died 10 days after last count. Second row: not recorded. Third row: dead 27 hours after last count; dead 20 hours after last count; dead 23 hours after last count; dead 16 hours after last count; dead 22 hours after last count; died on 11th day. Fourth row: died 2½ hours after last count (1,200,000); dead 16 hours after last count; died on 12th day; dead 7 hours after last count; died on 11th day.

The counts at the time of treatment expressed as logarithms to the base 10 were as follows: First (top) row 4.94, 4.62, 4.74, 4.84. Second row: See text. Third row: 5.06, 5.22, 4.93, 5.33, 5.24, 5.18. Fourth row: 5.64, 5.73, 5.68, 5.62, 5.68.

when there were about 50,000 trypanosomes per cm. of blood. The four rats receiving 40 mg. per kg. were all dead by the next day. The six rats receiving 20 mg. per kg. were cured judged by their survival from August 28th, when they were treated, to November 6th and beyond. Blood was examined from these rats several times and no trypanosomes were found. The history of four rats given 10 mg. per kg. and three rats given 5 mg. per kg. on August 28th is as follows:

Of the four rats given 10 mg. per kg., one died September 10th, one was cured by the initial dose (alive November 6th), one was cured by an additional dose of 20 mg. per kg. given September 13th following a second relapse, the first lapse having been treated with 10 mg. per kg. on September 5th and 6th, and one rat gave rise to a resistant strain following additional doses of 10 on September 6th and 20 mg. per kg. On September 11th and 12th. This resistant strain is designated Strain D.

Of the three rats given 5 mg. per kg., one was cured by additional doses of 10 mg. per kg. on September 5th and 6th, and 20 mg. per kg. on September 13th; one gave rise to a resistant strain following additional doses of 10 on September 5th and 6th, and 20 mg. per kg. on September 11th and 12th, and the third rat died between September 21st and October 22nd following additional doses of 10 on September 8th, and 20 on September 20th. The second rat was cured with Bayer 205 at a dose of 22.5 mg. per kg. after the second resistant strain was obtained on September 13th. The degree of resistance to antimony tartrate was at least 4-fold for a dose of 20 mg. per kg. failed to clear the blood as 5 mg. per kg. had done with Strain A.

Resistance to antimony tartrate was also encountered in a second experiment in which the strain was different from the one used above, but was derived from it in the following way: A series of rats were immunized to Strain A by curing them with pararosaniline hydrochloride given at various levels of infection. When these rats were challenged with Strain A, evidence was obtained that Strain A consisted of at least three different antigenic types. The two minor types made their appearance in immunized rats after 66 hours if the antigen dose (intensity of parasitemia at time of cure) did not exceed 10^4 trypanosomes per cmm. or after 101 hours if the antigen dose was above 10^5 trypanosomes per cmm. These delays correspond to incidences of 1 in $2^{13.7}$ and 1 in 2^{21}, if the generation time is taken to be 4.8 hours. For a fuller discussion of the antigenic heterogeneity of these populations and a description of the method for estimating incidences from the delay seen in immunized animals see (3).

A strain designated Strain C was established from one of the challenged rats carrying the scarcer minor type. Strain C was used to infect a group of rats which were treated with 20 mg. per kg. of antimony tartrate with the intention of curing them. Only one of 27 was cured. The others were all dead of the infection within 11 days. This behavior of strain C should be contrasted to that of strain A in which 20 mg. per kg. cured six of six rats. We conclude that strain C contained a component of antimony-tartrate-resistant cells, and since strain C was obtained in rats immunized by cure with pararosaniline, it appears that we have observed increased resistance to antimony tartrate in an arsenic resistant strain without contact with either arsenic nor antimony but following immunological manipulation of the population. The commoner minor type was also saved in rats and designated Strain B.

The resistance to oxophenarsine of strain D (also resistant to antimony tartrate) was investigated. The results are illustrated in the figure. Infected rats were treated with doses of 10, 20 or 40 micromoles per kg. intravenously. The effect of 10 micromoles per kg. was a slight slowing of the progress of the infection in which death was delayed a few hours at best. Such an effect was obtained with the unmodified strain with less than 1 micromole per kg. The infection in one of the rats given 20 micromoles per kg. behaved like the infections in the rats given 10 micromoles per kg. In the other rat, given 20 micromoles per kg., the course of the infection was greatly changed. The blood was cleared of trypanosomes for about a week and death was delayed about 10 days. One of the rats given 40 had about the same course. The other rat given 40 micromoles per kg. died within a few hours while the parasite density was declining, probably from drug toxicity rather than the disease.

The two minor components isolated from Strain A were also tested for resistance to oxophenarsine. The results are illustrated in the figure under the designations Strain B and Strain C.

The intensity of the infection at the time of treatment has an effect in the outcome. This is nicely shown in the curves for the unmodified strain where at every dose level the more lightly infected rat responded better to treatment.

The counts in these rats immediately before treatment were: 193,000 and 380,000 (½ micromole dose); 139,000 and 405,000 (¾ micromole dose); 155,000 and 340,000 (1 micromole dose); and 200,000 and 479,000 (1½ micromole dose).

DISCUSSION

We have examined, in all, four strains, for oxophenarsine resistance, including 3 strains that differed antigenically and one antimony-tartrate-resistant strain. It may be best to point out that the major type of strain C occurs as a minor type in strain B, so that although these strains can be said to differ antigenically, they do have a common component which is at a high incidence in one strain and at a low incidence in the other. As a result, animals immunized with high doses of a vaccine prepared from B are protected against C, but not vice versa.

The characteristic of oxophenarsine resistance was found in each of the four strains at about the same degree of development. The results with the different antigenic types indicate that large populations of oxophenarsine-resistant trypanosomes are composed of several different antigenic varieties each of which is resistant to oxophenarsine. From the result of the second experiment, it appears that some populations of oxophenarsine-resistant trypanosomes include a component of antimony-tartrate-resistant cells. In other populations, as in the first experiment, such antimony-tartrate-resistant cells occur so infrequently that their presence is revealed only after repeated subcurative treatments. A strain obtained by this process (Strain D) was found to retain its oxophenarsine resistant character.

Resistance to antimony tartrate presents a peculiar situation in that it has never been observed to develop directly in a perfectly normal strain of trypanosomes, but only in strains already resistant to some other drug, usually an arsenical. The present results are in accord with this, for the strain that gave rise to the antimony-resistant strains was first rendered resistant to oxophenarsine. The surprising aspect of the present results is the ease with which antimony-resistant strains were obtained in the rat. Schnitzer and co-workers (1) have described the difficulty experienced in obtaining drug-resistant strains in the rat. By comparison, the mouse is a far more satisfactory host for such studies, and most of the known strains have been developed in mice. Even in the mouse, however, it requires more prolonged treatment to develop appreciable resistance to oxophenarsine or pararosaniline than was the case in the present experiments with antimony tartrate.

The fact that antimony resistance was encountered in one instance following the selection of a minor antigenic type by immunological methods is not regarded as evidence that some antigenic types are more likely to give rise to antimony resistance than others. The change in antigenic type *per se* may be less important than the fact that such manipulation involves reducing the total number of the population to a low level at times, so that mutants may, by chance, be raised to incidences that they would not ordinarily attain without the selective action of the drug. The more interesting point is that a striking change in resistance to antimony occurred without previous contact with antimony. This result is opposed to the idea that changes in resistance to drugs are induced by the drugs, and supports the hypothesis that such changes occur spontaneously.

In a footnote to a paper devoted to another subject, Schnitzer and Kelly (**4**) wrote, ''According to the literature and our own experience, 20 percent of all strains rendered maximally resistant to arsenicals also acquire tartar emetic fastness.'' Perhaps the 80% that do not acquire tartar emetic fastness acquire the potentiality of being converted to tartar emetic fastness with relatively little selection, even in a host not well adapted to such a purpose. This suggests that antimony tartrate resistance may result from a mutation occurring at a relatively high rate in populations of oxophenarsine-resistant trypanosomes. By relatively high rate we mean as compared to the mutations determining oxophenarsine resistance.

SUMMARY

1. In the rat, antimony-tartrate resistance was encountered repeatedly in a strain of *Trypanosoma equiperdum* resistant to oxophenarsine. Increased resistance in antimony tartrate was observed in two different situations: (a) after a short period of subcurative treatment with antimony tartrate; (b) after immunological manipulation of the population.
2. An antimony-tartrate-resistant strain retained its resistance to oxophenarsine.
3. A population whose major antigenic type was resistant to oxophenarsine was found to contain at least two minor antigenic types

which were also resistant to oxophenarsine, while one of the minor types proved to be more resistant to antimony tartrate than was the major type.

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A Study of the Conductances of Some Potassium and Sodium Salts in N,N-Dimethylbutyramide at 25°C.*

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THE CONDUCTANCE behavior of potassium and sodium salts in N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA) and N, N-dimethylpropionamide (DMP) has been described in previous papers from this Laboratory (1,2,3). These amides, which have dielectric constants of 36.7, 37.8 and 33.1 respectively at 25°, have been shown to be potentially very useful media in which many electrolytes are not only soluble but also completely dissociated in dilute solutions. In DMF, the potassium and sodium ions exhibit their conventional behavior, *i.e.*, the effective sizes of the solvated ions are inversely related to their crystallographic radii resulting in the potassium ion being more mobile and consequently more conducting than the sodium ion. However, DMA and DMP in the same homologous series uniquely represent the first known solvents in which the limiting equivalent conductance of a sodium salt is greater than that of the corresponding potassium salt. A comparison of the conductance behavior of potassium and sodium salts with that of several tetraalkylammonium salts provides evidence that the alkali metal ions are solvated in DMA and DMP; nevertheless, unusual relative solvation effects must be operative. Owing to both the size and the structure of the solvent molecules, steric factors may contribute significantly to the possible extents of solvation through an ion-dipole mechanism.

The objective of the work which is described in this paper was to extend the previous studies by investigating the behavior of potassium and sodium salts in N,N-dimethylbutyramide (DMB) and comparing the results with those for DMF, DMA, and DMP solutions. This further represents the initial use of DMB as an electrolytic solvent.

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EXPERIMENTAL

1. *Preparation and Purification of Solvent.*—Approximately four liters of DMB was prepared by the well-known procedure of reacting two moles of gaseous dimethylamine per mole of butyryl chloride in a benzene solution at 0°C. After the precipitated dimethylamine hydrochloride was removed by filtration, the DMB was separated from the benzene by distillation. The crude amide was treated then with a cold saturated aqueous solution of KOH to remove the remaining dissolved dimethylamine hydrochloride which could not be separated by distillation owing to its thermal decomposition and subsequent recombination in the distillate. Final purification of the DMB was accomplished by refluxing it for several hours in contact with calcium oxide prior to fractional distillation through an efficient column at reduced pressure. The DMB which was used in the preparation of solutions had the following properties at 25°: conductivity, $2\text{-}5 \times 10^{-8}$ ohm^{-1} cm.$^{-1}$; d_4^{25}, 0.9063 g./ml.; n_D^{25}, 1.4397; viscosity, 1.271 centipoise; dielectric constant (at ten megacycles), 28.0. The values for the refractive index and density show good agreement with those reported by Ruhoff and Reid (**4**). Comparable DMB was recovered subsequently from the solutions of electrolytes for re-use by a single efficient fractional distillation.

2. *Purification of Salts.*—This phase of the work has been described previously (**1**). A substantial quantity of each salt in purified form was available from earlier studies.

3. *Procedure and Apparatus.*—Detailed descriptions of the bridge assembly, temperature bath and control, and the procedures utilized in the calibration of equipment, the preparation of solutions and the measurement of resistances are readily available elsewhere (**1**, **5**).

RESULTS AND DISCUSSION

Corresponding values of the equivalent conductance, Λ, and the concentration in gram-equivalents per liter, C, for each of six salts

TABLE I. EQUIVALENT CONDUCTANCES OF SALTS IN DMB AT 25°C.

$C \times 10^4$	Λ	$C \times 10^4$	Λ	$C \times 10^4$	Λ
(a) NaI		(b) $NaClO_4$		(c) NaSCN	
3.647	42.30	1.320	44.55	1.560	47.35
9.709	40.89	5.130	43.22	4.768	45.87
17.03	39.84	10.52	42.10	10.98	44.01
26.24	38.87	17.07	41.11	17.37	42.63
37.19	37.89	25.60	40.18	25.69	41.13
47.09	37.24	35.51	39.22	34.24	39.96
(d) KI		(e) $KClO_4$		(f) KSCN	
2.405	42.16	1.453	43.86	1.847	47.04
5.213	41.36	4.096	42.88	5.132	45.91
9.352	40.49	9.626	41.70	10.40	44.71
14.11	39.72	14.62	40.87	17.77	43.54
21.37	38.85	21.58	39.97	23.98	42.67
28.73	38.07	29.19	39.18	32.52	41.70

in DMB are presented in Table I. Comparable data for potassium and sodium nitrates and bromides were unobtainable by conventional procedures owing to their very limited solubilities in DMB.

The phoreograms (6) for KI, NaI, $KClO_4$ and $NaClO_4$ in DMB are parallel and slightly anabatic. Those for KSCN and NaSCN are parabatic and catabatic respectively and intersect at a concentration of approximately 1×10^{-3} N. The limiting slope of the phoroegram for each salt except NaSCN agrees within 5% with the theoretical slope, $-[1.076\Lambda_0 + 71.0]$, as predicted by the Onsager equation (7) for solutions of uni-univalent electrolytes in DMB at 25°C. This excellent agreement with theory indicates that the most straightforward analysis of the data for these salts should be based on the Shedlovsky rearrangement of the Onsager equation (8) which may be written in simplified form for DMB solutions at 25° as follows:

$$1. \qquad \Lambda'_0 \equiv \frac{\Lambda + 71.0\sqrt{C}}{1 - 1.076\sqrt{C}} = \Lambda_0 + B\,C$$

Values of Λ'_0 were calculated directly from the equivalent conductance and concentration data. Values of Λ_0 and B were obtained from the intercepts and slopes respectively of linear plots of Λ'_0 *versus* C. The results for the five salts are summarized in Table II.

TABLE II. DATA PERTINENT TO PLOTS OF EQUATION (1) FOR SOME ELECTROLYTES IN DMB AT 25°C.

Salt	Λ_0	B x 10^2
NaI	44.4	2.3
KI	43.9	1.9
$NaClO_4$	45.9	1.6
$KClO_4$	45.3	1.3
KSCN	48.7	0.1

The slope of the phoreogram for NaSCN is approximately 30% numerically greater than that predicted by theory thereby indicating some extent of ionic association. To be consistent with previous analyses of similar data (2,3), the results were treated by the Fuoss-Shedlovsky method (9). From the values of the ordinate-intercept and the slope of the plot of $S\Lambda$ *versus* $Cf^2S^2\Lambda^2$, values of the limiting equivalent conductance, Λ_0, and the dissociation constant, K, for

NaSCN in DMB were calculated to be 49.2 ohm^{-1} cm.^{2} equiv.$^{-1}$ and 2.2×10^{-2} respectively.

From the combined results is is discernible that the limiting equivalent conductance of a sodium salt in DMB is 0.5 ± 0.1 ohm^{-1} cm.^{2} equiv.$^{-1}$ greater than that of the corresponding potassium salt. This constant difference not only substantiates the additivity of limiting ionic equivalent conductances but also indicates that unusual relative solvation and mobility effects, similar to those observed previously for DMA and DMP solutions, are operative with the potassium and sodium ions also in DMB solutions. These three dimethyl amides are unique in being the only known solvents in which the limiting equivalent conductances of sodium salts are greater. A possible explanation for this behavior has been presented in previous papers (**2**,**3**).

The $\Lambda_0\eta_0$-product for a common salt such as NaI is 0.652, 0.622, 0.555 and 0.565 ohm^{-1} cm.^{2} equiv.$^{-1}$ poise for DMF, DMA, DMP, and DMB respectively as the solvent. This indicates that the value for the product passes through a minimum. Before making deductions based upon considerations related to Stokes' law, however, it would be advantageous and preferable to have the product for NaI or another suitable electrolyte in one or two more dimethyl amides. Such a conductance study involving either or both of the next two members of the homologous series of dimethyl amides would be feasible inasmuch as both are liquids at room temperature (**4**) and their viscosity probably do not exceed 2 or 3 centipoise.

A recent paper from this Laboratory (**10**) has described the high dielectric constants of some liquid N-methyl- and N,N-dimethylamides of methane sulfonic and benzene sulfonic acids and has discussed the possible utility of these as electrolytic solvents. It would be quite worthwhile not only to obtain information concerning the conductance behavior of some potassium and sodium salts in these virgin media but also to compare the results with those for the same salts in the mono- and di-N-substituted amides of the carboxylic acids.

SUMMARY

1. Values of several physical properties of DMB are reported for the first time.
2. In the initial use of DMB as an electrolytic solvent, the equivalent conductances of potassium and sodium iodides, perchlorates and

thiocyanates have been determined at several concentrations in the range of 1-50 × 10^{-4} N.

3. Five of the salts are completely dissociated in dilute DMB solutions and the observed conductance behavior for them compares excellently with that predicted theoretically by the Onsager equation. NaSCN solutions are characterized by a slight extent of ionic association.
4. Unusual conductance behavior is displayed by DMB solutions in that the limiting equivalent conductance of a sodium salt is greater than that for the corresponding potassium salt. This unique result has been observed previously only for DMA and DMP solutions.
5. The pattern of conductance behavior for salts in DMB completely parallels that for salts in DMA and DMP; however, salts become less soluble and less conducting as the molecular weight (or organic nature) and viscosity of the solvent molecules increase.

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The Effect of Pitressin on Lymph Drainage in the Frog

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INTRODUCTION

IN 1952 EWER (1) reported a new response to injection of pituitrin on fluid distribution in the toad, *Bufo regularis* Reuss. The experimental procedure was essentially to keep the animals in water and to measure the rates of water uptake and of urine output. Ewer found that when the animals were injected with pituitrin and then kept dry, instead of being replaced in water, the lymph sacs of the thighs became distended with fluid. The magnitude of the effect was variable but, nevertheless, was often of a quite marked degree.

Water exchange and dorsal lymph drainage in the frog, *Rana pipiens* Schreber, as affected by Pitressin have been investigated in the present study. Drainage of fluid from the lymph areas as affected by this hormone has been ascertained by following the urinary excretion of glycine which was injected into the dorsal lymph sac. Thus, in a sense the fluid flow from the dorsal lymph sac was tagged with this amino acid.

METHODS

This problem has been conducted as follows: The cloaca of each frog was ligated with thread to prevent urine escape during the course of each experiment. One group of frogs was injected subcutaneously with 50 milliunits of Pitressin solution (Parke-Davis). Another group, which served as a control was injected subcutaneously with distilled water. The volume of fluid used was one-half ml. in each instance. Each frog, in addition, was injected into the dorsal lymph sac with 30 mg of glycine in one and one-half ml. of solution.

Each animal was weighed and placed in 75 ml. of distilled water in a vessel so that about one-half of its body was submerged. The temperature of the chamber in which the vessel was placed was regulated to about 30°C., and the frog remained in this environment for two hours. Then the animal was weighed a second time to determine net water gain or loss. Following this, the ligature was released, the urine was expressed and collected for analysis of excreted glycine. Finally, the animal was weighed a third time to determine more precisely the volume of urine that had been formed. The urine was analyzed for amino nitrogen according to a modified spectrophotometric ninhydrin method of Troll and Cannon (**2**). That the amino acid glycine was being excreted has been indicated pre-

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viously by using paper chromatographic technique (**3**). Correction was not made for about ten per cent of the color, developed by ninhydrin, which had been determined previously (**3**), since it was felt this excretion was fairly uniform in both the experimental and control groups.

RESULTS

In Table I are shown the averaged data collected from frogs injected with Pitressin, as compared with data from frogs not injected with Pitressin but otherwise treated similarly. The uptake of water across the integument in the former case was 6.9 per cent of the body weight, and in the latter it was 6.4 per cent. The difference is negligible. The urine production was 10.6 per cent and 9.7 per cent respectively. The greater volume of urine over skin uptake volume is due most likely to the attempt of the organism to rid itself of glycine. Although there was a slight decrease in urine production in the Pitressin-injected animals, the decrease does not seem significant.

TABLE I. WATER EXCHANGE IN PITRESSIN-INJECTED FROGS*

	CONTROL		EXPERIMENTAL	
	# Animals	% Body Weight	# Animals	% Body Weight
Intake Across Skin...	20	6.9%	20	6.4%
Urine..............	20	10.6%	20	9.7%

Clearly there was at least no marked antidiuretic effect of the Pitressin in these experiments. This agrees with much of the literature which has revealed many species differences in responses to the several extracts of the posterior hypophysis.

Finally the data in Table II seem to point conclusively to the Pitressin having an effect of inhibition of flow of fluid from the dorsal lymph sac of the frog. It may be observed that the glycine excreted in the urine was drastically decreased, there being a decrease of approximately fifty per cent. The P value for these data is $< .05$, indicating significance. This resembles Ewer's (**1**) finding of Pituitrin exerting a similar action in *Bufo regularis* Reuss. This investigator observed an increased reabsorption of fluid from the bladder of the above animal under the action of Pitressin, but, although there was a slight decrease in urine formation in the present experiments this

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* The species was *Rana pipiens* Schreber.

does not seem very likely to be a factor that was operative here. Apparently under the influence of Pitressin lymphatic drainage, at least dorsal lymph, was depressed, there being a sort of lymphatic stasis.

TABLE II. GLYCINE EXCRETED FROM DORSAL LYMPH SAC

	# Animals	Mgs./Gm. Body Weight x 100
Control	20	6.7
Pitressin Injected	20	3.4

CONCLUSION

The interpretation of data collected in these experiments points to the following observation. The Pitressin apparently suppressed drainage of fluid from the dorsal lymph sac of the frog. This action is similar to that reported for Pituitrin in *Bufo regularis* Reuss. The present effect was evident even though the animal was kept in water. Pitressin excreted no or little anti-diuretic action as it was used in the present study.

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New Species of Land Snails from the Eastern United States

Leslie Hubricht

Stenotrema angellum, new species.

Shell imperforate, lens-shaped, spire convexly conoid, whorls 5 to 5.5, nearly flat; base convex; periphery bluntly angular in front, becoming somewhat rounded behind the lip. Color cameo brown to snuff brown. Embyronic sculpture of minute radially elongate granules, later whorls with irregular radial striae and short hairs which on the upper surface have radially elongated bases, on the lower surface the bases of the hairs are spirally elongated. Aperture narrow, parietal tooth brownish, long, gently curved, high, leaning towards the basal lip, its outer end curving into the aperture, axial end curving towards the basal lip but separated from it by a deep sinus. A distinct buttress connects the parietal tooth with the termination of the outer lip. Basal lip wide, adnate, inner edge thick, pale brown, with a rather small V-shaped median notch. Interdenticular sinus rather narrow and moderately deep. Outer lip with a very low tooth. Fulcrum well developed.

Height 6.4 mm.	diameter 10.9 mm.	whorls 5.3	Holotype.
Height 6.5 mm.	diameter 10.8 mm.	whorls 5.5	Paratype.
Height 6.0 mm.	diameter 10.0 mm.	whorls 5.4	Paratype.
Height 6.0 mm.	diameter 11.0 mm.	whorls 5.3	Paratype.

Localities.—INDIANA: Jefferson Co.: Ohio River Bluff, 1 mile north of Marble Hill. Clark Co.: hillside, 1.5 miles northeast of Charlestown. Harrison Co.: Blue River Bluff, 1 mile west of White Cloud. Crawford Co., Wyandotte. KENTUCKY: Trimble Co.: ravine, near Milton. Franklin Co.: Frankfort. Meade Co.: Ohio River bluff, 5 miles east of Brandenburg; ravine, below Morgans Cave, Otter Creek Park. Anderson Co.: Kentucky River Bluff, just north of Tyrone. Woodford Co.: Kentucky River bluff, west of Versailles. Mercer Co.: Kentucky River Bluff, 1 mile northeast of Shakertown; Kentucky River bluff, near Brooklyn Bridge. Jessamine Co.: Jessamine Creek bluff, at Glass Mill, southeast of Wilmore cedar woods, Camp Nelson, holotype 622091 and paratypes 622092 U. S. National Museum, other paratypes 19036, collection of the author. Gerrard Co.: Kentucky River bluff, north of Bryantsville. Edmonson Co.: Green River bluff, near Mammoth Cave; base of Indian Hill, 1 mile northeast of Brownsville. Hart Co.: Green River bluff, 1.5 miles south of Linwood. Green

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Co.: creek bluff, 4.5 miles south of Exie. Adair Co.: upland oak woods, 5 miles southeast of Columbia. Russell Co.: mountainside, near Wolf Creek Dam. Pulaski Co.: Cumberland River bluff, Burnside. Warren Co.: Barren River bluff, at Ky-101, south of Three Forks; ravine, Friendship Spring, 1.4 miles south of Alvaton. Logan Co.: edge of cedar woods, 3 miles northeast of Russellville. Cumberland Co.: roadside, 1 mile northeast of Dubre; near Dutch Creek Cave, 2.2 miles north of Waterview. TENNESSEE: Clay Co.: fossil, talus, 4 miles southwest of Celina. Jackson Co.: fossil, talus, 10 miles northeast of Gainesboro.

Stenotrema angellum may be readily distinguished from *S. stenotrema* (Pfr.), with which it is frequently found, by the depressed shape, subangular periphery, and by the radially expanded bases of the hairs on the upper surface of the shell.

Mesodon burringtoni, new species.

Mesodon mitchellianus downieanus (Bland), Hubricht, 1950, *Nautilus 64:* 7.

Shell depressed-globose, with conoidal spire, translucent pale corneus to coppery, glossy, imperforate. Whorls 4 to 5, somewhat flattened above with impressed sutures. Embryonic whorls smooth, with fine close rib-striae beginning near the end of the second whorl, microscopic impressed spiral lines beginning about the middle of the third whorl. Last quarter whorl rapidly expanding, decending in front, with a furrow behind the lip. Peristome white, reflected, thickened within. Parietal callus thin and transparent.

Tip of the everted penis with a lobed fan-shaped process, the lobes lavender colored.

Height 10.4 mm.	diameter 14.3 mm.	Holotype.
Height 8.6 mm.	diameter 11.9 mm.	Paratype
Height 11.1 mm.	diameter 14.4 mm.	Paratype.
Height 11.3 mm.	diameter 16.6 mm.	Greendale.
Height 8.6 mm.	diameter 11.7 mm.	Altavista.

Localities.—VIRGINIA: Campbell Co.: near the river, Altavista. Pittsylvania Co.: bluff along Staunton River, opposite Altavista. Staunton River bluff, Smith Mountain Gorge, 5 miles north of Sandy Level. Montgomery Co.: near the railroad, Radford. Smyth Co.: near North Fork Holston River, Chatham Hill, holotype 202184, and paratypes 202183 Academy of Natural Sciences of Philadelphia, other paratypes A9020, collection of the author; Locust Cove Creek bluff, 4 miles east of Broadford. Washington Co.: ravine, 1.4 miles northwest of Greendale; bluff along North Fork Holston River, 2 miles southeast of Hyters Gap. WEST VIRGINIA: Fayette Co.: railroad embankment, Belva. Mercer Co.: Spanishburg (M.C.Z.). TENNESSEE: Hamilton Co.: Chattanooga; Sawyer Springs (A.N.S.P.).

Mesodon burringtoni is most closely related to *M. wheatleyi* (Bland) which has a similar fan-shaped process on the tip of the everted penis, but it is pigmented a dark slaty-blue.

This species has been confused with *M. downieanus* (Bland) and *M. mitchellianus* (Lea). It differs from *M. downieanus* in having finer rib-striae and a larger more globose shell. *M. mitchellianus* does not have the fan-shaped process on the tip of the everted penis.

This species, distinguishable by anatomical characters, is named in honor of the land snail anatomist, Dr. Horace Burrington Baker.

Triodopsis burchi Hubricht

Triodopsis tennesseensis burchi Hubricht, 1950, *Nautilus 64:* 8.

Shell depressed with low conoidal spire, buffy brown in color, glossy, umbilicate, the umbilicus contained about 7 times in the diameter of the shell. Whorls 4.5 to 5.5, body whorl with rounded periphery, not decending in front, channeled behind the lip. First two whorls of spire smooth or nearly so, third whorl with numerous growth wrinkles, last two whorls finely rib-striate, spiral lines absent. Aperture somewhat triangular in outline, lip flatly reflected, rather narrow, thickened within, with a very small conical tooth on the upper lip and a similar tooth on the basal lip. Parietal tooth rather short but high.

Height 5.5 mm.	diameter 10.9 mm.	whorls 4.8	Holotype.
Height 6.7 mm.	diameter 12.5 mm.	whorls 5.0	Paratype.
Height 4.1 mm.	diameter 7.6 mm.	whorls 4.5	Brier Mtn.
Height 7.8 mm.	diameter 17.4 mm.	whorls 5.5	Bent Mtn.

Localities.—VIRGINIA: Montgomery Co.: Lafayette (A.N.S.P.). Roanoke Co.: Poor Mtn., 2.2 miles northwest of Airpoint; near dripping spring, Bent Mtn., near head of Back Creek; 3700 ft., Poor Mtn., south of Glenvar; Salem; near mouth of Dry Hollow Cave, 1.5 miles south of Olaf. Bedford Co.: Summit of Smith Mtn., 20 miles south of Bedford. Franklin Co.: 6.5 miles northeast of Figsboro; 6 miles southeast of Henry Fork; Bald Knob, Rocky Mount; Ferrum. Patrick Co.: 3.5 miles southwest of Nettleridge; low woods near creek, 1.5 miles northwest of Patrick Springs; 1 mile west of Penns Store. Henry Co.: ravine, Turkeycock Mtn., head of Terry Creek, 4 miles west of Callands; Ridgeway; Martinsville. Pittsylvania Co.: Smith Mtn. Gorge, 5 miles north of Sandy Level; summit of Smith Mtn., 3.5 miles northwest of Sandy Level; Staunton River bluff, 3 miles northwest of Brights; hills above Staunton River, 2 miles northeast of Hurt; Brier Mtn., north of Callands; summit, Judy Bird Mtn., 3.5 miles southwest of Bachelors Hall; Sandy River bluff, 4 miles northeast of Bachelors Hall, woods, 4 miles east of Bachelors Hall; along US-58, 3 miles west of Danville, holotype and paratypes 186178 A.N.S.P., other paratypes A8422, collection of the author; woods, 1.5 miles west of Schoolfield; 1 mile north of Schoolfield; oak ridge, along

Mt. Cross road (Va-750), 1 mile northwest of US-58, west of Danville; Danville; along Cascade Creek, 2 miles south of West Fork. NORTH CAROLINA: Rockingham Co.: 1.8 miles northeast of Mayodan; summit of Mayo Mtn., 1 mile east-northeast of Mayodan; 5 miles east of Draper. Guilford Co.: 5.5 miles northwest of Julian. Davidson Co.: 4.5 miles south of Handy; 3.8 miles south of Fair Grove. Randolph Co.: 1.3 miles north of Staley. Montgomery Co.: 2 miles northwest of Eldorado.

Triodopsis burchi is a species of the western Piedmont, which has spilled through the Roanoke Gap in the Blue Ridge Mountains, into the Great Valley. It has climbed the mountains in a small area in Roanoke County, where it has become quite large. In North Carolina it occupies a narrow belt between the range of *T. juxtidens* (Pilsbry) on the east, and *T. pendula* Hubricht on the west. Within its range it is the dominant dentate *Triodopsis* of the upland oak woods.

Additional collecting has shown this snail to be a distinct species rather than a subspecies of *Triodopsis tennesseensis* (Walker) as originally treated. It has been felt advisable, therefore, to describe it more fully. It is probably more closely related to *T. complanata* (Pilsbry) than to *T. tennesseensis.*

Triodopsis picea, new species

Shell depressed-globose, with convexly conoid spire, dull snuff brown in color, periphery rounded, sutures well impressed. Umbilicate, the umbilicus contained about 6.5 to 7 times in the diameter of the shell. Sculpture of rather coarse rib-striation, which continues onto the base of the shell; entire surface, except nuclear whorls, covered with papillae. Whorls slowly increasing, periphery of body whorl rounded, decending slightly in front, with a deep furrow behind the lip. Aperture somewhat dished, lip reflected, with a heavy white callus within, outer lip-tooth broad, blunt, only slightly inflected, basal tooth narrow, seated on a nearly straight callus ridge. Parietal tooth high, long, nearly straight.

Height 8.6 mm.	diameter 13.7 mm.	Holotype.
Height 7.9 mm.	diameter 11.9 mm.	Paratype.
Height 8.1 mm.	diameter 14.2 mm.	Paratype.
Height 9.7 mm.	diameter 14.8 mm.	Webster Co.

Localities.—WEST VIRGINIA: Pendleton Co.: 4400 ft, Spruce Knob, holotype 202186, and paratypes 202185 A.N.S.P., other paratypes 11895, collection of the author. Webster Co.: wooded hillside, 1 mile east of Three Forks of William River. Fayette Co.: mountainside, 4.5 miles northwest of Rainelle.

Triodopsis picea is related to *T. fraudulenta* (Pils.) and *T. juxtidens* (Pils.), appearing intermediate between the two. The teeth are similar to those of *T. fraudulenta* but not as large. It resembles *T. juxtidens* in the smaller umbilicus. It is often higher spired than either of the above species, and is unique in having papillae over the upper surface of the shell.

Triodopsis palustris, new species.

Shell depressed, of 5.5 whorls, snuff brown in color, somewhat glossy. Umbilicate, the umbilicus contained about 6.5 times in the diameter of the shell. Embryonic 1.5 whorls smooth or with very fine radial striae, later whorls closely rib-striate. Last whorl well-rounded with the periphery above the middle, decending in front, contracted behind the lip. Lip reflected, with a thin white callus within; with a small tooth, a little immersed on the outer margin; and a small basal tooth, with a callus extending towards the columella. Parietal tooth of moderate size, curved, tongue-shaped.

Height 7.0 mm.	diameter 13.2 mm.	Holotype.
Height 8.4 mm.	diameter 15.2 mm.	Paratype.
Height 6.2 mm.	diameter 11.9 mm.	Paratype.

Localities.—SOUTH CAROLINA: Berkeley Co.: flood-plain of Santee River, 5.5 miles northwest of St. Stephens, holotype 202187, and paratypes 202188 A.N.S.P., other paratypes 10410, collection of the author. Williamsburg Co.: flood-plain of Santee River, 2 miles north of Jamestown. Georgia: Altamaha Swamp (A.N.S.P.). Screven Co.: flood-plain of Savannah River, 17 miles northeast of Sylvania.

Triodopsis palustris resembles *T. hopetonensis* (Shuttleworth), but this seems to be due more to similar degeneration than to close relationship. It is probably more closely related to *T. pendula* Hubricht. It is a species of the flood-plains of the larger rivers of South Carolina and Georgia.

Mesomphix ruidus, new species.

Shell perforate, depressed, dull pale Saccardo's umber above, glossy ucru-olive below. Whorls 5 to 5.5, well rounded, sutures moderately impressed. First whorl nearly smooth, later whorls rib-striate, last whorl distinctly but finely rib-striate above and on the sides, but becoming very weakly so below. Very weak papillose spiral threads beginning on the second whorl, becoming more strongly developed on later whorls, well developed on the last whorl above and on the sides, but weak or wanting below. Lip thin, aperture a little wider than high.

Height	Diameter	Aperture Ht.	Aper. Diam.	
14.3 mm.	20.8 mm.	10.6 mm.	11.2 mm.	Holotype.
13.0 mm.	19.7 mm.	9.4 mm.	10.2 mm.	Paratype.
13.9 mm.	20.7 mm.	10.0 mm.	11.0 mm.	Paratype.
14.9 mm.	22.0 mm.	10.8 mm.	11.9 mm.	Paratype.

Penis rather short, club shaped, with a slender terminal appendage which is about one-half as long as the penis. Interior of penis uniformly papillose, with a single pilaster extending downward from the opening of the epiphallus. Retractor muscle moderately heavy. Epiphallus rather stout, a little longer than the penis, entering the penis about one-third from the end. Spermathecal duct short, spermatheca large, ovoid, Free oviduct about as long as the penis.

Localities.—INDIANA: Harrison Co.: near Mosquito Creek, 1 mile north of New Boston. KENTUCKY: Meade Co.: Ohio River flood-plain, just east of Brandenburg, holotype 202182, and paratypes 202181 A.N.S.P., other paratypes 16451, collection of the author. Edmondson Co.: Green River flood-plain, near River Styx, Mammoth Cave National Park. TENNESSEE: Montgomery Co.: near Dunbar Cave, near Clarksville.

Mesomphix ruidus may be distinguished from *M. vulgatus* H. B. Baker, by the higher spire and coarser sculpture of the shell, and by the presence of a distinct appendage on the penis.

Helicodiscus triodus, new species

Shell discoidal, the spire flat or nearly so; dull pale corneus, opaque; umbilicus wide and shallow, showing all the whorls, contained from 2 to 2.5 times in the diameter of the shell. Whorls 4.5, well rounded, very narrow and slowly increasing, sculptured with numerous spiral threads. Aperture lunate, the peristome thin. Occurring at irregular intervals within are pairs of small conical teeth on the outer and basal walls, on the parietal wall in front of each pair of teeth is a broad flat-topped tooth, butressed behind. There are usually three or four groups of teeth in the last whorl. The teeth in the preceding whorls are absorbed as the shell grows.

Height 1.5 mm.	diameter 4.0 mm.	Holotype.
Height 1.6 mm.	diameter 4.2 mm.	Paratype.

Type locality.—WEST VIRGINIA: Fayette Co.: in shale talus, below Cane Branch Falls. 1.3 miles southeast of Gauley Bridge, holotype 202180, and paratypes 202179 A.N.S.P., other paratypes A8765, collection of the author.

Helicodiscus triodus may be readily distinguished from *H. parallelus* (Say) by its larger size, shallower umbilicus, coarser sculpture on the nuclear whorls, finer but more numerous spiral threads on the later whorls, and by the presence of a tooth on the parietal wall in front of the paired teeth on the outer and basal walls.

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The Substitution of Mannich Groups on Some Halogenated Phenols*

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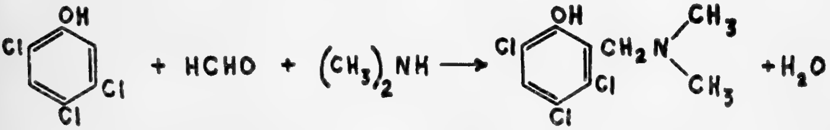
SINCE 1953 a large number of synthetic amino phenols and bisphenols have been prepared in this laboratory (1,2) with the idea that possibly some of them might possess useful physiological properties. This work has been carried out in cooperation with the Geschickter Fund for Medical Research, Washington, D. C., where the physiological testing program has been conducted.

In the synthesis work and testing of some of the single-ring phenolic derivatives containing halogen atoms in the ring, it was observed in a few instances that a halogenated phenol possessing a Mannich group showed some activity similar to that of a bronchial dialator. Consequently, several compounds containing Mannich groups and halogen atoms attached to a single phenolic ring have been prepared. Only the synthesis part of the work is reported here.

Using a secondary amine, formaldehyde, and the appropriate halogenated phenol as starting materials, Mannich derivatives of phenols were prepared in a way similar to that described in a previous article (2). Halogenated phenols used were as follows: 4-bromophenol, 4-chlorophenol, 6-chlorothymol, 3,5-dimethyl-4-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol. The following secondary amines were employed: dimethylamine, diethylamine, morpholine, pyrrolidine, piperidine, and N-methylpiperazine. A typical example is the reaction between 2,4,5-trichlorophenol, formaldehyde, and dimethylamine.

These compounds are listed as free bases in Tables I and II. The yields in most cases were satisfactory and ranged from 75 to 95%,



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* A portion of the thesis submitted by D. S. Byrd, Jr., to the Graduate School, University of Kentucky, in partial fulfillment of requirements for the M.S. degree.

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TABLE I. MANNICH DERIVATIVES OF SOME MONO-HALOGEN PHENOLS[a]

Mannich Group added to phenol	M.p., °C.	Formula	Nitrogen, %		Neutralization Equiv.		Ring Positions Substituted by Mannich Groups[b]
			Calc'd	Found	Calc'd	Found	
From 6-Chlorothymol							
Dimethylaminomethyl[c]	54.5–5.5	$C_{13}H_{20}ClNO$	5.79	5.86	242	240	2
Diethylaminomethyl	26–7	$C_{15}H_{24}ClMO$	.....	.....	270	269	2
Morpholinomethyl	88–9	$C_{15}H_{22}ClNO_2$	4.94	5.05	284	283	2
N-methylpiperazinomethyl[d]	87–7.5	$C_{16}H_{25}ClN_2O$	.....	.....	148	148	2
Piperidinomethyl	85–6.5	$C_{16}H_{24}ClNO$	4.97	5.08	282	280	2
Pyrrolidinomethyl	58–9	$C_{15}H_{22}ClNO$	5.23	5.25	268	267	2
From 3,5-Dimethyl-4-Chlorophenol							
Dimethylaminomethyl (mono)	63–4	$C_{11}H_{16}ClNO$	6.56	6.25	214	213	2
Dimethylaminomethyl (di)	41–3	$C_{14}H_{23}ClN_2O$	10.35	7.85	135	161	2,6(?)[f]
Morpholinomethyl (mono)	127–8	$C_{13}H_{18}ClNO_2$	5.48	5.60	256	255	2
Morpholinomethyl (di)[e]	174–6	$C_{18}H_{27}ClN_2O_3$	7.89	7.62	177	176	2,6
N-methylpiperazinomethyl (mono)	132–2.5	$C_{14}H_{21}ClN_2O$	10.42	10.70	134	137	2
Piperidinomethyl (mono)[e]	148.5–9	$C_{14}H_{20}ClN_2O$	5.52	5.35	254	254	2
Pyrrolidinomethyl (mono)[g]	44–5	$C_{13}H_{18}ClNO$	.....	.....	240	238	2
Pyrrolidinomethyl (di)	103.5–4.5	$C_{18}H_{27}ClN_2O$	8.68	8.55	161	161	2,6
From 4-Bromophenol							
Piperidinomethyl	60–2.5	$C_{12}H_{16}BrNO$	.....	.....	270	268	2
Pyrrolidinomethyl	75–6	$C_{11}H_{14}BrNO$	5.47	5.35	256	254	2
From 4-Chlorophenol							
Pyrrolidinomethyl	69–71	$C_{11}H_{14}ClNO$	6.62	6.39	212	212	2

[a] All derivatives prepared and reported as free amine bases. They were recrystallized from absolute methanol except where otherwise indicated. [b] Mannich groups (R_2N-$CH,_2$) were introduced ortho to the OH group. [c] Full name of compound: 6-Chloro-2-(α-dimethylamino)methylthymol. [d] Recrystallized from ethanol-water mixture. [e] Recrystallized from absolute ethanol. [f] Probably a mixture of the mono-and di-derivatives. [g] Recrystallized from methanol-water mixture.

TABLE II. MANNICH DERIVATIVES FROM DI- AND TRI-SUBSTITUTED HALOGENATED PHENOLS[a]

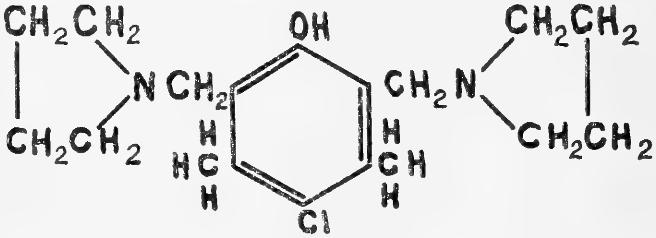
Mannich Group added to phenol	M.p. °C.	Formula	Analysis, %		Neutralization Equiv.	
			Calc'd	Found	Calc'd	Found
From 2,4-Dichlorophenol						
Dimethylaminomethyl[b]	60.5–1.5	$C_9H_{11}Cl_2NO$	49.38 (C); 5.06 (H)	49.57 (C); 5.04 (H)	220	220
Morpholinomethyl[c]	91.5–2	$C_{11}H_{13}Cl_2NO_2$	50.60 (C); 5.02 (H)	50.40 (C); 4.87 (H)	262	261
Piperidinomethyl	80.5–1	$C_{12}H_{15}Cl_2NO$	55.60 (C); 5.83 (H)	55.90 (C); 5.87 (H)	260	261
Pyrrolidinomethyl	46.5–7.5	$C_{11}H_{13}Cl_2NO$	53.68 (C); 5.34 (H)	53.71 (C); 5.38 (H)	246	246
From 2,4,5-Trichlorophenol						
Diethylaminomethyl	81–2.5	$C_{11}H_{14}Cl_3NO$	4.95 (N)	4.80 (N)	283	284
Morpholinomethyl	138.5–9.5	$C_{11}H_{12}Cl_3NO_2$	4.72 (N)	4.53 (N)	297	294
N-methylpiperazinomethyl[d]	88–9	$C_{12}H_{15}Cl_3N_2O$	9.05 (N)	8.86 (N)	155	155
Piperidinomethyl	110–1.5	$C_{12}H_{14}Cl_3NO$	4.75 (N)	4.93 (N)	294	295
Pyrrolidinomethyl	80–2	$C_{11}H_{12}Cl_3NO$			281	278

[a] All compounds in this table are mono-Mannich derivatives reported as the free base with a single Mannich group ortho to the OH in each case. [b] This and other derivatives were recrystallized from absolute methanol unless otherwise stated in notes. [c] From 95% ethanol. [d] From methanol and water mixture.

depending on impurities present, solubility of product and type of solvent used.

With 6-chlorothymol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol only a single Mannich group can be introduced into the ring since only one ortho position is open. However, with 4-bromophenol, 4-chlorophenol, and 3,5-dimethyl-4-chlorophenol, it is possible that di-Mannich derivatives could be prepared since two ortho positions are open in these compounds for the introduction of Mannich groups. No di-Mannich derivatives of 4-bromophenol and of 4-chlorophenol could be prepared, and only two mono-Mannich derivatives of each of these two phenols could be isolated as pure crystalline compounds (those from pyrrolidine and piperidine). Only one of these four mono-Mannich compounds, the 2-(∝-piperidyl)methyl-4-chlorophenol from 4-chlorophenol, HCHO, and piperidine, has been reported previously (**3**); the other three are described in Table I. Attempts to make other derivatives of 4-bromophenol and of 4-chlorophenol resulted in the formation of gels or oils which failed to crystallize and purification was not possible.

With 3,5-dimethyl-4-chlorophenol the situation was somewhat different. Nice crystalline mono-Mannich derivatives were obtained with five different amines, and di-Mannich derivatives resulted when the attempt was made with morpholine and pyrrolidine; dimethylamine, however, produced a mixture of the mono-and di-Mannich compounds (M.p., 41-43°) which could not be separated. The second Mannich group entered more slowly and with more difficulty than the first one. Neither N-methylpiperazine nor piperidine under conditions used would produce a di-Mannich derivative. This may be due to a kind of steric effect with larger entering groups. Formula for the di-Mannich pyrrolidine derivative is given below:



2,6-Bis-(∝-pyrrolidinyl)methyl-3,5-dimethyl-4-chlorophenol
M.p., 103.5-4.5°C (see Table I)

EXPERIMENTAL

All melting points were taken with a Fisher-Johns apparatus, and are uncorrected. The analytical results for carbon, hydrogen, and nitrogen were furnished by the Weiler and Strauss Microanalytical Laboratory, Oxford, England. Neutralization equivalents were determined with standard perchloric acid in glacial acetic acid solution according to the method of Seaman and Allen (**4**).

Preparation of Mono-Mannich Derivatives

2-(α-Pyrrolidinyl)methyl-3,5-dimethyl-4-chlorophenol is representative of the group of mono-Mannich derivatives prepared. A mixture of 9.36 g. (0.06 mole) of 3,5-dimethyl-4-chlorophenol, 4.4 g. (0.06 mole) of pyrrolidine, and 15 ml. of absolute ethanol was cooled in an ice bath, and 5.4 g. (0.06 mole) of aqueous formaldehyde (37%) was added very slowly over a 15-minute period with shaking and cooling. After addition of the formaldehyde the solution was allowed to stand one hour at room temperature and then refluxed on a steam bath for two hours. By using an aspirator the excess solvent was withdrawn leaving an oily residue Crystals weighing 11.75 g. (82.5% of theoretical yield) were obtained by adding 10 ml. of fresh absolute alcohol and allowing to cool overnight. Recrystallization from a 90% methanol (and 10% water) solution gave white crystals melting sharply at 44-5°C. (See Table I for analytical data.) The product was easily soluble in dilute mineral acids.

Preparation of Di-Mannich Derivatives

2,6-Bis-(α-pyrrolidinyl)methl-3,5-dimethyl-4-chlorophenol was prepared from 2-(α-pyrrolidinyl)methyl-3,5-dimethyl-4-chlorophenol, formaldehyde, and pyrrolidine. In a 100 ml. flask 4.3 g. (.018 mole) of 2(α-pyrrolidinyl) methyl-3,5-dimethyl-4-chlorophenol, and 3.8 g. (.053 mole) of pyrrolidine were slowly mixed with 15 ml. of absolute ethanol. After cooling in an ice bath, 3.2 g. (.04 mole) of aqueous formaldehyde (37%) was added dropwise over a 15-minute period and the mixture allowed to stand about one hour at room temperature. The flask was then connected to a vertical condenser and the contents refluxed for five hours on a steam bath. The excess solvent was then removed by distillation at reduced pressure, cooled, and allowed to solidify. About 10 ml. of warm absolute methanol was added to the solidified crude product. White crystals weighing 5.2 g. (88.8% of theory) were obtained. A second recrystallization from absolute methanol gave white needles melting at 103.5-104.5°C. (See Table I for analytical data.) The product dissolved readily in dilute acids.

Note: An alternate procedure for preparing this derivative would be to start with the equivalent of one mole of 3,5-dimethyl-4-chlorophenol and add a minimum of four to five moles of pyrrolidine plus four to five moles of formaldehyde, and reflux for at least six to eight hours. Because of purification difficulties with some new compounds of this type, the other method would, in general, be preferred.

ACKNOWLEDGMENTS

The authors wish to thank the Geschickter Fund for Medical Research, Washington, D.C., for help and cooperation in carrying out the present research program. Especially do they appreciate the helpful suggestions of Dr. C. F. Geschickter of the Georgetown Med-

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The Male of Pseudanophthalmus audax (Coleoptera, Carabidae)

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OF THE six species of eyeless carabid beetles known to inhabit the caves of the Mammoth Cave region in Edmonson and Barren Counties, Kentucky, the rarest and most aberrant is perhaps *Pseudanophthalmus audax.* This species was described by Horn (1883) from material collected by Hubbard and Schwarz in "Ronalds Cave, Ky." About 4.0 to 4.5 mm in length, *P. audax* is distinguished from all other known *Pseudanophthalmus* by the absence of the first and second elytral discal setae. The aedeagus of the male has not been described previously. Since 1928 several unsuccessful attempts to relocate Ronalds Cave (Bolívar and Jeannel, 1931; Henrot, 1949; J. M. Valentine, pers. comm.) have been made.

In the present paper the number, sex, and present location of the original series of *P. audax* collected by Hubbard and Schwarz is clarified, and an aedeagus of a male from this series is described and figured (Fig. 1). The location of the type locality cave is given, though several visits to the cave have yielded no additional examples of this unusual cavernicole.

Jeannel (1931, 1949) described and figured the female *P. audax* quite adequately, though he was mistaken in the belief that the type series consisted of three females. At least six specimens, three of them males, were collected by Hubbard and Schwarz. The type, in the LeConte collection at the Museum of Comparative Zoology, Harvard University, is a male (P. J. Darlington, pers. comm.), as Horn's original description clearly states. Two males are in the Horn collec-

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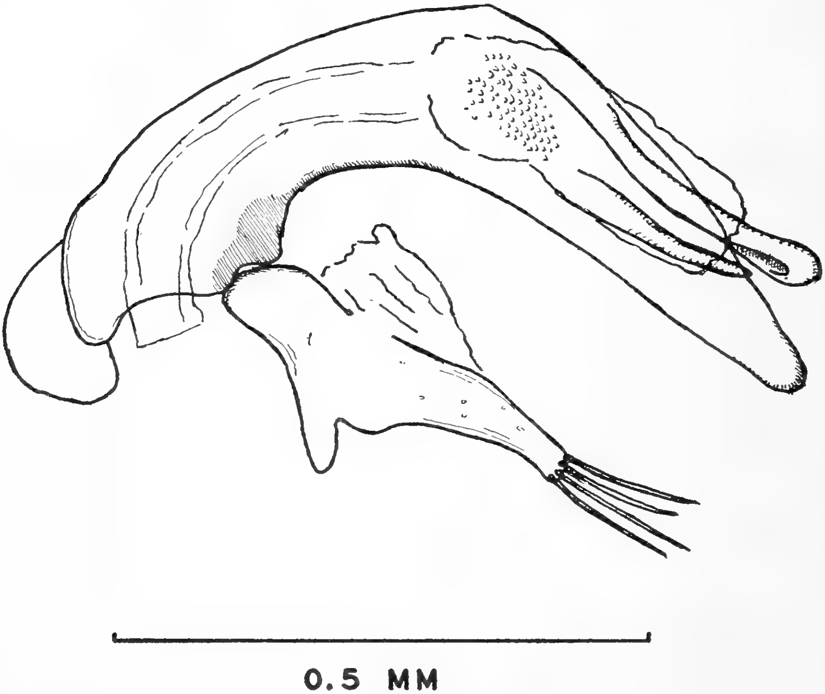

FIGURE 1. Aedeagus of *Pseudanophthalmus audax* (Horn), Ronalds Cave, Kentucky

tion (H493) at the Academy of Natural Sciences of Philadelphia; these specimens were apparently not available to Horn at the time he described the species. Two females are in the U. S. National Museum collections, and one female is in the Museum National de l'Histoire Naturelle, in Paris.

The aedeagus of one of the males in the Horn collection was removed and studied (Fig. 1). It measures 0.75 mm in length. The median lobe is thick, strongly arcuate, with a large, terminal basal plate and a blunt apex. The transfer apparatus consists of a shorter, stout, rod-like left copulatory piece with pointed apex, and a longer, clavate right piece which is apparently medially grooved. The internal sac is armed with oblique rows of small, rounded scales. The parameres are stout, with four setae and a prominent ventral apophysis.

The rod-like nature of the copulatory pieces would appear to further Jeannel's (1949) implied suggestion, based on external morphology, that *audax* is a rather curious offshoot from the *menetriesi-robustus* branch of the genus.

Ronalds Cave, the type locality, is situated two and a half miles northeast of Cave City at the edge of Hart County, Kentucky, in the floor of Hatcher Valley. The cave is owned by Robert Hogan. The mouth is a small hole which opens vertically downward. The cave consists of a single gallery 225 feet long, 20 feet wide, and 15 feet high, with a damp irregular floor. Four other species of cave trechines—*Neaphaenops tellkampfi, P. menetriesi, P. striatus,* and *P. pubescens*—have been collected here, but no *audax* have been discovered, despite collecting trips at all seasons of the year.

The writer is indebted to: Dr. P. J. Darlington, Museum of Comparative Zoology, Harvard University, for verification of the sex of the type; Dr. O. L. Cartwright, U. S. National Museum, for loan of specimens; and Dr. Harold J. Grant, Jr., Academy of Natural Sciences of Philadelphia, for loan of specimens.

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Solar Energy Measurements at Lexington, Kentucky, for 1951

E. B. Penrod, W. W. Walton and H. O. Knight
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Research in geophysics is being done by members of the mechanical engineering staff of the University of Kentucky. In 1949, the thermal diffusivity of a clayey soil was determined at a depth of about 5.5 ft. by the use of an earth heat pump (**1**,**2**). Two years later, the thermal conductivity, thermal diffusivity, specific heat, and density of a fine, oven-dried clay were determined simultaneously by performing a single laboratory experiment (**3**). Both of the above methods were of the transient heat flow type. Using a third method, the thermal diffusivity of the same soil was measured by recording soil temperatures at depths of 0, 2, 4, 6, 8, and 10 ft., regularly every two hours throughout the year of 1952 (**4**).

Theoretical calculations show that the cost of operating a solar-earth heat pump compares favorably with the cost of heating houses with conventional furnaces (**5**). This type of heat pump system will operate efficiently in cold weather on days during which the sun does not shine, and is suitable for heating or cooling houses in rural areas. Therefore, it is essential to know how much solar energy is available for operating solar-earth heat pumps.

Geophysical studies at the University of Kentucky include the determination of solar energy that falls on a horizontal surface. A pyrheliometer was mounted on the top of Anderson Hall in 1950 to measure the vertical component of solar radiation (**4**,**6**). The instrument is located at Lat. 38° 02′ N., Long. 84° 30′ W. at an elevation of 1026.6 ft.

In this research a 50-junction type Eppley pyrheliometer is used. It is a form of the U. S. Weather Bureau type, and is a specialized thermopile made with wire of an alloy of 60% gold and 40% palladium against wire of an alloy of 90% platinum and 10% rhodium (**7**,**8**). The wires are 0.0016 inch in diameter. Alternate junctions are in thermal contact with concentric silver rings 0.01 inch thick, but are electrically insulated from them. The receiving surface of the inner

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ring is black, and that of the outer ring is white. The receiving element is hermetically sealed in a lamp bulb of soda-lime glass which transmits about 10% of the incident radiation at 2900A. The bulb, 3 inches in diameter, is mounted on a metal base with leveling screws. Heavy copper leads are provided for connecting the instrument to the wires leading to a recorder located on the second floor of Anderson Hall. The lead-in wires are each 80 ft. in length, and consist of No. 14 solid copper wire housed in weatherproof rigid conduit.

By the use of this pyrheliometer, solar energy falling on a horizontal surface at Lexington has been measured since October 14, 1950. Data are obtained in graphical form by the use of a Leeds & Northrup strip-chart recorder especially designed and calibrated to record solar energy in gram calories per min. per sq. cm. The strip-chart is 9.9 inches wide, having 10 major divisions, each of which is divided into 10 subdivisions. A radiation intensity of 2 gram calories per min. per sq. cm. is required to drive the recording pen through maximum deflection. The chart speed is 2$\frac{1}{16}$ inches per hour, and the chart is divided into 15-minute intervals. The area under the curve on the chart is proportional to the total radiation on a horizontal plane surface in gram calories per sq. cm., since it is a plot of gram calories per min. per sq. cm. versus minutes. The unit of one gram calorie per sq. cm. is defined as one Langley.

If the pen deflection is x inches and the chart movement is y inches, then the product xy represents an actual area on the chart. The total area under the curve for a given period of time can be expressed by the integral $\int xdy$. Now:

$F_x = 2\ \frac{\text{gm. cal.}}{\text{min. cm.}^2} \cdot \frac{1}{9.9\ \text{in.}} = 0.2020\ \frac{\text{gm. cal.}}{\text{min. cm.}^2\ \text{in.}}$, where F_x is the intensity of solar radiation required to move the recording pen one inch in the x direction. And:

$F_y = \frac{60\ \text{min.}}{2\frac{1}{16}\ \text{in.}} = 29.09$ min. per inch, where F_y is the time required for the chart to move one inch in the y direction. The product F_xF_y is set equal to K, a new factor. Then:

$$K = F_xF_y = 0.020\ \frac{\text{gm. cal.}}{\text{min. cu.}^2\ \text{in.}} \times 29.09\ \frac{\text{min.}}{\text{in.}} = 5.8762\ \frac{\text{gm. cal.}}{\text{cm.}^2\ \text{in.}^2}$$

The value of K is the total radiation for each square inch of the chart. When K is multiplied by the area under the curve on the chart for any given period (*i.e.*, for a day) the product is the total solar radiation normal to a horizontal plane surface for that day. In British units K

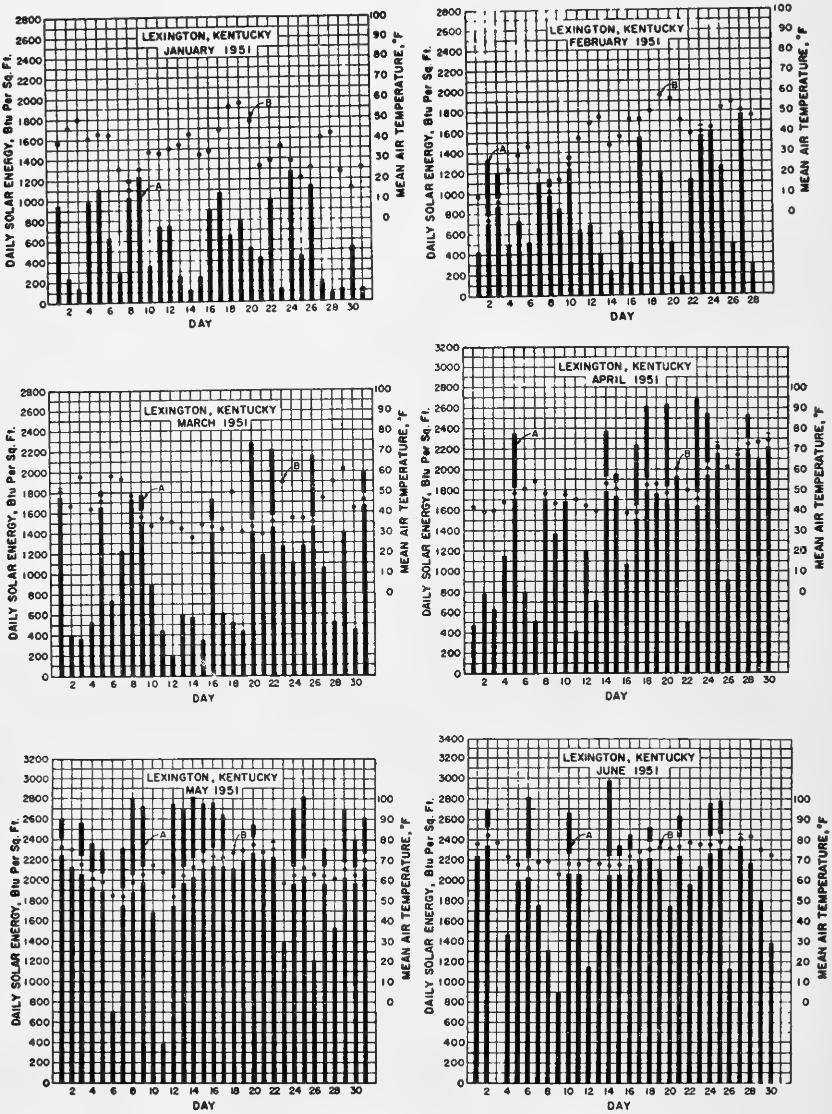

Figure 1. Data for January-June, 1951. A. Solar energy. B. Mean daily air temperature.

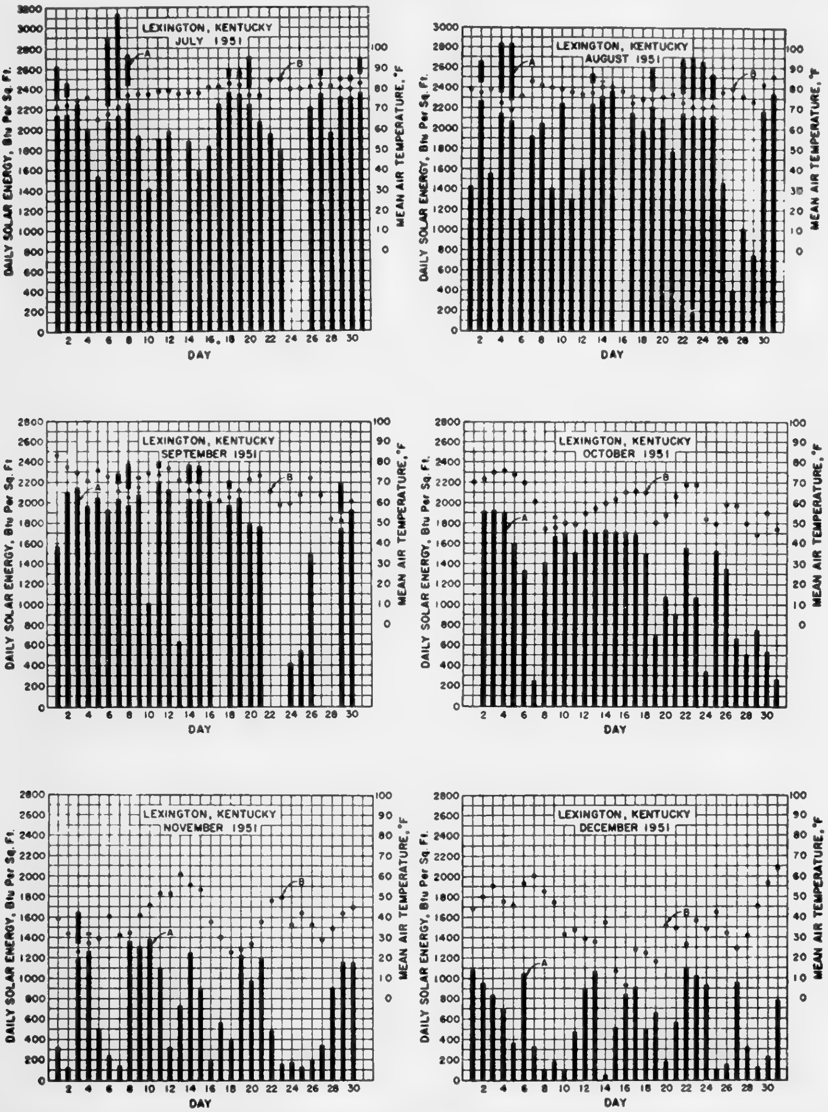

FIGURE 2. Data for July-December, 1951. A. Solar energy. B. Mean daily air temperature.

is equal to 21.6513 Btu. per sq. ft. per sq. in. If Q is the solar energy on a horizontal surface for one day, and A is the area under the curve for that day, then $Q = KA$.

The area A, under one curve is, for example, 134.14 square inches. Then the solar energy for that day is equal to 21.6513 times 134.14, or 2,904.30 Btu. per sq. ft.

Calculated values of Q are shown graphically for each month of the year 1951, in Figures 1 and 2. Mean air temperatures at the Blue Grass Airport are also shown in these figures. Monthly average values of solar energy and air temperatures are shown graphically in Fig. 3. Total and average vales of solar energy for each month are listed in Table I. Also, average air temperatures and total precipitation are given in this table.

From Table I it can be seen that the monthly average solar energy and total solar energy are 41,914 Btu. per sq. ft. (or 1,827,000,000

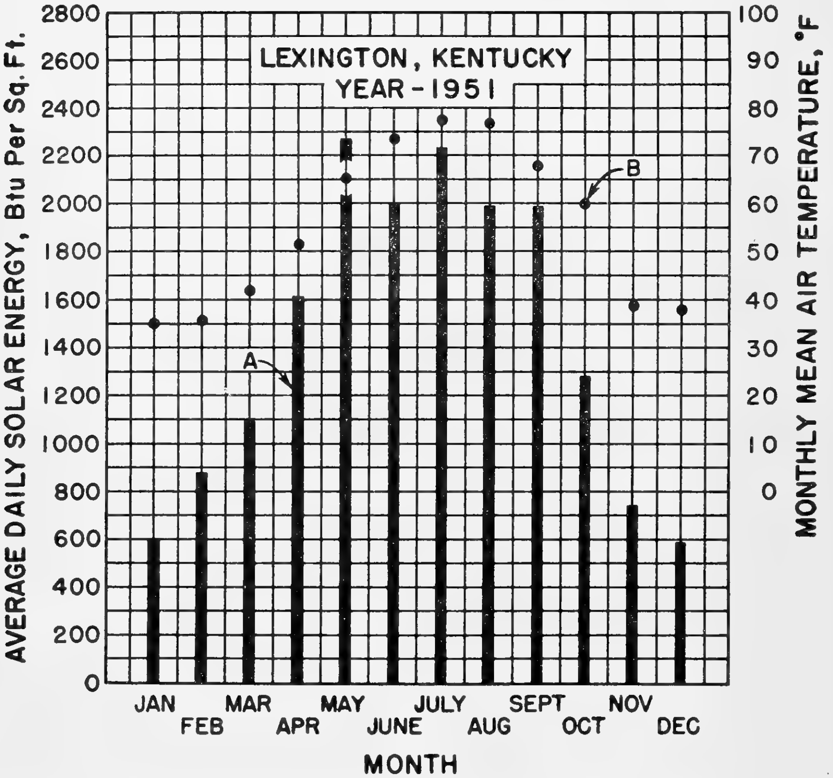

FIGURE 3. Solar energy and average air temperatures for 1951. Solar data were taken and recorded at the University of Kentucky. Air temperatures were taken and recorded at the U.S. Weather Bureau Station at the Blue Grass Airport, Lexington, Kentucky.

Table I. Total and average solar energy for 1951.

Month	Total Solar Energy Btu/sq. ft.	Daily Average Solar Energy Btu/sq. ft.	Average Air* Temperature °F	Precipitation* Inches
January	18,645	601	34.9	11.15
February	24,526	876	35.4	3.64
March	33,706	1,087	41.9	4.96
April	48,465	1,616	51.7	2.49
May	70,232	2,266	65.3	2.16
June	60,330	2,080	73.4	4.38
July	62,546	2,234	77.4	1.83
August	59,714	1,990	76.5	2.98
September	46,130	1,845	67.4	3.60
October	38,435	1,281	59.1	3.10
November	22,168	739	38.4	6.87
December	18,071	583	37.6	7.30
Totals	502,968	17,198	659.0	54.46
Averages	41,914	1,433	54.92	4.54

* U. S. Weather Bureau Data, Blue Grass Airport, Lexington, Kentucky.

Btu. per acre) and 502,968 Btu. per sq. ft. (or 21,909,000,000 Btu. per acre), respectively. There was less than 500 Btu. per sq. ft. of solar energy for 63 days of the year and more than 2,500 Btu. per sq. ft. for 45 days of the year. There was the least solar energy during December, and the most during May and July.

Part of the solar radiation is absorbed and stored temporarily in the earth's crust and the rest is transferred to the atmosphere and outer space by convection and radiation. Some of the incident solar radiation, however, can be trapped and converted into thermal or electrical energy as desired. Direct solar radiation can be converted into electrical energy by the use of thermoelectric generators made of p- and n-type semiconductors. When the sun shines, the electricity can be used to operate a Peltier refrigerator, for example, and when the sun does not shine, the Peltier refrigerator can be made to operate on solar energy stored in solar batteries. Also, trapped solar radiation can be used to heat water and to serve as a heat source for solar-earth heat pumps to provide continuous air conditioning for homes. It is apparent, therefore, that information concerning the amount of solar energy available, and its variation from day to day is becoming increasingly more important.

This paper is an initial report on a ten-year research project designed to establish a norm on solar energy available for doing useful work in this locality. Additional solar data will be released in future papers.

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The Herpetofauna of Clemons Fork, Breathitt County, Kentucky

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DURING THE SUMMERS of 1955 and 1956 a study was undertaken to determine the species of amphibians and reptiles occurring at Clemons Fork, Breathitt County, Kentucky. Some ecological life history data were accumulated on 19 species of amphibians and 20 species of reptiles. An annotated list is presented giving the most salient features of habitats, local distribution, and breeding habits. All specimens are deposited in the collections of the University of Kentucky.

Clemons Fork is located at 83° 13′ West longitude and 30° 27′ North latitude in the southeastern part of the county. The physiography of the area is rough. Elevations in the locale range from approximately 850 feet in the bottomlands to summits of 2200 feet. All collections were made within a radius of five-miles from Camp Robinson, owned and operated by the University of Kentucky.

The periods of most fruitful collecting seemed to be marked by falling temperatures and moderate precipitation. Cool weather and intermittent showers, June 2-10, 1955, resulted in the collection of many herpetological forms. Similar circumstances prevailed June 20-28, 1956, and multitudes of frogs voiced from rain pools. Bishop (1926) commented on the evening rains on June 24, 1925, at Quicksand, Breathitt County, Kentucky, and the throngs of singing frogs.

No previous studies of this nature have been conducted at Clemons Fork. Some herpetofauna species were collected by Dury and Williams (1933) compiled from three discrete locations in Breathitt County. In relation to Clemons Fork, the areas which they studied were: South Fork (25 airline miles N.W.); Lost Creek (9 airline miles W.); and Troublesome Creek (5 airline miles S.W.). In addition, Bishop (1926) reported a few amphibians and reptiles from Quicksand (11 airline miles N.W.), and Barbour (1956) listed one reptile species from the Clemons Fork study area.

The aforementioned authors gave accounts of 16 amphibian and

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10 reptilian forms as existing in the county. During the present study 21 of the 26 species they recorded were secured, and furthermore, 18 new species for the county were found. Because of the proximity of the study areas, probably the other five species extend their range into Clemons Fork.

New records for Breathitt County, Kentucky, are denoted by an asterisk in the annotated list which follows.

ACCOUNT OF SPECIES

Necturus maculosus maculosus Rafinesque. A specimen was reported by Dury and Williams (1933) from the Troublesome Creek area.

Ambystoma opacum Gravenhorst.* Two immature males were taken on June 29, 1955, from an area burned over by fire. An adult male collected on June 4, 1956, was removed from beneath a fragment of sandstone 50 feet from the same location.

Diemictylus viridescens viridescens Rafinesque. Individuals were reported by Dury and Williams (1933) from the Lost Creek area.

Desmognathus fuscus welteri Barbour. The status of this subspecies in Kentucky has recently been clarified by Barbour (1957). The Black Mountain Dusky Salamander was plentiful in all small tributaries of Clemons Fork. It was frequently collected from moist leaves and rocks in the streams. Seventeen egg-bearing females had an average number of 21 eggs. The average egg diameter was 1.5 mm.

Desmognathus ochrophaeus ochrophaeus Cope.* A single female was taken from the bark of an overhanging log in a narrow ravine on June 14, 1955.

Desmognathus monticola monticola Dunn. The Seal Salamander was not too abundant in the study area. Five individuals were taken from a spring in a woodland thicket. Two females collected on June 14 and 16, 1955, had 24 eggs each. The average egg diameter was 1.5 mm.

Plethodon cinereus (Green). A subadult listed by Bishop (1926) represents the only county record.

Plethodon glutinosus glutinosus (Green). All specimens fit the characters ascribed to *P. g. glutinosus* as reviewed by Clay, Case, and Cunningham (1955). It was commonly found in places of high humidity, usually being taken from beneath rocks and logs on wooded slopes. A few were collected as they foraged openly. Bishop (1943) stated, "The dissection of females indicates, farther north, the egg laying season may be in the early spring months.'' Egg laying at Clemons Fork seems to occur about early June. Four females collected on April 21, 1956, had an average of 25 eggs. The average egg diameter was 1.9 mm. A female taken on June 12, 1956, had eight eggs. The average egg diameter was 3.1 mm. Many egg-bearing females taken between these dates all contained eggs. Fifteen mature females collected after June 12 had no eggs.

Gyrinophilus porphyriticus porphyriticus Green. Bishop (1926) collected a larva of this species from Quicksand, Kentucky.

Pseudotriton montanus diastictus Bishop.* This species was not very abundant, largely being distributed at lower elevations beneath logs and rocks. Noble (1931) reported, ''This species, unlike its relative *P. ruber,* is partial to muddy springs.'' Habitat differences between this form and *P. ruber* were not evident. A large female taken on April 21, 1956, had 81 eggs; average egg diameters were 0.8 mm. A smaller female taken on June 20, 1956, had 81 eggs; average egg diameters were 2.1 mm. This individual was collected from a pond teeming with breeding *Hyla versicolor, Pseudacris brachyphona,* and chorusing *H. crucifer.*

Pseudotriton ruber ruber Sonnini. A female collected on April 21, 1956, had numerous eggs; average egg diameters were 0.5 mm. Another female taken on June 21, 1956, contained 40 eggs; average egg diameters were 1.5 mm.

Aneides aeneus (Cope).* A single adult male was collected from beneath the bark of a decaying *Juglans cinerea.* The habitat was a steep slope, considerably shaded by an overstory of *Fagus grandifolia* and *Liriodendron tulipifera.*

Eurycea bislineata rivicola (Green). The Midwest Two-lined Salamander was not especially plentiful. Individuals were taken occasionally from dry woods; however, most were collected from streams.

Eurycea longicauda longicauda (Green). Individuals of this species lived in rather distinct habitats. All specimens were collected from under loose flaky fragments of shale and sandstone; intermittent streams traversed the habitats.

Bufo terrestris americanus Holbrook.* The American Toad frequently was taken as it fed near flood lights at the camp site. Adult females containing eggs were caught on June 6, 14, 16, 18, and July 11, 1955.

Bufo woodhousei fowleri (Hinckley). Fowler's Toad was a puddle visitant after showers had flooded roadside ditches. Females collected on June 22 and July 14, 1956, had eggs.

Hyla crucifer crucifer Wied. Spring Peepers were first heard calling late in the afternoon of June 22, 1956. Four specimens were collected from a rain pool later that evening.

Hyla versicolor versicolor (LeConte). Chorusing Gray Tree Frogs were collected from the limbs of *Salix nigra* and trunks of *Betula nigra* on June 21, 1956. Several more were collected during the following week. A female caught on June 22, 1956, had numerous eggs.

Pseudacris brachyphona (Cope).* Mountain Chorus Frogs appeared abundantly at breeding areas of roadside ditches and field puddles during rainfalls in June 1955 and 1956. Two specimens were taken in dry woods from the bark of a decaying stump. Three egg-bearing females were collected on June 21, 1956.

Rana catesbeiana Shaw.* Deeper pools of water seemed to be the favorite habitat of this common species. Often they were collected in company with *R. c. melanota.*

Rana clamitans melanota Latreille. Mecham's study (1954) refers to this as the Kentucky subspecies of *Rana clamitans.* Green Frogs found harborage in shallow stagnant pools of water at Clemons Fork. Egg-bearing females were collected on June 5 and 6, 1955, June 17 and June 28, 1956.

Rana sylvatica sylvatica LeConte.* An adult female was uncovered from beneath a log on June 25, 1956. A subadult male was collected on July 2, 1956, from moist litter on the forest floor.

Rana pipiens sphenocephala Cope. Two Leopard Frogs were heard calling on June 8, 1956. A single male was collected on June 12, 1956.

Rana palustris LeConte. Dury and Williams (1933) reported the collection of this species in the county. Wright and Wright (1949) reported that, "These frogs breed from April 23 to May 15." I was not in the study area between these dates.

Chelydra serpentina serpentina Linnaeus.* Carr (1952) reported that, "This turtle shows almost no discernible habitat preference." Two males were caught from an exceedingly shallow pool of water partially covered by algal growth. A juvenile female was taken from an intermittent stream which flowed over a gravel road.

Terrapene carolina carolina (Linnaeus). A bottomlands dweller, the Box Turtle was common throughout the study area. On June 21, 1956, a day of rainy weather, many individuals appeared along the roadside. Egg deposition occurs at mid-June. Carr (1952) stated, "The eggs are from two to seven in number, most often 4 or 5." The average egg clutch of 12 females collected during the deposition period averaged 3.6; average egg lengths were 37.8 mm.

Trionyx ferox spinifera LeSueur.* An adult female was collected as it basked in the sun on June 15, 1956; it contained 13 fully developed eggs. The average egg length was 27.1 mm.

Sceloporus undulatus hyacinthinus (Green). This lizard was collected from logpiles, stumps, and pieces of loose tree bark. According to Smith (1946), "Egg laying has been reported for May through early August in various parts of the range, but probably in any one area the season normally includes only about a 6-week period." Egg deposition begins in late May or early June at Clemons Fork. The latest date of a female containing depositional sized eggs was July 11. Smith (1946) reported that the young in Maryland appear at any time from late July to Mid-September, and post depositional development is estimated to be about 10 weeks under normal conditions and 12 weeks in captivity. On June 16, 1955, six *Sceloporus* eggs were collected from beneath a rock. The eggs were placed in moist sand, and hatched on August 21, 1955. The average clutch of eggs for 18 females was 8.4; average egg length was 12.7 mm.

Lygosoma laterale Say.* Most captures were made during the cooler days of June. Frequently individuals were caught as they skittered into woody thickets along the road. According to Smith (1946), "The eggs are 1 to 5, usually 3 in number, laid in humus, rotten stumps, and logs, etc. The egg laying period extends from early June to early August. Hatching occurs in late August and probably throughout most of September." Based on a comparison of lengths of fully developed eggs as given by Smith, egg laying at Clemons Fork attains a seasonal peak in mid-July. Ten females had an average egg number of 3.9; average egg length was 5.9 mm. Three *Lygosoma* eggs were unearthed on July 13, 1955. These were placed in moist sand and hatched on July 24, 1955. Females taken in late July during both summers had no eggs.

Eumeces fasciatus (Linnaeus). The Five-lined Skink occupied a rock-and-log type of habitat. Egg laying in this region seemed to occur in late May and early June. Newly hatched young first appeared on July

27, 1956. Measurements of some young taken in July compared to data gathered by Smith (1946) indicated that the eggs were deposited in early June. Supporting the above conclusions, three *E. fasciatus* eggs collected on July 13, 1955, hatched in a terrarium on July 24, 1955. The average egg clutch of five females was 9.2; egg lengths averaged 8.2 mm.

Eumeces laticeps (Schneider).* A single male was collected from a steep slope in a dry woods on June 25, 1956.

Eumeces anthracinus anthracinus (Baird). Barbour (1956) has recently reported an adult male collected on June 22, 1955.

Natrix sipedon sipedon (Linnaeus). The Northern Water Snake was fairly common along the edges of streams. Generally it sought refuge in the deeper pools of water.

Storeria occipitomaculata occipitomaculata (Storer).* This secretive snake was taken entirely from road kills. It was collected quite commonly in the study area. One adult contained six eggs that had an average length of 13.1 mm.

Thamnophis sirtalis sirtalis (Linnaeus).* Two males were collected at elevations of 1550 feet in dense tangles of *Smilax* sp. and *Vaccinium* sp. Apparently this snake is not abundant.

Heterodon platyrhinos Latreille.* Males taken on June 17, and July 11, 1955, represent the only specimens in my collections. These were caught in a stand of dry pine.

Diadophis punctatus edwardsi Merrem. Rocks and logs offered adequate habitat sites for this abundant species. Two females collected on June 15 and June 17, 1955, had three eggs each. The average egg length was 24.5 mm. Newly hatched young first appeared on August 30, 1955, from eggs kept in captivity.

Carphophis amoenus amoenus Say. Thirty-eight Worm Snakes from this locality fit the characters ascribed to *C. a. amoenus* Say. Five bear the fused internasal-prefrontal characters of *C. a. helanae* Kennicott; five others were intermediate (*i.e.* had one side fused but not both). Egg laying seemed to take place about mid-June. Seven females having depositional-sized eggs had an average clutch of 3.2; average egg length was 24.2 mm. Ten females taken between June 19 and July 2 had no eggs.

Coluber constrictor constrictor Linnaeus. The Black Racer was fairly plentiful. It was collected mostly from wooded areas.

Opheodrys aestivus Linnaeus. This species was frequently collected from open level areas and roadside ditches. A large female was caught as it attempted to climb a large tree. Schmidt and Davis (1941) stated, "The eggs are laid in July and hatch in September." Two females containing depositional-sized eggs were caught on July 11, 1955, and July 15, 1956. The female collected earlier had four eggs with an average length of 34.9 mm.; the other had five eggs with average egg lengths of 30.2 mm.

Elaphe obsoleta obsoleta Say. This snake was common in the study area, and most individuals were collected in pine stands. Schmidt and Davis (1941) stated that, "Matings have been reported for May 25 and June 19, and eggs laid in July in loose earth, damp sawdust or manure piles." On June 22, 1956, a male and female were found mating in a thicket of *Alnus* sp. The female contained 10 eggs; average egg length was 35.4 mm.

Lampropeltis getulus niger Yarrow.* A specimen was taken from a gravel road on June 23, 1956. The snake was badly damaged and the sex was not determined.

Ancistrodon contortrix mokeson (Daudin).* Open, level areas offered fruitful collecting sites for the abundant Copperhead. Gravid females were taken on June 8 and 13, 1955, and June 14 and 16, 1956. The average egg clutch was 5.3; average egg length was 39.3 mm.

Crotalus horridus horridus Linnaeus.* A few individuals were collected at elevations ranging from 1300 to 2100 feet. Klauber (1956) listed a maximum clutch of 17 young for this species. A female collected on June 13, 1955, contained 30 eggs. The average egg length was 6.9 mm. No attempts were made to ascertain the fertility of the eggs. A female taken on June 14, 1955, had 10 eggs; average egg length was 7.1 mm.

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Response of Dwarf and Normal Vertebrates (Cattle and Mice) to Physiological Stress, as Measured by Differential Leucocyte Counts

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INTRODUCTION

THE LEUCOCYTE COUNT has been of value as a diagnostic aid in medical practice for a good many years. An elevated lymphocyte count indicates a generalized toxic condition, an elevated polymorph count is accepted as evidence for a number of bacterial infections, and eosinophilia is of importance to clinicians in diagnosing several conditions. It is known also that, in many instances, a decrease in the numbers of the various leucocytes is evidence for a failure of the body's defensive mechanisms to cope with several conditions.

In more recent years, coincident largely with the work of Hans Selye (1), it has been shown that the differential leucocyte count is a reliable indicator of the effectiveness of the body's homeostatic mechanisms (2). It is known also that the adrenal cortex affects, in a definite way, the response of leucocytes to various kinds of stress. This phenomenon has been used by various workers to indicate the degree of response by the body mechanisms to adrenocortical hormones, ACTH, insulin, and many other forms of stress.

Our own work in this direction grew out of our studies on the forms and manifestations of dwarfism, first in beef cattle (3,4,5), later in mice. These studies were originally morphological, but have become directed toward functional changes. The dwarf animal exhibits many peculiarities of form and function, and is an easy prey to digestive and respiratory disturbances. Furthermore, invariably there are disturbances in the sex mechanisms of dwarfs. From these facts and the fairly consistent variations in the anatomy of the glands of internal secretion, it is natural to postulate physiological abnormalities in the endocrine systems of dwarfs.

Lasley and his coworkers at the University of Missouri (6) have demonstrated differences in the leucocyte response to insulin injections

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between normal, dwarf, and "carrier" calves. In their studies total white, and differential lymphocyte and neutrophil counts were made. They then injected insulin intravenously and repeated blood-cell counts at regular intervals until the counts returned to normal, or very nearly so. They found that in normal animals the total leucocyte count rose sharply within about two hours after insulin injection, and then fell rapidly, returning to a normal level in roughly three more hours. However, in dwarfs the increase was very slight, was lower in occurring, and many hours passed before the return to a normal count. Coincident with these changes were quite definite and constant differences in relative lymphocyte and neutrophil counts in the dwarf and normal animals. The "carriers" (heterozygotes) were approximately midway in their responses. No differences in eosinophil or basophil counts were reported.

EXPERIMENTAL AND RESULTS

We repeated the experiments of the Missouri investigators with a few modifications, and achieved results (Fig. 1) closely comparable to theirs. A combined Giemsa-Wright stain was used, and the total granulocyte count was contrasted with the lymphocytes. Protamine Zinc Insulin was used throughout with a dosage of one unit per kg. of body weight. As the total white cells increased, the relative lymphocyte count decreased and the granulocytes increased. This contrast was much greater in normals than in dwarfs. In normal calves a relative eosinopenia occurred, and in dwarfs a large number of immature granulocytes were noted.

We then made comparable studies using a strain of dwarf mice procured from the Roscoe B. Jackson Laboratory of Bar Harbor, Maine. Most workers who have studied the dwarf mouse (7,8) class it as "pituitary" and not "achondroplastic." There is general agreement that dwarf calves are of the achondroplastic type. However, leucocyte responses to insulin injection in dwarf mice closely parallel those exhibited by dwarf cattle. This is consistent with previous observations (9,10) that the various kinds of dwarfs in different species have many conditions in common, and that dwarfism varies but little, morphologically and physiologically, in the animals studied so far.

Because of the small size of the mice, and especially the dwarfs, it was necessary to inject the insulin intraperitoneally. This may account for some slight differences in response when compared to cattle.

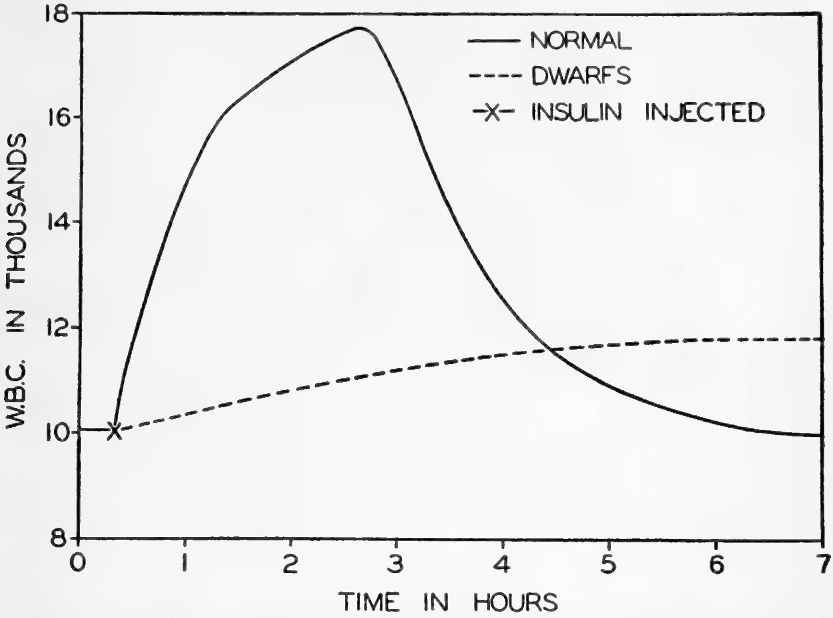

FIGURE 1. Total white-blood-cell count in dwarf and normal calves, showing difference in response to insulin. (6 normals, 6 dwarfs).

Zinc insulin was administered at the rate of 0.04 unit per normal mouse, weighing 28-30 g., and reduced to as little as 0.01 for dwarfs weighing 8-10 g. each. The response of normal mice to insulin (Figs. 2, 3) was rapid and marked, the total white cell count doubling within about two and one-half hours and returning to normal in about the same time. However, the dwarfs showed no more than a 15 per cent increase in total white count, required a longer time to reach this "peak," and did not return completely to a normal count within eight hours. Relative responses of lymphocytes and granulocytes were also similar to those obtained in dwarf cattle, and there was a high incidence of immature granulocytes in dwarf mice as in cattle. In normal mice there was a conspicuous eosinopenia, with no change in basophils, but this was not obvious in dwarfs. These phenomena also parallel those in cattle. Thus the contrast between lymphocytes and neutrophils used by the Missouri group seems logical. The significance of the contrasting eosinophil counts is not clear.

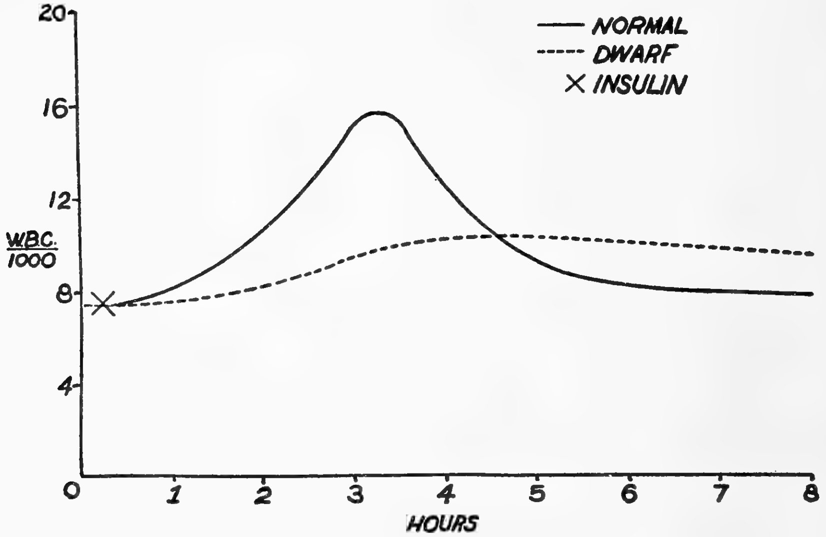

FIGURE 2. Total white-blood-cell count in dwarf and normal mice, following intraperitoneal injection of insulin. (10 normals, 5 dwarfs).

DISCUSSION

The differential leucocyte count is an important aid in measuring the response of the vertebrate homeostatic mechanism to physiological stress. As a result of its use there is additional evidence that the different kinds of dwarfs have several common denominators. Not the least of these is the lowered ability of dwarf animals to respond to stress on the hypothalamic-hypophyseal-adrenocortical axis.

This work could be expanded profitably with further careful studies of the response of each type of leucocyte under many stress conditions, and with histological studies on the hypothalamus and the endocrine glands of the animals subjected to stress.

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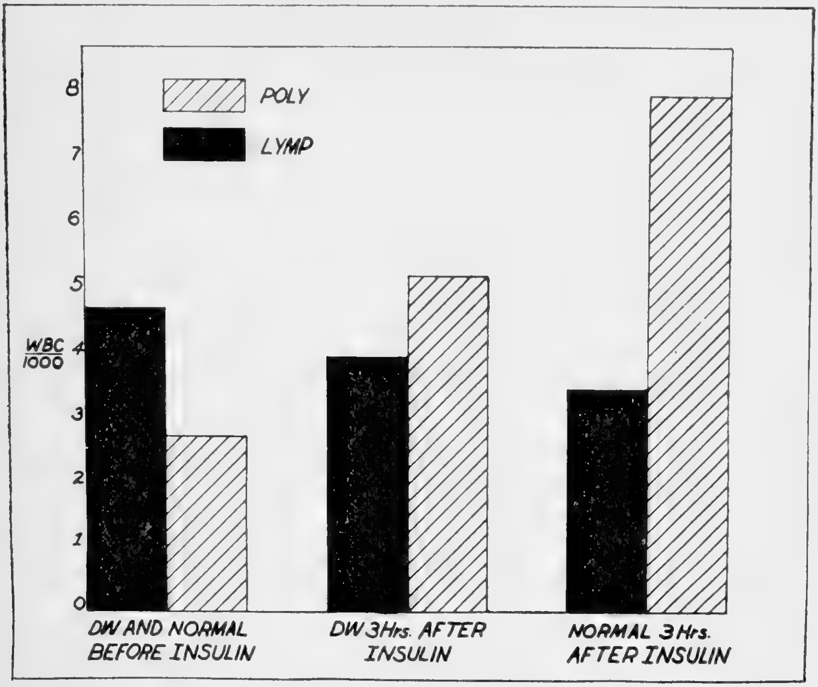

FIGURE 3. Differential white-blood-cell count in dwarf and normal mice 2½-3 hrs. following insulin injection; at peak of total count. (same animals as in Fig 2).

SUMMARY

1. Dwarf and normal cattle and mice were subjected to the stress of insulin injection, and changes in relative leucocyte counts were noted. Responses were similar in both species.
2. Following insulin injection, the total leucocyte count increased, but the lymphocytes decreased. There was a gradual return to normal levels. The initial responses and the return to normal were much more rapid and pronounced in normal animals than in dwarfs.
3. The normal animals exhibited a relative eosinopenia in response to insulin stress, but this was not obvious in dwarfs. In dwarfs many immature granulocytes were noted following insulin injection.
4. Because the responses of dwarf mice and cattle were similar, it is tentatively concluded that the various types of dwarfs in different species have several common denominators. One of these is a low-

ered ability to respond to stress on the hypothalamic-hypophyseal-adrenocortical axis.

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Selective Clemmensen Reduction of 1,2 Diketones

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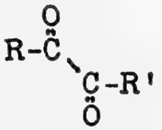
ALTHOUGH the Clemmensen method of reduction has been applied to a variety of carbonyl compounds, the reduction of 1,2-diketones has been neglected. In view of the reduction of alpha-hydroxyketones to ketones (1) by the Clemmensen method without subsequent reduction of the ketone group, it was of interest to determine if one ketone group of a 1,2-diketone could be selectively reduced. The selective reduction of only one ketone group in an unsymmetrical 1,2-diketone also presents interesting possibilities.

In the present work we have studied the reduction under Clemmensen conditions of two aliphatic, straight chain *alpha*-diketones and of two cyclic diketones.

When 2,3-pentanedione was reduced the main product was shown to be a mixture of methyl *n*-propyl ketone and diethyl ketone. That both ketones were present was shown by preparation and separation of derivatives of each ketone. On the basis of the refractive index of the mixture and of the pure monoketones, the mixture appears to consist of equal parts of the two possible isomeric ketones.

In the case of the reduction of 2,3-octanedione (I) in which the alkyl groups attached to the diketone group differ markedly from each other, only 2-octanone was formed. Identification of the product is based on the refractive index of the product (which is different than that of 3-octanone) and on the preparation of the 2,4-dinitrophenylhydrazone. It thus appears that in this case the Clemmensen reduction is subject to certain selective steric effects.



$R-C(=O)-C(=O)-R'$

R=n-amyl, R'=methyl

I

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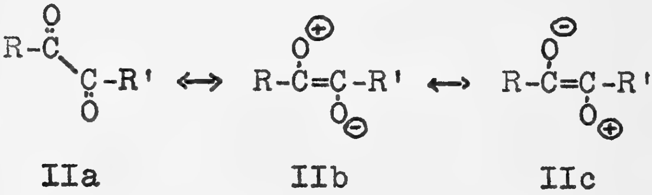
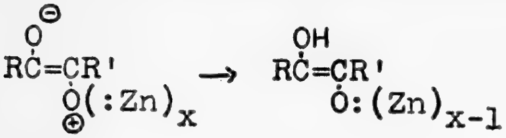
In order to explain the preferential reduction at the 3-position it is necessary to delve into the mechanism of the reduction. At first glance it appears that the carbonyl at the 3-position, being adjacent to the larger alkyl group, would be the more hindered and thus reduced with greater difficulty than the carbonyl at the 2-position. The Brewster-Noller (2,3) mechanism of the Clemmensen reduction does not offer a ready explanation of the selective steric effects observed with 2,3-octanedione. We believe that these effects indicate that a different mechanism is operating in the reduction of 1,2-diketones. This different mechanism results primarily from the fact that in 1,2-diketones the carbonyl groups are conjugated and that in the acyclic diketones the most stable conformation is as shown in IIa, in which the angle between the carbonyl groups is large, due to the repulsion between the carbonyl dipoles.

An acyclic, 1,2-diketone may also be represented in the following way.

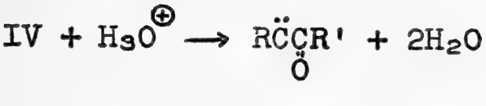

IIa IIb IIc

The relative importance of forms IIb and IIc in which the charges are on oxygen rather than on carbon (as they might be in a monoketone) suggests that the attachment of the diketone to the metal surface is not at the carbon (as it would be for a monoketone) but is at the oxygen. This is shown in III. Moreover, the tendency toward a *trans* conformation of the diketone requires that when one of the oxygens is attached to the metal the other oxygen will be held away from the surface of the metal. The oxygen which preferentially is attached to the metal surface will be the one which is least hindered. In the case of 2,3-octanedione this will be the oxygen of the 2-carbonyl. The oxygen at the 3-position is not close to the metal surface but it is in a position to be readily protonated by the acidic medium to give IV. Reaction of IV with a hydronium ion will give a carbene intermediate V which can either react with two atoms of nascent hydrogen to give a monoketone, or be absorbed on the surface of the zinc where it could take on two electrons and two protons.



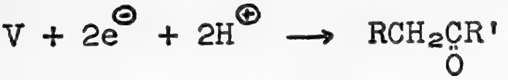
$$R\overset{\overset{\ominus}{O}}{\dot{C}}=\underset{\underset{\oplus}{\dot{O}}(:Zn)_x}{C}R' \longrightarrow R\overset{OH}{\dot{C}}=\underset{\dot{O}:(Zn)_{x-1}}{C}R'$$

III IV



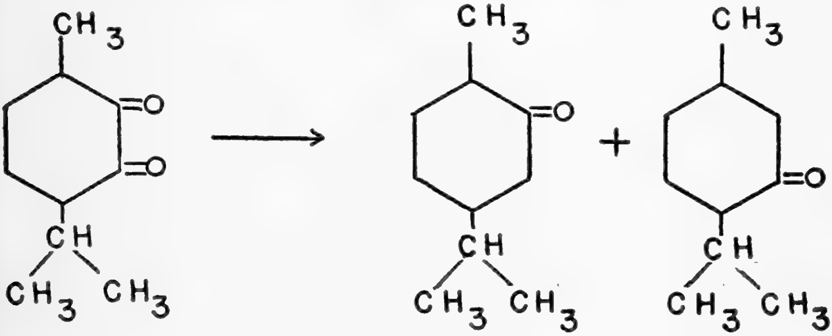
$$IV + H_3O^{\oplus} \longrightarrow RC\underset{\ddot{O}}{\ddot{C}}R' + 2H_2O$$

V



$$V + 2e^{\ominus} + 2H^{\oplus} \longrightarrow RCH_2\underset{\ddot{O}}{C}R'$$

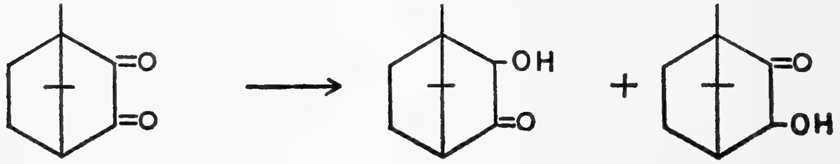
The reduction of the cyclic diketone, diosphenol (VI), gave a mixture of carvomenthone (VII) and menthone (VIII).



VI VII VIII

The refractive index of the mixture indicates that both ketones are formed in approximately equal amounts. Thus it appears that the steric effects of the methyl and isopropyl groups are not markedly different in this compound. It should also be noted that these groups are removed by one carbon from the carbonyl groups and are not attached directly to the carbonyl carbons.

The reduction of camphorquinone (IX) provides the most interesting results of all the ketones investigated in this study. The Clemmensen reduction of camphorquinone was found to give a mixture consisting mainly of hydroxyepicamphor (X) and hydroxyepicamphor (XI).



IX X XI

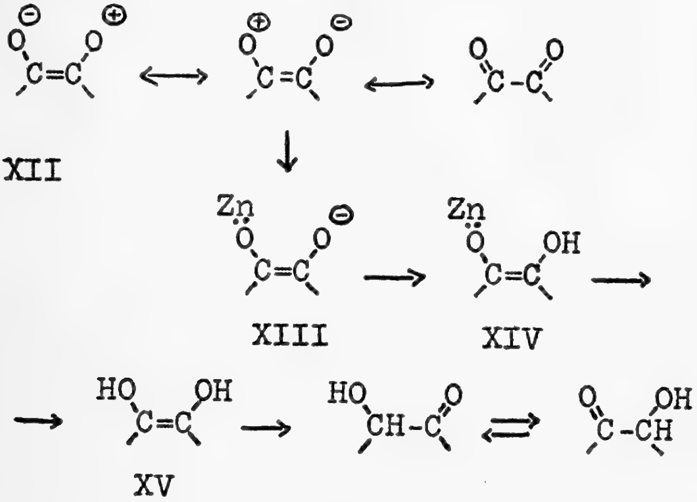
This mixture of products could not be readily separated, but could be converted to a semicarbazone in good yield. This semicarbazone was shown by both melting point and analysis to be the semicarbazone of 2-hydroxyepicamphor. This result indicates that the mixture of X and XI is an equilibrium mixture which reacts with semicarbazide to give only the derivative of X. To demonstrate that such a mixture does give only one semicarbazone, a pure sample of hydroxyepicamphor semicarbazone was hydrolyzed. The hydrolysis product was a mixture similar to that isolated from the reduction of camphorquinone and from which the semicarbazone had been prepared. This mixture, on treatment with semicarbazide, was reconverted to the semicarbazone of hydroxyepicamphor.

In the reduction of camphorquinone as described above, the camphorquinone is largely insoluble in the aqueous reaction mixture. To show that the failure to reduce camphorquinone to a monoketone was not a solubility effect, the reduction was carried out under essentially the same conditions except that some of the water was replaced by ethanol so that the camphorquinone was in solution during the reaction. The product was still a mixture of hydroxyepicamphor and hydroxycamphor, just as in the run in which the ethanol was not added. A reduction in the presence of toluene was slower than the reductions in water or alcohol but the reduction products were the same as in the other cases described above.

Prolongation of the reaction time from four hours to ten or twenty-four hours had no appreciable effect on the yield or nature of the products. Apparently hydroxycamphor and hydroxyepicamphor are not easily reduced to the simple ketones. This is in contrast to the rather facile reduction of acyclic and large ring *alpha*-hydroxyketones (1,4).

The reduction of a cyclic 1,2-diketone in which the ring is six-membered (diosphenol) can be explained by essentially the same mechanism that was postulated for an acyclic 1,2-diketone, such as 2,3-octanedione. The angle between the carbonyl groups cannot be as large in the six-membered ring diketone as in the acyclic diketone but spectral evidence indicates that the angle can be as large as 60° (5). This angle may become even larger in the activated state.

In the five-membered ring diketone, such as camphorquinone, the angle between the carbonyl groups must be less than 10°. The carbonyl groups will thus be in close proximity to each other and the reduction may proceed in the following manner.



XII

XIII

XIV

XV

Intermediate XIV is a zinc alkoxide derivative of the enediol XV and is converted to XV in the presence of acid. In IV the carbonyl group not attached to zinc is more exposed to the solvent and to attack by hydronium ion than is the corresponding carbonyl group in XIII.

Prevention of complete reduction of even one carbonyl group in camphorquinone is no doubt aided considerably by the presence of the other ring of the bicyclic system. The marked difference between the reduction of a six-membered ring diketone and a five-membered ring diketone would probably not be so pronounced if the five-membered ring diketone were a simple monocyclic compound rather than the bicyclic camphorquinone.

EXPERIMENTAL

Camphorquinone.—This compound was prepared by the selenium dioxide oxidation of camphor (**6**).

Diosphenol.—This compound was prepared by the ferric chloride oxidation of menthone in acetic acid (**7**).

Reduction of 2,3-Pentanedione.—A mixture of 85 g. (1.31 moles) of mossy zinc, 6 g. (0.022 moles) of mercuric chloride, 4 ml. of concentrated hydrochloric acid, and 106 ml. of water was shaken for five minutes. The aqueous solution was then decanted, and the amalgamated zinc was covered with 64 ml. of water and 85 ml. of concentrated hydrochloric acid. Then 34.1 g. (0.341 moles) of 2,3-pentanedione was added and the mixture was heated under reflux for twenty-four hours. The mixture was cooled and the two layers separated. The aqueous layer was extracted five times with 25-ml. portions of ether. Removal of the ether and distillation of the residue yielded 2.9 g. of material boiling at 90-98° and a residue weighing 3.2 g. Distillation of the organic layer, previously separated, yielded 11.1 g. of material boiling at 90-98°, 6.2 g. of material boiling at 104-106°, and a residue weighing 2.1 g. The residues were discarded and the fractions obtained from the ether extract and the oil were combined, dried over anhydrous sodium sulfate, and redistilled. Two fractions were obtained, (a) 13.6 g. boiling at 96-112°, and (b) 5.9 g. boiling at 105-107°. The refractive index at 27° for fraction (a) was 1.3920 and for fraction (b) was 1.3999.

A 2,4-dinitrophenylhydrazone was prepared from fraction (a) which, on fractional crystallization gave two products. The first melted at 143-144° and gave no depression when a mixed melting point with the 2,4-dinitrophenylhydrazone of an authentic sample of methyl *n*-propylketone was taken. The second melted at 155-156° and showed no depression when a mixed melting point with the 2,4-dinitrophenylhydrazone of an authentic sample of diethyl ketone was taken.

A semicarbazone was made from this fraction, which also separated into two fractions on fractional crystallization. The first melted at 108-109° and showed no depression of the melting point when mixed with the semicarbazone from an authentic sample of methyl *n*-propyl ketone. The second melted at 136-138° and showed no depression of the melting point when mixed with the semicarbazone of an authentic sample of diethyl ketone.

From the refractive index of this fraction (1.3920) and the known refractive indices at 27° of methyl *n*-propyl ketone (1.3895) and diethyl ketone (1.3939), it is judged that this fraction, representing 40% of the starting material, is composed of equal amounts of methyl *n*-propyl ketone and diethyl ketone.

An oxime prepared from fraction (b) melted at 68-69° and showed no depression of the melting point when mixed with an oxime prepared from an authentic sample of 2,3-pentanedione. This fraction represents a recovery of 17.3% of the original starting material.

Reduction of 2,3-Octanedione.—A mixture of 18.5 g. of mossy zinc, 1.3 g. of mercuric chloride, 1 ml. of concentrated hydrochloric acid, and 22 ml. of water was shaken for five minutes. The aqueous solution was decanted and the amalgam was washed with a small amount of water. The amalgamated zinc was covered with 14 ml. of water and 18.5 ml. of concentrated hydrochloric acid and then 10.65 g (0.075 mole) of 2,3-octanedione was added. The mixture was heated under reflux for twenty-four hours and then allowed to cool to room temperature. The oil which formed was removed and the aqueous solution was extracted three times with 25-ml. portions of ether. The ether solutions and the oil were combined and dried. Removal of the ether and distillation of the residue gave (a) 6.0 g. boiling at 78-86° (27 mm.), (b) 1.5 g. boiling at 86-106° (27 mm.), and a residue weighing 2.5 g.

The refractive index of fraction (a) at 29° was 1.4140. At the same temperature the refractive index of 2,3-octanedione was 1.4200, 2-octanone was 1.4139, and 3-octanone was 1.4130. The ultraviolet absorption spectra indicated that fraction (a) consisted essentially of a mixture of monoketones and contained no diketone.

A 2,4-dinitrophenylhydrazone prepared from fraction (a) melted at 55-56° and showed no depression of the melting point when mixed with an authentic sample.

Reduction of Diosphenol.—A mixture of 27.6 g. of mossy zinc, 6.1 g. of mercuric chloride, 2.5 ml. of concentrated hydrochloric acid, and 35 ml. of water was shaken for five minutes. The aqueous solution was decanted and the amalgam was washed with a small amount of water. The amalgamated zinc was covered with 42 ml. of water and 56 ml. of concentrated hydrochloric acid, and then 12.5 g. (0.075 mole) of diosphenol was added. The mixture was heated under reflux for twenty-four hours. The aqueous mixture was decanted from the zinc into a separatory funnel and extracted four times with 50-ml. portions of ether. Removal of the ether and distillation of the residue yielded (a) 2.5 g. boiling at 40-60° (19 mm.), (b) 6.5 g. boiling at 95-99° (19 mm.) and a residue weighing 1.5 g. The refractive index of fraction (b) at 20° was 1.4528. This value is that expected for a mixture of equal parts of menthone (n_D^{20}1.4505) and carvomenthone (n_D^{20}1.4553).

Reduction of Camphorquinone.—A mixture of 22.5 g. of mossy zinc, 5 g. of mercuric chloride, 2 ml. of concentrated hydrochloric acid and 28 ml. of water was shaken for five minutes. The supernatant liquid was decanted and the amalgamated zinc was covered with 35 ml. of ethyl alcohol and 45 ml. of concentrated hydrochloric acid. Camphorquinone (10.1 g., 0.061 mole) was added, and the mixture was heated under reflux for ten hours. The cooled mixture was diluted with 100 ml. of water and extracted with ether. Evaporation of the ether gave an oily residue which solidified to a white crystalline mass when shaken with an excess of ice cold water. The product melted at 180-197° and weighed 8 g. Since this product could not be effectively fractionated from aqueous alcohol it was converted to the semicarbazone. The crude semicarbazone melted at 203-212°, but after two recrystallizations from methanol it melted at 216-217°. Bredt (**8**) reports the melting point of the semicarbazone of 2-hydroxyepicamphor as 215-216°. Our sample was found to contain 18.4% N. (Calculated, 18.65% N).

When the above experiment was repeated, except that the refluxing times were four hours and twenty-four hours, the results were essentially the same as in the ten hour run. In each case a mixture was obtained which could be converted to the semicarbazone of hydroxyepicamphor.

A mixture of zinc amalgam (11.2 g., prepared as described above), camphorquinone (5 g.), water (17.5 ml.) and concentrated hydrochloric acid (22.5 ml.) was refluxed for twenty-four hours. Complete reduction was indicated by the disappearance of the yellow color typical of camphorquinone. There was obtained four grams of product melting at 140-178°. This product was converted to the semicarbazone of hydroxyepicamphor melting at 216-217°.

The above experiment was repeated with the exceptions that the water was replaced with an equal volume of toluene and the refluxing was continued for a total of thirty-two hours. A bright yellow color in the organic layer indicated incomplete reduction. The yellow-white product weighed 3.7 g. Recrystallizations from Skellysolve B gave 1.5 g. of almost colorless material melting at 155-175°. This material also gave the semicarbazone of hydroxyepicamphor.

Hydrolysis of Hydroxyepicamphor Semicarbazone.—Hydroxyepicamphor semicarbazone (2 g.) was heated under reflux with 20 ml. of 18% hydrochloric acid for four hours. Extraction with ether followed by distillation of the ether gave 1.2 g. (80% yield of material which melted at 155-182° after one recrystallization from aqueous alcohol. A semicarbazone prepared from this mixture weighed 1.3 g. It gave identical fractions when fractionally crystallized from methanol and was identified by melting point and mixed melting point as hydroxyepicamphor semicarbazone. No fraction corresponding to the semicarbazone of hydroxycamphor was obtained.

SUMMARY

The Clemmensen reduction of 2,3-pentanedione and diosphenol gives a mixture of the two possible isomeric monoketones. 2,3-Octanedione is reduced selectively to 2-octanone, while camphorquinone gives a mixture of hydroxycamphor and hydroxyepicamphor. This latter mixture is not reduced further to the monoketones under conditions of the Clemmensen method. A mechanism is advanced to explain both the results with camphorquinone and the reduction of the apparently more hindered carbonyl group of 2,3-octanedione. This explanation is based on the probable conformations of 1,2-dicarbonyl systems in acyclic and in five- and six-membered ring diketones and on the consideration that when conjugation is possible between the 1,2-dicarbonyl groups, the point of attachment of the diketone to the zinc will be at the oxygen rather than at the carbon.

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Preparation of Anhydrous Metal Compounds by Neutralization with Methyl Carbonates

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THE PREPARATION of iron(III)nitrilotriethoxide has been reported recently (**1**). The compound is hydrolyzed by excess water, and anhydrous iron(III)chloride and nitrilotriethanol (triethanolamine) hydrochloride were required as starting materials. The reaction is essentially:

$$1. \qquad Fe^{3+} + (HOC_2H_4)_3N \rightleftharpoons Fe(OC_2H_4)_3N + 3H^+$$

Triethylamine served as proton acceptor to complete the reaction:

$$2. \qquad H^+ + (C_2H_5)_3N \rightleftharpoons (C_2H_5)_3NH^+$$

The by-product, triethylammonium chloride, could be extracted from the reaction mixture due to its greater solubility.

The method cannot be applied to metal nitrilotriethoxides with solubilities similar to that of the by-product. Experiments, therefore, were continued in order to find a more general method of preparing nitrilotriethoxides and other metal complexes in nonaqueous reaction media.

In aqueous solution, an elegant method yielding only volatile byproducts is the metathesis of metal carbonates:

$$3. \qquad 2\,H^+ + CO_3^{2-} \rightarrow CO_2 + H_2O$$

In nonaqueous media, monoalkyl carbonates, yielding carbon dioxide and alcohols, should be useful:

$$4. \qquad H^+ + CH_3OCO_2^- \rightarrow CO_2 + CH_3OH$$

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Methyl carbonic acid has been reported (**2**) to be about as weak as carbonic acid. Therefore, methyl carbonate precipitates of weakly basic metals could be expected to lose carbon dioxide as readily as do corresponding carbonate precipitates. Nevertheless, the preparation of the methyl carbonates of chromium(III) and iron(III) was attempted. The product containing iron was allowed to react with triethanolamine, and both chromium and iron precipitates were tested qualitatively for reaction with a number of weak acids and complexing reagents.

EXPERIMENTAL

1. *Materials.*—The metal chlorides were of analytical reagent purity, the solvents of practical grade, and the sodium methyl carbonate of technical grade (85%). Microanalyses were carried out in the Microanalytical Laboratory, Max Planck-Institut für Kohlenforschung, Mülheim (Ruhr), Germany. The averages of two determinations are given.

2. *Preparations.*—(a) Sodium methyl carbonate (13.7 g., 0.14 mole) was added to 50 ml. of ethylene glycol and 50 ml. of methanol. The solution was filtered by suction, the remaining sodium carbonate was washed with methanol. Chromium(III)chloride 6-hydrate (10.66 g., 0.04 mole) was warmed with 34 ml. of 2,2-dimethoxypropane (**1,3**). After the solution had changed color from green to red it was cooled and dropped slowly into the solution of sodium methyl carbonate. Liberation of carbon dioxide was observed. The green precipitate was separated by centrifugation and washed with ethylene glycol—methanol (1:1) until free of chloride, and with methanol to remove the ethylene glycol. It was dried in a vacuum desiccator over calcium chloride. The amount obtained was 10.5 g. Anal. Found: C, 27.48; H, 5.85; Cr. 24.06; C/Cr ratio 5.0. Calc. for $CrOCH_3(OCOOCH_3)_2$: C, 25.76; H, 3.89; Cr, 22.31.

(b) Sodium methyl carbonate (13.7 g., 0.14 mole) was dissolved as described above; the insoluble sodium carbonate was separated and washed by centrifugation. Iron(III)chloride 6-hydrate (10.81 g., 0.04 mole) was warmed with 34 ml. of 2,2-dimethoxypropane. The solution was left to cool and then dropped into the solution of sodium methyl carbonate. A considerable amount of carbon dioxide was liberated. The yellow-brown precipitate was washed and dried as described before. The amount obtained was 8.65 g. Anal. Found: C, 17.47; H, 3.76; Fe, 40.55; C/Fe ratio, 2.0. Calc. for $FeOH(OCH_3)_2$: C, 17.80; H, 5.23; Fe, 41.39.

(c) The iron precipitate (0.50 g.) was heated with 1.0 g. of triethanolamine in an oil bath for one hour with occasional stirring. The temperature was allowed to rise from 100 to 150° C. in the first 30 minutes. The mixture was left to cool and then washed with absolute ethanol by centrifugation until the wash liquid remained practically colorless. The yellowish-white precipitate was washed with ether and dried in a vacuum desiccator over calcium chloride. The amount obtained was 0.78 g., the yield was 74.9%. The product showed all the properties of anhydrous iron(III) nitrilotriethoxide (**1,4**).

Small amounts were added to 2 ml. of boiling sec.-butanol until no more of it dissolved. The solution was separated by centrifugation and ether was added slowly. The typical yellow hexagonal crystals of iron(III)nitrilotriethoxide monohydrate (**1,4**) were formed.

3. *Qualitative tests.*—Samples (1 – 2 mg.) of the chromium and iron precipitates were heated with representatives of different classes of compounds containing replaceable hydrogen (Table I). Colored complexes formed in an excess of the reagent. A solvent, ethylene glycol, was used to lower the melting point of ethylenediaminetetraacetic acid, and to avoid an excess of diphenylthiocarbazone which has an intense color of its own.

TABLE I. COMPLEXING AGENTS AND WEAK ACIDS REACTING WITH THE METHYL CARBONATE PRECIPITATES OF CHROMIUM(III) AND IRON(III).

Carboxylic acids	Acetic, stearic, benzoic, salicyclic acids; ethylenediaminetetraacetic acid in ethylene glycol.
Phenols	Phenol, 2,4,6-trichlorophenol, pyrocatechol, salicylaldehyde, methyl salicylate, salicylamide, 8-quinolinol.
Enolic compounds	Acetylacetone, thenoyltrifluoroacetone, ethyl acetoacetate.
Aminoalcohols	Ethylenediaminetetraethanol, 3-amino-1-propanol.
Imino compounds	1,5-Diphenylcarbohydrazide, diphenylthiocarbazone, both in ethylene glycol.

RESULTS AND DISCUSSION

The liberation of carbon dioxide and the analytical data prove that normal methyl carbonates, $M(OCOOCH_3)_3$, of chromium and iron with a carbon/metal ratio of 6 do not form under the experimental conditions. It was the aim of the investigation to test the reactivity of precipitates formed under ordinary conditions and regardless of their composition. Therefore, no attempt was made to prepare normal methyl carbonates under more favorable conditions, for instance under a higher pressure of carbon dioxide.

The precipitates obtained are probably mixtures of uncertain composition as are many metal carbonate precipitates used in metathetical reactions. Recrystallization was not possible as no neutral solvent could be found. The carbon/chromium ratio of 5.0 suggests that product (a) is essentially a basic chromium methyl carbonate with a methoxo group. The carbon/iron ratio of 2.0 in product (b) indicates complete loss of carbon dioxide and partial hydrolysis by water from the atmosphere producing a basic iron methoxide.

The iron compound reacted readily with triethanolamine:

5.
$FeOH(OCH_3)_2 + (HOC_2H_4)_3N \rightarrow Fe(OC_2H_4)_3N + 2\,CH_3OH + H_2O$
Since all by-products are volatile and easy to remove, this method of preparing iron(III)nitrilotriethoxide may be regarded as preferable to those reported before (1, 3, 4). The qualitative tests show the precipitates to be reactive with a number of other weak acids. In spite of their varying composition, intermediates produced with sodium methyl carbonate should be useful for the preparation of anhydrous metal compounds, in particular, when a time-consuming separation of by-products must be avoided. Their use in spot tests of organic compounds is also suggested.

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Solar Energy at Lexington, Kentucky for 1952

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Solar energy received on a horizontal surface has been measured at the University of Kentucky since October 1950. The pyrheliometer used in the research is mounted on the top of Anderson Hall, and is located at Lat. 38° 02′N., Long. 84° 30′W. at an elevation of 1026.6 ft. (1)

Data obtained for 1951 have been recorded and presented graphically (2). The total solar energy received on a horizontal surface was 502,968 Btu. per sq. ft. or 21.9 billion Btu. per acre. The daily average for the whole year was 1,433 Btu. per sq. ft. or 62.4 million Btu. per acre.

Total and average solar energy for 1952 and pertinent weather data are listed in Table I. Daily solar energy and mean daily air temperature are presented graphically for each month in Figures 1 and 2. The average daily solar energy for each month and the monthly average mean air temperature for the entire year are presented in Figure 3. Monthly average solar energy, mean air temperature and precipitation for 1951 and 1952 are listed in Table II.

The total solar energy received on a horizontal surface for 1952 was 539,350 Btu. per sq. ft. or 23.5 billion Btu. per acre. The daily average for the whole year was 1,535 Btu. per sq. ft. or 66.8 million Btu. per acre.

Maxima of solar energy were obtained in May and July, 1951 and in June and July, 1952 (2). Two-year norms of solar energy and air temperature are presented in Figure 4.

These data will be useful to the agricultural engineer in determining whether it is economical to use direct solar energy in Kentucky for heating water for domestic use and other purposes in rural areas (3). It is possible to design a solar—earth heat pump water heater that will heat from 2 to 6 times as much water as an electric resistance heater for the same cost. Furthermore, solar energy may be used as a heat source for a solar earth heat pump system to provide continuous air conditioning for homes in rural area (1, 4, 5). Pilot heat pumps

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TABLE I. Solar energy and weather data for 1952.

Month	Total Solar Energy Btu./sq. ft.	Daily Average Solar Energy Btu./sq. ft.	$t_{max.}$* °F	$t_{min.}$* °F	$t_{avg.}$ °F	Precipitation* Inches/Mo.
January	17,412	562	46.4	30.5	38.5	5.62
February	26,929	929	47.7	30.0	38.9	2.72
March	43,884	1,416	54.4	34.7	44.6	7.36
April	48,394	1,669	66.0	43.3	54.7	2.06
May	61,411	2,118	76.9	53.5	65.2	3.15
June	73,637	2,455	90.9	66.6	78.8	4.43
July	69,050	2,466	91.4	67.9	79.7	3.01
August	56,640	2,098	87.4	64.5	76.0	2.80
September	58,526	1,951	81.1	55.6	68.4	1.58
October	43,033	1,388	63.6	38.5	51.1	1.17
November	25,308	844	55.4	36.3	45.9	3.51
December	15,128	522	44.6	32.0	38.3	3.58
Totals	539,352	18,418	805.8	553.4	680.1	40.99
Averages	44,946	1,535	67.2	46.1	56.68	3.42

* U. S. Weather Bureau Data, Blue Grass Airport, Lexington, Kentucky

TABLE II. Two year average solar energy and weather data for 1951 and 1952.

Month	Average Solar Energy Btu./sq. ft./day	Average Mean Air Temperature °F	Average Precipitation Inches/Mo.
January	582	36.7	8.39
February	902	37.2	3.18
March	1,251	43.3	6.16
April	1,642	53.2	2.28
May	2,192	65.3	2.66
June	2,267	76.1	4.40
July	2,350	78.5	2.42
August	2,044	76.2	2.89
September	1,898	67.9	2.59
October	1,335	55.1	2.14
November	791	42.1	5.19
December	552	38.0	5.44
Totals	17,806	669.6	47.74
Averages	1,484	55.8	3.98

should be built to determine maintenance and operating cost because the demand for electric power has doubled every ten years for the last three decades.

According to Farrington Daniels we have used up about 5 per cent of all our coal, oil and gas—most of it since 1900 (6). The time left before the exhaustion of these natural resourses depends on the

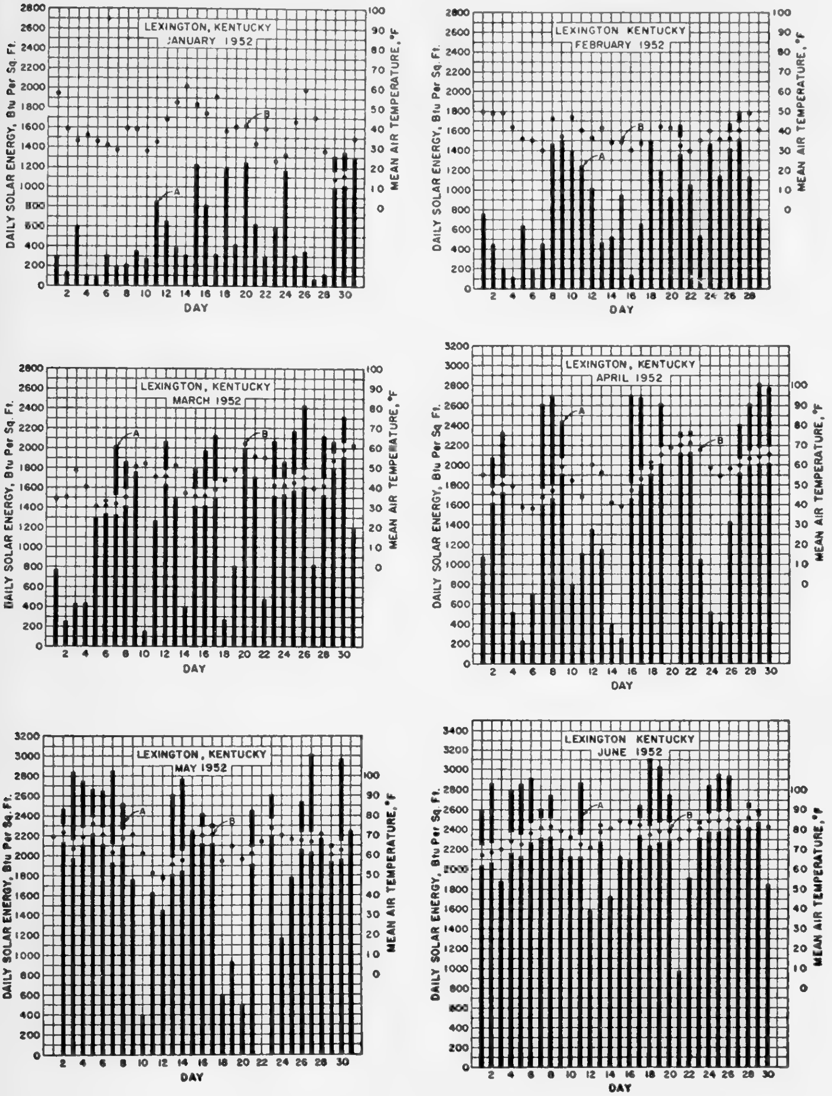

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FIG. 1. Data for January-June, 1952. A, solar energy. B, mean daily air temperature.

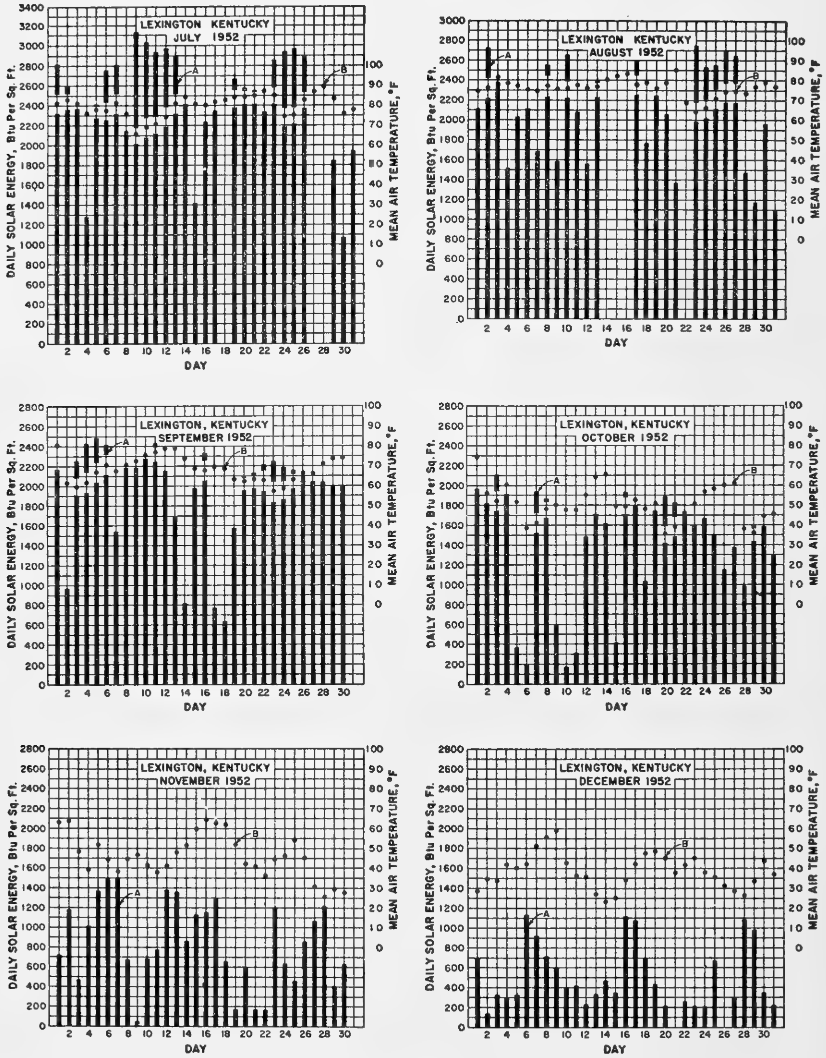

FIG. 2. Data for July-December, 1952. A, solar energy. B, mean daily air temperature.

increase of the rate of consumption. It has been estimated that oil and gas cannot last a century, and that a few thousand years will see the end of all our fossil fuels. The consumption of coal will be accelerated if it is processed and put on our highways.

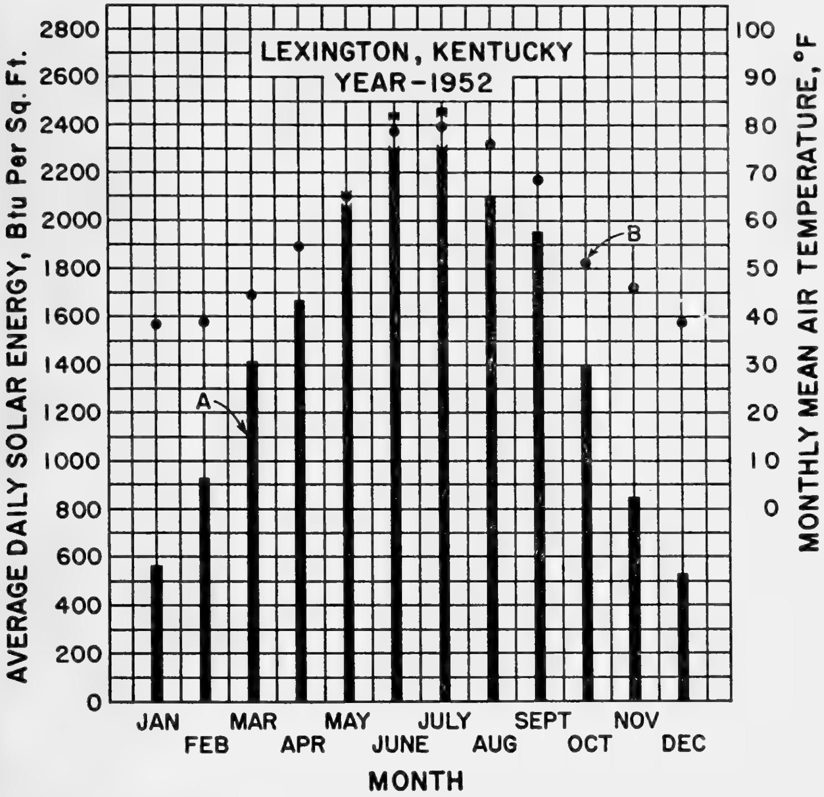

FIG. 3. Solar energy and average air temperature for 1952. Solar data were taken and recorded at the University of Kentucky. Air temperatures were taken and recorded at the U. S. Weather Bureau Station at the Blue Grass Airport, Lexington, Kentucky.

Furthermore, a knowledge of the amount of solar energy received at the earth's surface will be useful to the climatologists, agronomists and plant pathologists in their studies of crop production and plant disease.

This research is sponsored by the Engineering Experiment Station of the University of Kentucky.

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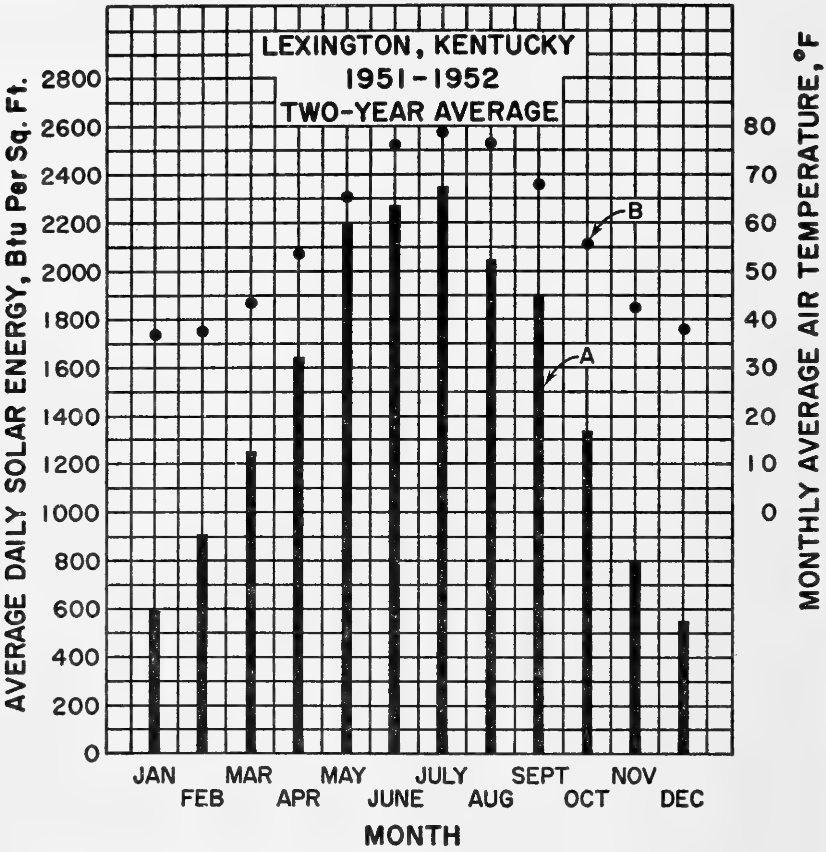

FIG. 4. Two-year norms of solar energy and air temperature.

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Glutathione Distributions During Early Development of *Callosobruchus maculatus* Fabr. (Coleoptera) Eggs, as Determined by the Nitroprusside Reaction

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INTRODUCTION

GLUTATHIONE is a sulfhydryl compound, a tripeptide, containing the amino acids cysteine, glutamic acid, and glycine. It is present in practically all active animal tissues and the concentrations are exceptionally high in embryonic and cancerous tissue. The actual physiological function of this compound remains obscure in the framework of cell respiration. Several concepts have been postulated as to the role which it plays, but most authors seem to agree that it exerts no general but only certain special functions. The substance is autoxidisable, and due to the changes that take place in a redox reaction, it performs both as a hydrogen acceptor and donor. It is possible that this compound serves in the maintenance of functional mechanisms of enzymes that are essential factors for growth and respiration (Elvehjem and Wilson, 1942; Oppenheimer and Stern, 1939).

The nitroprusside reaction is one of the most suitable methods for demonstrating the histological distribution of this compound. Apparently, the glutathione in tissues forms a complex with the nitroprusside molecule when the reaction is carried out in an alkali medium. As a result, a vivid purplish-red color is produced which affords a qualitative estimate of the amount of glutathione present. Absolute quantitative values, however, are unobtainable with this procedure (Hammett and Chapman, 1938; Pearse, 1953).

The effects of potassium cyanide and ultraviolet irradiation treatments on developing eggs of *Callosobruchus maculatus* Fabr. have been elucidated (Brauer, 1938 and 1949). In this paper, the effects of these treatments on the developing eggs and their glutathione distributions are compared and discussed in relation to susceptibility and early developmental pattern.

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METHODS AND MATERIALS

Eggs of *Callosobruchus maculatus* were collected at fifteen minute intervals to allow for approximately equal age. The eggs were then immersed in M/100 and M/1000 concentrations of potassium cyanide for periods of 10, 20, 30, 40 and 50 minutes. After treatment part of the eggs were returned to the incubator for further development; another portion was tested immediately with nitroprusside reagent.

For the second part of the study, the eggs were collected similarly and subjected to treatments with ultraviolet irradiation (2537 Angstroms) for periods of 5, 10, 15 and 20 minutes. After treatment,

TABLE I

M/100 KCN—Re-incubation

Age (hrs.) of ea. group (A-E) at treatment					Length of Treatment (minutes)				
					10	20	30	40	50
A	B	C	D	E	A	B	C	D	E
2	2	1½	1½	2	+++	+++	+	+++	+
10	10	9	12	9	+	++	+	++	++
14	15	13	16	13	++	++	+	+	+++
19	20	21	20	23	++	++	+	+	++
26	30	32	26	33	++	++	+	+	++
41	40	46	46	47	++	++	++	++	++
M/1000 KCN—Re-incubation									
4	2	2	2	1	+++	+	+	+	+
11	10	10	10	9	+++	++	++	++	++
14	14	13	13	14	++	++	+	++	+
22	21	21	20	22	++	+	+	+	−
26	26	33	26	33	++	+	−	+	+
43	43	46	46	45	++	+	−	−	++

+++ Abundant coloration
++ Moderate coloration
+ Trace amount of color
− Negative reaction

TABLE II

M/100 KCN—No incubation

Age (hrs.) of ea. group (A-E) at treatment					Length of Treatment (minutes)				
					10	20	30	40	50
A	B	C	D	E	A	B	C	D	E
3½	5	5½	5	6	++	++	+	++	++
10	11	9½	9½	11	++	+	++	+	+
16	16	17	17	17	+++	++	++	+	++
21	23	23	21	21	+++	+++	+++	+++	+++
34	34	34	34	34	+++	+++	+++	+++	+++
44	45	45	45	45	+++	+++	+++	+++	+++

M/1000 KCN—No Incubation

3	4½	4	3½	3	++	+	+	+	+
8½	8½	8	8½	8½	++	++	++	+	+
14	14	14	14	14	++	+	++	++	++
21	22	22	22	22	++	+++	+++	+++	+++
34	35	35	35	35	+++	+++	+++	+++	++++
41	41	41	41	41	+++	+++	+++	+++	+++

+++ Abundant coloration
++ Moderate coloration
+ Trace amount of color
— Negative reaction

one-half of each group was allowed to reincubate; the remaining half was tested immediately.

The control series for this work consisted of eggs that were of equal age to the treated groups. These controls received neither cyanide nor ultraviolet treatments. They were subjected only to the nitroprusside test.

The technique employed in the application of the nitroprusside reaction was a modified version of Rapkine's Nitroprusside Test. The reagent was a 2% sodium nitroferricyanide (nitroprusside) solution. This test was carried out in an alkali medium of ammonium sulfate crystals and concentrated ammonium hydroxide solution.

Results

The various time periods are of primary importance in the development of this insect's egg. Complete descriptions of its chronology have been thoroughly presented (Brauer and Taylor, 1936). For this particular study, the earliest periods have not been considered as extensively as they probably should have been. This reflection should be borne in mind when interpreting the data presented.

For recording purposes, each reaction was classified as it appeared. Four different categories of resultant colors were distinguished and indicated with plus symbols, or the appropriate sign in the case of negative reactions.

Table III

Ultraviolet—Re-incubation

Age (hrs.) of each group (A-D) at treatment				Length of Treatment (minutes)			
				5	10	15	20
A	B	C	D	A	B	C	D
5	4½	3½	3	+++	+++	+++	++
8	7	8	7	+++	+++	+++	+++
17	17	17	17	+++	+++	+++	+++
23	23	23	23	++	++	+++	+++
31	31	31	32	+++	+++	+++	+++
42	42	43	43	+++	+++	+++	+++

Ultraviolet—No Incubation

5	4½	3½	3	++	++	++	+
8	7	8	7	++	++	+	+
17	17	17	17	+	++	++	++
23	23	23	23	++	++	+	++
31	31	31	32	+++	+++	+++	+++
42	42	43	43	+++	+++	+++	+++

+++	Abundant coloration
++	Moderate coloration
+	Trace amount of color
−	Negative reaction

TABLE IV

Crontrols							
Age (hrs.)..........	3	8	13	19	25	40	48–
Result.............	++	++	++	++	+++	+++	+++

+++ Abundant coloration
++ Moderate coloration
+Trace amount of color
– Negative reaction

Malformations were regularly produced as a result of the cyanide and ultraviolet treatments; the most susceptible periods for obvious anomalous development ranged from oviposition to eighteen hours of age. Eggs that were incubated after cyanide treatments did not show a consistent reaction pattern (Table I). This may have been due to a preliminary treatment with a 10% solution of potassium cyanide, which according to some authors intensifies the color produced by the nitroprusside reaction. The procedure, however, necessitated extra washing which increased the loss of material. However, in view of the fact that the majority of the reactions were qualitatively positive, these particular tests were not repeated.

For the remainder of the study, the results showed a generally consistent pattern throughout (Tables II, III, and IV). With increasing age of the developing egg, a corresponding increase in the amount of color was obtained.

In all cases the color concentrations in those eggs prior to blastoderm formation were found to be scattered (Fig. 1). After blastoderm formation the concentrations were more localized midventrally (Figs. 2 and 3). Following this period, the observable concentrations were most prominent in the anterior and posterior regions with slightly lesser amounts distributed in the midgut region (Figs. 4 and 5).

ANALYSIS AND DISCUSSION

This study to determine the distribution of glutathione has shown that the compound is readily observed during development of this insect. The early periods of development are marked by increased mitotic activity. With this progression of development, there is a corresponding increase in the amount of color produced by the reaction, thus showing more glutathione to be present. The largest amount of coloration is observed during and after the period of 24-36 hours of development.

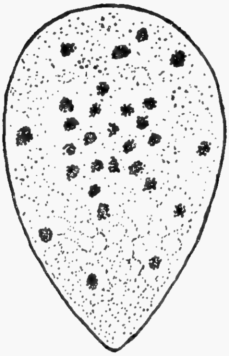

Fig. 1

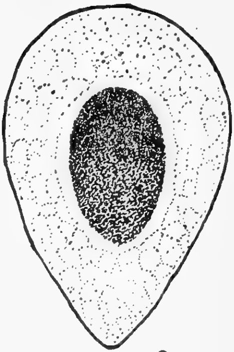

Fig. 2

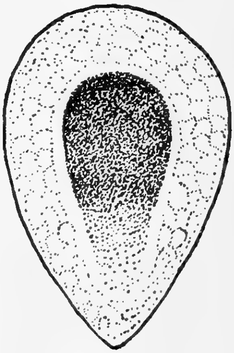

Fig. 3

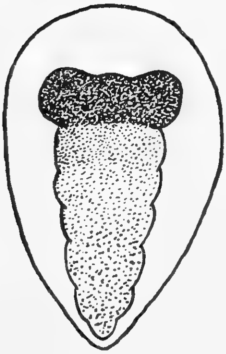

Fig. 4

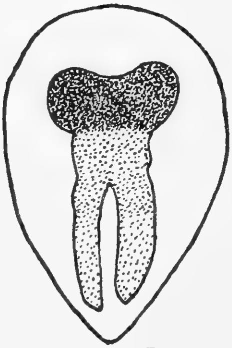

Fig. 5

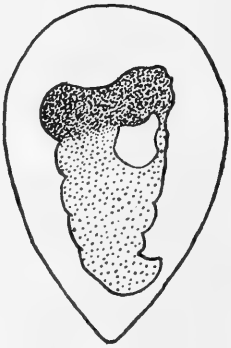

Fig. 6

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Fig. 1. An egg of 3½ hours. Treated with ultraviolet irradiation for 10 minutes. Heavily stippled portion represents color distribution. Light stippling throughout egg represents yolk material.

Fig. 2. An egg of 8 hours age. Treated for 10 minutes in M/1000 KCN and tested immediately with nitroprusside reagent. Color distribution is shown by heavily stippled area. Light stippling around blastoderm represents yolk material.

Fig. 3. An egg of 11 hours age. Treated with ultraviolet irradiation for 20 minutes and tested immediately. Stippling shows gradual diminution of color. Light stippling around ventral plate represents yolk material.

Fig. 4. A normal, untreated embryo of 72 hours. Ventral view. Stippling shows distribution of color.

Fig. 5. A partial twin. Cephalic lobes are unequal. Coloration is same as normal. Eggs were approximately 17 hours old when treated with M/100 KCN for 30 minutes.

Fig. 6. An embryo of 76 hours age. Treated *in toto* with ultraviolet irradiation for 20 minutes at 14 hours of age. Stippling shows color distribution. Injury effect is registered in left side and left cephalic lobe.

The periods of development prior to twenty-four hours are most susceptible to cyanide treatments, and the malformations produced are typical for the periods in which the eggs were treated. Explanations for these phenomena have been thoroughly presented (Brauer, 1938).

Ultraviolet irradiation which produced deformities and in some cases death, did not inactivate glutathione. Positive reactions were obtained throughout this portion of the study. Portions of the embryos which were destroyed did not show a reaction whereas the rest of the embryo exhibited a positive result (Fig. 6). Possible causation of this may be the result of metabolic cessation and not of glutathione destruction. On the basis of results obtained, it is concluded that the embryo was malformed or dead because of cellular injuries and not as a result of glutathione inactivation.

The control series show the expected pattern during development. The lesser reactions during the earliest phases are primarily due to the relatively few cells in the egg, excluding the yolk cells which do not contain glutathione. In the later phases, however, with increase in metabolic activity, the localization of distributions are found to be more pronounced in the head region, and a diminution of the color is gradual from anterior to posterior. This indicates an existent physiological gradient, which is in accordance with the theory of Child (1940).

SUMMARY AND CONCLUSIONS

1. Developing eggs of *Callosobruchus maculatus* Fabr. were subjected to treatments with potassium cyanide and ultraviolet irradiations (2537A) to determine the effect on glutathione distribution in the developing embryo.

2. The eggs exhibit highest susceptibility to these treatments during the stages of early development. This is evidenced by the number and types of malformations that are produced.

3. As development of the egg progresses, there is also an increase in the amount of glutathione produced. The presence of glutathione is shown by the nitroprusside reaction.

4. The greatest amounts of color produced by the reaction appear after the twenty-fourth hour of development. The distributions are most prominent in the anterior region of the embryo, and they diminish gradually from anterior to posterior. The glutathione con-

centrations parallel the intensity of metabolic activity, thus showing a physiological gradient.

5. Cyanide and irradiation treatments of adequate dosage to produce malformations in the embryos do not appear to be concomitantly sufficient to produce an inhibitory effect on the glutathione content in these embryos.

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Cytochrome Oxidase Concentrations in *Callosobruchus maculatus* Fabr. (Coleoptera) Eggs During Early Development as Shown by the Nadi Reaction

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INTRODUCTION

THE CELLS of aerobic organisms contain a group of iron porphyrin proteins other than hemoglobin, catalase and peroxidase, referred to collectively as the cytochromes. These compounds were discovered by MacMunn in 1886, by a microspectroscopic technique. It was not until 1925, however, that the physiological significance of the cytochrome system was brought to light by Keilin.

The cytochrome system (the catalysis by hematin enzymes) provides the major pathways of respiration in cells of higher animals and the same is true for practically all lower animals, plants and microorganisms (Lemberg and Legge, 1949).

The chief biological function of cytochrome oxidase is the activation of molecular oxygen. The pattern in which it is found seems to correspond with the physiological gradient pattern of C. M. Child. Moog (1943) traced the appearance of cytochrome oxidase in the developing chick embryo from the very first day of development. Previously some investigators had stated that the enzyme was not present in embryonic tissues, or at any rate not in detectable amounts. Statements appear in the literature of embryology to the effect that a specific enzyme is responsible for such processes as determination, morphogenesis and differentiation, but so far this has not been proven for any enzyme. Nevertheless enzyme development and functional differentiation during development go hand in hand (Willier, Weiss and Hamberger, 1955).

The functional pattern of development of the *Callosobruchus maculatus* Fabr. embryo was shown by demonstrating the concentrations of cytochrome oxidase by the Nadi reaction, with the aid of ultraviolet energy and potassium cyanide. Both ultraviolet energy and potassium cyanide have been used to block the developmental

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pattern of the Bruchid embryo and both have been shown to have a detrimental effect upon the cytochrome oxidase (Brauer, 1938 and 1949, and Yost, Robson, and Spiegleman, 1956).

METHODS

Eggs of the pea beetle, *Callosobruchus maculatus* Fabr. deposited on sterilized peas over 15 minute intervals were removed from the peas and at the desired ages were immersd in potassium cyanide solutions of M/100 or M/1000 or irradiated with ultraviolet energy with a wave length of 2537 Angstroms. Both of these treatments were used to block the normal development of the embryo so that the effects on the cytochrome oxidase could be seen. After treatment with potassium cyanide or ultraviolet energy groups of eggs were immediately treated with the Nadi reagent while other groups were reincubated before the Nadi reagent was applied.

The Nadi reagent was made up just before use. It was composed of freshly prepared one percent solution of alpha naphthol and freshly prepared one percent solution of N,N-dimethly-p-phenylenediamine monohydrochloride (p-aminodimethylaniline monohydrochloride) in a 50:50 ratio.

The dorsal chorion of each egg was perforated with a fine pointed needle so that the reagent could come in contact with the embryo. The eggs remained in the Nadi reagent about ten minutes and then were removed and washed in 70 percent alcohol and mounted in Zwemer's "Glychrogel."

Eggs that were reincubated were placed in the incubator with a temperature of 29 to 30 degrees Centigrade and a relative humidity at near saturation. The reincubation periods ranged from 40 hours upward, depending on the age of the embryo at the time of the potassium cyanide treatment or ultraviolet irradiation. After reincubation the eggs were treated in the same manner as those that were immediately treated with the Nadi reagent.

RESULTS

Eggs of 0 to 6 hours of incubation in which the Nadi treatment immediately followed irradiation showed very little indophenol blue or the color indicating cytochrome oxidase. The reason is that at this early stage there are relatively few cleavage cells and they are still deep in the yolk material. The ultraviolet energy received by each of them is very small because of the small area of the cell and

TABLE I

Ultraviolet irradiation of Bruchid eggs at different ages, immediately followed by Nadi treatment.

Age at time of treatment (in hours)	Length of irradiation (in minutes)					
	5		10		15	
	No. Eggs	Color React.	No. Eggs	Color React.	No. Eggs	Color React.
0-6	29	+	18	+	23	+
6-12	6	++	6	+	19	+
12-24	15	+++	10	++	13	++
24-36	12	+++	8	+++	16	+++
36-48	14	+++	12	+++	10	+++
Ultraviolet irradiation of Bruchid eggs at different ages, followed by a reincubation period.						
0-6	13	+++	23	++	15	+
6-12	14	+++	9	++	17	++
12-24	16	+++	16	+++	14	++
24-36	16	+++	16	+++	10	+++
36-48	14	+++	25	+++	11	+++

\+ Trace amounts of coloration
++ Moderate coloration
+++ Abundant coloration

because the yolk material, vitelline membrane and the chorion absorb some of the radiation. Thus, the amount of color present very nearly indicates the exact amount of the enzyme present in the cleavage cell stage (Bodine and Boell, 1936 and Allen, 1940).

The blastoderm is formed in the period of 6 to 12 hours incubation. The eggs of this period show small amounts of color, but it is difficult to distinguish since the blastoderm is only one cell layer thick. Ultraviolet irradiation has a noticeable effect upon the embryo at this stage but the increase in the number of cells in the blastoderm causes an increase in the amount of cytochrome oxidase to be expected. The blastoderm now is more vulnerable to irradiation because it is on the periphery of the egg. At the same time there is an increase in the cytochrome oxidase because of the increased number of cells in the blastoderm, resulting in an increase in indophenol blue produced by the Nadi reaction. An increase in color from a trace to a moderate amount was noticed. Consequently the amount of irradiation which produced modifications in the embryo at this stage was not sufficient to produce a noticeable difference in the cytochrome oxidase.

The period of development between 24 and 36 hours incubation is the time during which the greatest increase in the color was found. From this period on, the increase in coloration is less noticeable except in old embryos, eg. 90 hours. At that age the embryo is nearly grown and the Nadi reagent cannot penetrate it, so the surface of the embryo is only slightly colored. During the period between 24 and 36 hours incubation the definite increase in color could be correlated with the period of maximum constant percentage increase of oxygen consumption as described by Romanoff (1941).

After 36 hours development the amount of color showed no marked increase. Since no great increase in the coloration was noticed it can be said that there was no great increase in the amount of cytochrome oxidase produced after the period of maximum constant percentage increase of oxygen consumption.

Reincubation following ultraviolet irradiation showed an abundance of color in all cases; however, the modifications were progressively more pronounced. Indophenol blue found in the malformed embryos was in approximately the same amounts as found in the older embryos that were not reincubated. This suggests that the ultraviolet energy which produced the malformations in the embryo has a lesser effect upon the enzyme.

Eggs that were treated with potassium cyanide showed little variation between the amount of color present in them and eggs that were not treated. M/100 potassium cyanide is very detrimental to the embryo at early stages of development. The exact amount of potassium cyanide which produces the modifications is not known because the permeability of the vitelline membrane and the chorion is unknown. Since M/1000 potassium cyanide has produced complete inactivation of the cytochrome oxidase in an extract form, apparently the amount of potassium cyanide which is able to cause anomalies in development is not sufficient to cause inactivation of the enzyme.

The results showed that the highest concentrations of cytochrome oxidase were found in the head and caudal plate regions of the embryo of 24 hours and older. From these results, cytochrome oxidase may

TABLE II

Potassium cyanide treatment on Bruchid eggs at different ages, immediately followed by Nadi treatment.

Age at time of treatment (in hours)	Concentration of potassium cyanide			
	M/100		M/1000	
	No. Eggs	Color React.	No. Eggs	Color React.
0-6	22	+	33	+
6-12	16	++	36	++
12-24	14	++	29	+++
24-36	9	+++	23	+++
36-48	19	+++	30	+++

Potassium cyanide treatment on Bruchid eggs at different ages, followed by a reincubation period.

Age at time of treatment (in hours)	M/100 No. Eggs	M/100 Color React.	M/1000 No. Eggs	M/1000 Color React.
0-6	60	+++	23	+++
6-12	51	+++	14	+++
12-24	55	+++	30	+++
24-36	49	+++	17	+++
36-48	18	+++	18	+++

+ Trace amounts of coloration
++ Moderate coloration
+++ Abundant coloration

be expected to be found in the physiological head, namely the region of the first neural ganglion which is slightly forward to the center of the egg at this stage. The high cytochrome oxidase concentrations found in these areas indicates a high metabolic activity, thus in the very early stages of development it seems probable that the enzyme would be found in highest concentrations in the areas of highest metabolic activity, or the areas of the greatest development. The enzyme, according to the physiological gradient pattern, would be found in progressively decreasing amounts going away from the center of the pattern. The amount of the enzyme present is proportional to the amount of oxygen consumed, so if the Nadi reaction has gone to completion the amount of color indicates the amount of the enzyme present. The center of the gradient pattern accordingly should be colored in the highest concentrations.

CONCLUSIONS

Cytochrome oxidase was found in the highest concentrations in the head and caudal plate areas or the areas of the greatest metabolic activity.

Ultraviolet energy showed that the cytochrome oxidase is progressively inactivated with irradiation, but with an increase in the cells of the embryo an increase in cytochrome oxidase results, thus the indophenol blue is increased in concentration as the embryo becomes older and the length of the irradiations remain constant.

No difference in the amount of cytochrome oxidase was found between eggs treated with potassium cyanide and untreated eggs. This is probably due to the permeability of the vitelline membrane and the chorion. The amount of potassium cyanide which produces malformations in the developing embryo is not sufficient to inactivate the cytochrome oxidase.

The Nadi reaction indicates that the period of maximum constant percentage of oxygen consumption is between 24 and 36 hours development; and the highest concentrations of the cytochrome oxidase are found in the areas where the greatest amount of differentiation is taking place. Therefore, the indophenol blue is expected to be found in the areas of the highest metabolic activity or the areas of the greatest amount of development. These areas in the very early eggs are the centers of the physiological gradient patterns and in the case of the Bruchid egg the area is in the region where the first neural ganglion originates.

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A Summary of Our Knowledge of Kentucky Crustaceans

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INTRODUCTION

ORIGINAL descriptions of more than 20 species of Crustacea are based on material collected in Kentucky, yet there are many gaps in our knowledge of that arthropod class within the boundaries of the state. The purpose of the present paper is to summarize the existing body of information concerning the occurrence of crustaceans in Kentucky, to report species which have not been listed in earlier published accounts of the state fauna, and to discuss some taxonomic problems. A complete bibliography of the literature concerned with Kentucky crustaceans is not included, but this paper should serve as a guide to persons who might wish to assemble all the important references to any particular group.

Three lists of crustaceans have been omitted, although these omissions may not be justified entirely. Hoff (1943, 1944a) reported on the Crustacea of Reelfoot Lake, which overlaps the Kentucky line. His collections were made in Tennessee, but it is reasonable to assume that many of the reported species occur in the Kentucky part of the lake as well. Ward (1940) listed more than 50 species of entomostracans from ponds in the region around Cincinnati, Ohio, close to Kentucky, but I have not included her Ohio specimens in the present compilation.

I am indebted to Stanley Levy for his aid in searching for references to Kentucky crustaceans. Also, Louis A. Krumholz and Stuart E. Neff were kind enough to examine some papers which were not available to me, and their help is gratefully acknowledged. I am deeply grateful to Gabriel W. Comita, who provided valuable information concerning the status of certain copepod species. However, any omissions and errors in this paper must be attributed solely to the author.

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BRANCHIOPODA

ANOSTRACA. Weise (1957) reviewed our knowledge of Kentucky anostracans, and published a complete bibliography of them. Only three species of fairy shrimps have been reported from the state.

Fairy shrimps probably can be found in Kentucky during at least six or seven months of the year. *Eubranchipus vernalis* (Verrill) can be collected in Jefferson County during January, and during some winters as early as December. Weise (*loc. cit.*) found *Streptocephalus seali* Ryder as late as the third week of July in Warren County. By that time of year active *Eubranchipus* probably will have disappeared from Kentucky ponds.

There are interesting problems concerning the taxonomic and distributional status of *Eubranchipus neglectus*, described by Garman (1920) from ponds in "Bluegrass, Kentucky." Dexter (1956) reported some Ohio specimens in the U.S.N.M., and that report constitutes the only record of its occurrence since Garman's paper. Because *E. neglectus* is much like the common *E. vernalis*, the specific status of the former is problematical. If there is a specific difference between the two, here might be a rare opportunity to study a rare, endemic species as it approaches extinction, and comparative ecological studies of the two species would be valuable. Whatever the case may be, a concentrated effort should be made to find Garman's fairy shrimp in the temporary ponds around the type locality.

CONCHOSTRACA. Only one conchostracan definitely is known to occur in Kentucky. This is the widespread *Cyzicus mexicanus* (Claus), which has been included in most reports as a member of either the genus *Estheria,* or the genus *Caenestheriella.* The latest paper mentioning the occurrence of *C. mexicanus* in Kentucky is that of Mattox (1957).

Some undetermined conchostracans have been collected in Kentucky, and there may be more than one species in the state. Chambers (1874) reported an "*Estheria*" from the vicinity of Covington, and the writer collected two conchostracan specimens from a shallow, rocky pool in Otter Creek Park, Meade County, during May, 1951. Unfortunately, they were lost before being studied carefully, and subsequent searching at that station has been fruitless.

CLADOCERA. Records of 18 cladocerans in Kentucky have been published prior to this paper. Cole (1957b) listed 16 planktonic and microbenthic species from Tom Wallace Lake, Jefferson County.

Four of those had been reported before. Turner (1895) mentioned *Daphnia pulex* from Covington, and also *Chydorus sphaericus* (O.F.M.) from Kentucky. Ward (1940) collected *Macrothrix laticornis* (Jurine) in northern Kentucky across the Ohio River from Cincinnati, and Herrick (1883) reported "*simocephalus* (?)" from Swan Lake near Decatur. Also Herrick (*loc. cit.*) wrote of the presence of "*sida*" in Swan Lake. Although he did not capitalize the generic names of *Sida* and *Simocephalus,* he did refer to them as Cladocera. His is the only reference to the occurrence of *Sida* in Kentucky; one species, *crystallina* (O.F.M.), is present in the United States, and presumably Herrick referred to this. Herrick (*loc. cit.*) also stated that *Ceriodaphnia reticulata* (Jurine) was collected "back of Paducah, Ky."

However, there is some question as to whether Herrick actually collected in Decatur, Kentucky. In his paper of 1883, he wrote of a series of small pools in Decatur, Ky., where he collected copepod crustaceans. He also mentioned Swan Lake, near Decatur, without naming the state. In the same paper he spoke of collections from Paducah, Ky., and Decatur, Alabama. Decatur, Ky., is a small community in the northern part of Russell County. There is a Goose Creek near it, or running through it, but maps do not show any Swan Lake there. There is a good possibility that Herrick's references to Decatur, Ky., actually applied to Decatur, Alabama, and that all his collections in Kentucky were made near Paducah. This seems reasonable because Herrick (1895b, p. 257) wrote with reference to another cladoceran, "I have notes of it from Swan Lake near Decatur, Ala."

Some revealing words concerning Herrick's methods were written by Edward A. Birge (1910, p. 1021), and may have a bearing on the problem of where Herrick collected. After Herrick's death, Birge received all his original notes and sketches of entomostracans. According to Birge (*loc. cit.*), "The sketches rarely bear date or locality and very few notes, only a word here and there."

The references to *Daphnia pulex* (de Geer) and *D. longispina* (O.F.M.) in Cole's paper (1957b) must be evaluated in light of an important monograph (Brooks, 1957) on the genus *Daphnia,* which was not available at the time the Kentucky specimens were examined. Brooks has shown that *D. longispina* does not occur in North America, and that several species have been lumped as *D. longispina* and as *D. pulex* in the past. Thus, there must be a reexamination of Kentucky daphnids, before the cladoceran list from the state can be considered up to date.

In addition to the cladoceran species previously listed in published accounts, some others have been collected and will be reported here for the first time from Kentucky. A plankton collection from Cumberland Lake (15 Oct. 1954) included a few specimens of *Bosminopsis deitersi* Richard. This is probably the only cladoceran known from the state that can be considered a typical southern form. *Leydigia acanthocercoides* (Fischer), and *Moina affinis* Birge have been collected from the duck pond in Cave Hill Cemetery, Jefferson County, and samples from various farm ponds in the same county have included *Ceriodaphnia pulchella* Sars, *C. quadrangula* (O.F.M.), and *Alonella rostrata* (Koch).

Another new record from Kentucky is *Bosmina coregoni* Baird. Dewey L. Bunting, II, collected specimens of this from the plankton of Dale Hollow Lake during May, 1958. A few specimens of *B. longirostris* (O.F.M.) were in the same collection. Although most workers now believe these two cladocerans to be taxonomically distinct, very recently Jones (1958) expressed doubt concerning the specific distinction between them. The specimens from Dale Hollow seem to be separable morphologically into two species on the basis of mucrone lengths and the exact place of origin of the sensory hair near the antennules. However, in Tom Wallace Lake the *Bosmina* which has been called *longirostris* (Cole, 1957b) has characteristic short mucrones, but in most specimens the sensory hair originates near the base of the antennules, as in *B. coregoni*.

Copepoda

CALANOIDA. The first report of a calanoid copepod from Kentucky was that of Chambers (1881), who described a new species, *Diaptomus kentuckyensis*, from Covington. Chambers' species apparently is not referable to any known copepod.

In 1883 Herrick discussed the European species, *Diaptomus castor*, which he said was found in a series of small pools near Decatur, Ky. In that discussion Herrick stated that *D. sanguineus* Forbes, and *D. stagnalis* Forbes were forms of *D. castor*. However, at the present time it appears that *sanguineus* and *stagnalis* are separate, valid species, and that all reports of *D. castor* from America probably are in error. From Herrick's paper it is difficult to ascertain whether he found what he considered to be both the *sanguineus* type and the *stagnalis* type of *castor* in the Decatur pools. His drawings (*loc. cit.*; Plate V, 1, 1a, 4, 9a, 9b) could apply to both species, but he did not state whether they were based on specimens from Decatur, Ky. or from Decatur, Alabama. Perhaps Herrick had both *sanguineus* and *stagnalis* in his collections, because sometimes they do occur together (Cole, 1953). However there is the good possibility, as was mentioned in the previous section on Cladocera, that the specimens Herrick discussed were from Alabama. Later Herrick (1895a) stated that *D. stagnalis,* ''—has been found in Minnesota, Illinois, Ohio, Kentucky and Alabama.'' Following this, Marsh (1907) wrote that *stagnalis,* ''—is reported as occurring—in'' Kentucky, Alabama, and the three other states, and he defined the geographical range of *D. sanguineus* in such a manner that Kentucky would be included, although he did not mention the state.

In 1900 Kofoid wrote of an ''empty carapace'' of a *Diaptomus* found in a plankton sample from Echo River, in Mammoth Cave. This represented the last collection of Kentucky calanoid material until the paper of Cole (1953), which included records of *D. stagnalis, D. sanguineus, D. pallidus* Herrick, and *Osphranticum labronectum* Forbes from Jefferson and adjacent counties. No other calanoids have been reported from Kentucky since then, but some additional notations can be made now. Recent collections from the Ohio River at Louisville contain *D. pallidus,* the common calanoid of north central Kentucky. *Osphranticum labronectum* has been found at three more stations in Jefferson County, and *D. stagnalis* has been found there for the first time. In Kentucky, the last two species are typical of temporary, vernal pools.

CYCLOPOIDA. The chronological history of the study of cyclopoid crustaceans from Kentucky begins with the description of a new species, *Cyclops tenuissimus,* from Paducah (Herrick, 1883). Later Herrick (1895a, p. 96) placed *tenuissimus* in the synonymy of *C. leuckarti* Claus. Kiefer (1929, p. 82) assigned it, with some doubt, to the synonymy of *Mesocyclops oithonoides* (Sars). In 1943 Coker briefly mentioned Herrick's *C. tenuissimus,* retaining the earlier synonymy proposed by Kiefer. Apparently other authors have considered *C. tenuissimus* unrecognizable, and there are no recent references to it. However, at this time, it seems to the writer that Herrick's (1883) description is referable to *Cyclops edax,* described by Forbes (1891), and now assigned to *Mesocyclops* Sars. The reasons for this will be brought out in another manuscript, now in preparation, which will discuss in greater detail the taxonomic status of *tenuissimus.*

Kofoid (1900) reported four cyclopoids in a plankton sample from Echo River in Mammoth Cave. They were: *Cyclops bicuspidatus, Cyclops albidus, Cyclops serrulatus,* and *Cyclops viridis* var. *americanus.* These are probably referable to *Cyclops bicuspidatus thomasi* Forbes, *Macrocyclops albidus* (Jurine), *Eucyclops agilis* (Koch), and *Cyclops vernalis* Fischer, respectively.

Chappuis (1931) listed six cyclopoids from Mammoth Cave. Of these, *Eucyclops serrulatus* (Fischer) and *E. elegans* (Herrick) are probably referable to *Eucyclops agilis* (Koch), although his *elegans* might have been *Eucyclops speratus* (Lilljeborg). He also reported *E. prasinus* (Fischer), *Cyclops robustus* Sars, *Mesocyclops leuckarti edax* Forbes, and *Paracyclops finitimus* Kiefer. The first three of these are probably referable to *Tropocyclops prasinus* (Fischer), *Cyclops vernalis* Fischer, and *Mesocyclops edax* (Forbes), respectively. *Paracyclops finitimus* may be a valid species, but I know of no other record from North America for it. Copepodologists have either overlooked Chappuis' report of its occurrence, or have considered it invalid. Whatever the case may be, one of the latest papers which includes a notation on the species (Harding 1955, p. 220) does not include North America in its distribution on the basis of earlier reports in the literature. *Paracyclops finitimus* has eight antennal segments as does *P. fimbriatus* (Fischer), a species which has been reported several times from North America. Another species with eight antennal segments, *Paracyclops poppei* (Rehberg) has been included in the North American fauna (Kiefer 1929, p. 42). Did Chappuis have *P. fimbriatus* or *P. poppei* rather than *P. finitimus?*

Cole (1953) listed 10 cyclopoids from the Louisville region including an ovigerous *P. fimbriatus. Macrocyclops fuscus* (Jurine), *Microcyclops varicans rubellus* (Lilljeborg), and *Orthocyclops modestus* (Herrick) were reported for the first time from Kentucky in that list.

Since 1953 two other species have been taken in Jefferson County, Kentucky. *Macrocyclops ater* (Herrick) was represented by one ovigerous female in a sample from a pool in Bear Grass Creek on November 7, 1954. Samples from two other stations have contained *Cyclops latipes* Lowndes, which seems to be a vernal species, associated with a typical temporary-pond fauna.

HARPACTICOIDA. Four harpacticoid copepods have been reported from Kentucky, but two of them must be assigned to doubtful or uncertain status. Chambers (1881) described a new species, *Tachidius fonticola,* from Big Bone Springs, Ky., and stated that, in his opinion, it occupied no more than two square yards of the earth's surface. Chamber's description is not referable to any known species of copepod. The second questionable species is *Canthocamptus cavernarum* Packard (1889) found in Wandering Willie's Spring, a pool not far from the mouth of Mammoth Cave. Chappuis (1929b) placed *C. cavernarum* under the heading *Species incertae sedis,* and Coker (1934) stated that, "Packard's *cavernarum*———seems unidentifiable." A further complication exists because Kofoid (1900) erroneously stated that Packard had named the crustacean from Wandering Willie's Spring, *Canthocamptus stygius!* However, *C. cavernarum* may be identical to *Attheyella pilosa* described by Chappuis (1929a) from specimens collected in Indiana caves, and reported also from Mammoth Cave, Kentucky.

Chappuis (1929a) described a second subterranean harpacticoid from Kentucky. In collections from Horse Cave he found a copepod which he considered a subspecies of his *Echinocamptus morrisoni,* known only from cave waters of Indiana, and now assigned to the genus *Bryocamptus.* The Kentucky subspecies is known as *B. m. elegans* (Chappuis).

Harpacticoid copepods are common in the epigean waters of Kentucky, and there must be, conservatively, at least six or seven other species which have not yet been recorded from the state.

PARASITIC COPEPODA. The most interesting of the parasitic copepods known from Kentucky is the lernaepodid, *Cauloxenus stygius.* Cope (1872) gave an account of this species, accompanied by three text figures, on the basis of specimens collected from Wyandotte Cave, Indiana. Cope stated that it lives attached to the inner edge of the upper lip of the blind cave fish, *Amblyopsis.* Giovannoli (1933) reported that *C. stygius* had been found frequently in Mammoth Cave, Ky., attached to the mouth of "the blind fish." However, *Amblyopsis* is extremely scarce in Mammoth Cave, and one must assume that the alleged, frequent occurrence of the parasite there might involve a different host.

Several times the writer has observed members of the genus *Lernaea* attached to bluegills (*Lepomis macrochirus* Rafinesque) from Kentucky. In another instance a member of the genus *Argulus* was obtained from a home aquarium in Louisville. No other information on parasitic copepods from Kentucky seems to be available.

OSTRACODA

There is a great opportunity for studies on the ostracod fauna of Kentucky because only six species have been recorded from the state prior to this paper.

Sharpe (1897) reported *Cypris reticulata* Zaddach "from Cypress Swamp, Kentucky, in September." He may have been referring to immature *Cypricercus reticulatus* (Zaddach), a species which is extremely common in temporary, vernal pools in Jefferson County.

Other ostracods which have been reported are: *Limnocythere* sp. from Echo River in Mammoth Cave (Kofoid, 1900); and two species, *Physocypria pustulosa* Sharpe and *Cypridopsis vidua* (O.F.M.), from Tom Wallace Lake (Cole, 1957b). The last two species are rather common in Jefferson County.

A doubtful Kentucky record is that of *Candona jeanelli* Klie from Mammoth Cave. Klie (1931) described this species from specimens collected in an Indiana cave, and, in the same publication, mentioned an unidentified ostracod from Mammoth Cave. However, Tressler (1947) listed *C. jeanelli* from Mammoth Cave, citing Klie (1931) as the source.

Five other Kentucky ostracods are reported here for the first time. A species of *Cyprinotus* has been taken in some collections from a weedy pond in Jefferson County. *Darwinula stevensoni* (Brady and Robertson), *Cypria turneri* Hoff, and a species of *Candona* have been collected from Cherokee Lake, Jefferson County. In December 1954, several specimens of *Chlamydotheca unispinosa* (Baird) were brought to the writer's attention. The ostracods were living in a small aquarium in Louisville. *Chlamydotheca* is essentially a neotropical genus, but *C. unispinosa* has been reported from Ohio and from the Chicago area (Hoff, 1944b).

Ten species of the Ostracoda may represent no more than a third of those to be found in the state. Certainly, some investigation should reveal members of the genus *Entocythere* living in the branchial chambers of Kentucky crayfish, and there must be many more cyprids to be reported. Furthermore, in this group of the Crustacea we might well expect the discovery, in Kentucky, of a few species new to science.

MALACOSTRACA

ISOPODA. Five species of epigean, aquatic isopods found in various habitats near Louisville were discussed by Cole (1957a). Four of them had not been listed previously from Kentucky. They were *Asellus brevicaudus* Forbes, *A. intermedius* Forbes, *A. militaris* Hay, and an undescribed species of *Asellus*. The fifth, and most abundant species, *Lirceus fontinalis* Rafinesque, has a unique Kentucky history. In 1820 Rafinesque described and named this isopod from specimens collected in streams and springs around Lexington, Kentucky. Seventy years later Garman (1890), apparently unaware of Rafinesque's paper, described what he thought to be a new species of isopod, and assigned it to the genus *Mancasellus* Harger. He named it *M. macrourus,* and stated that it was abundant in spring-fed rivulets and ponds in eastern Kentucky. Garman's name was applied to this crustacean by several authors in subsequent years. However, Hubricht and Mackin (1949) established the priority of Rafinesque's name. The 1820 paper of Rafinesque had been brought to their attention, and Hubricht travelled to Lexington to collect from the springs and streams of that region. He discovered the common asellid there was *Mancasellus macrourus,* and decided that Rafinesque's earlier name must have applied to that crustacean. Thus, *Lirceus fontinalis* stands as the type species for the genus, which now contains a dozen or more species.

The subterranean Isopoda of Kentucky have been the object of much interest since Packard (1871) discovered a new, blind, white isopod, *Caecidotea stygia,* in Mammoth Cave. There has been much controversy concerning the generic name *Caecidotea,* which Packard coined. He believed that the isopod was closely related to the marine genus *Idotea,* but most modern workers question the generic distinction between isopods assigned to *Caecidotea,* and the various epigean species of *Asellus*. At this time Packard's isopod usually is referred to as *Asellus stygius* (Packard), and it is fairly common in some caves and wells in Kentucky, Tennessee, and Indiana. Some older references to *C. microcephala* Cope, *C. richardsonae* Hay, and *C. nickajackensis* Packard probably apply to it. However, because nothing has been published on extensive collections of subterranean isopods in Kentucky since the discovery of reliable taxonomic structures in the genus *Asellus,* it is possible that the state has other cave-dwelling asellids not yet described.

Very little work has been done with the terrestrial oniscoid isopods of Kentucky, and nearly all the species reported were probably introduced from Europe. Preliminary collections made in the mid-1950's by Milan Beasely, a student at the University of Louisville, revealed that a few species were rather common in Jefferson County. *Trachelipus rathkei* (Brandt), and *Porcellio scaber* Latreille are probably the two most abundant oniscoids there, but Beasely also collected *Armadillidium vulgare* (Latreille), *Porcellio laevis* Latreille, and *Porcellionides pruinosus* (Brandt).

Armadillidium nasatum Budde-Lund has been found by the writer in a wood pile at the campground in Cumberland Falls State Park. In most of the places in North America, and in cooler regions of Europe where this species has been collected it is confined largely to greenhouses and warmed buildings. Muchmore (1957) has reviewed the distribution, and the rare, outdoor occurrences of this species.

During late summer, 1959, near Brandenburg Station, Meade County, W. L. Minckley collected several isopods, which he kindly sent to the author. These specimens are referable to *Ligidium longicaudatum* Stoller, a species which has not been reported previously from the state. According to Minckley, "—the isopod is definitely semi-aquatic, scuttling into water or under leaves with no apparent regard for the medium involved."

Muchmore (*loc. cit.*) also cited *Haplophthalmus danicus* Budde-Lund as having been collected in Kentucky, bringing the state list of terrestrial isopods to eight species. In adjacent Indiana, 10 species have been reported (Eberly, 1954).

Parasitic bopyrid isopods, living in the gill chambers of palaemonid decapods, may be present in Kentucky, but there seem to be no references to them from the state.

AMPHIPODA. With the publication of a paper by Hubricht in 1943, five new species of amphipod crustaceans had been described from specimens collected in Kentucky. One of these, *Gammarus propinquus* Hay (1902), has been synonymized with *Gammarus minus* Say, described earlier from another state, but the other four have type localities in Kentucky. They are *Stygobromus vitreus* Cope, *S. exilis* Hubricht, *Crangonyx anomalus* Hubricht, and *Synurella dentata* Hubricht.

Hubricht (*loc. cit.*) also reported the previously known species, *Crangonyx shoemakeri* (Hubricht and Mackin), *C. gracilis* Smith, and *C. gracilis packardi* Smith from the state. Cole (1957a) listed six epigean amphipods from north central Kentucky, recording *Crangonyx obliquus* (Hubricht and Mackin), and *Hyalella azteca* (Saussure) for the first time from the state.

In a recent, detailed monograph Bousfield (1958) raised several questions about some of the above amphipods. He separated *C. shoemakeri* into two species, *C. shoemakeri* and *C. setodactylus,* a new species. Some so-called *setodactylus* specimens, collected in Kentucky, were sent to him by Hubricht. If Bousfield is correct, there is no authentic record of *C. shoemakeri* from Kentucky.

Furthermore, Bousfield stated that, in his opinion, *C. gracilis packardi* warrants specific rank as *C. packardi,* and he pointed out that *C. gracilis,* as reported in older literature, actually may be a complex of several species. Some similar ideas were expressed a few years ago by Mr. Hubricht (personal communication). He stated that specimens identified as *C. shoemakeri* might well include two species, and that at least two or three species have been reported as *C. gracilis.*

Very recently W. L. Minckley collected many amphipods from a cold stream in Meade County. These proved to be puzzling, and were sent to Dr. E. L. Bousfield for identification. Dr. Bousfield, in personal communication, has stated that they are members of an undescribed species of *Gammarus.*

DECAPODA. Among the collections in the Biology Department, University of Louisville, are several specimens of the glass shrimp, *Palaemonetes,* taken by H. Bishop from Floyds Fork, Jefferson County. One collection was made on October 30, 1938; the other is dated simply "1945." Although those specimens are labelled *P. paludosus,* they are actually *P. kadiakensis* Rathbun. In addition, there are some specimens from Murphy's Pond, Hickman County, collected by Paxton Gibbs during September 1954. Those animals also are referable to *P. kadiakensis,* rather than to *P. paludosus.* Distribution records in the monograph of Holthuis (1952) make it probable that *kadiakensis* is the only species of *Palaemonetes* in Kentucky, and it is undoubtedly rather common in suitable habitats. Creaser (1931) listed that crustacean from Kentucky under the synonym, *P. exilipes* Stimpson.

Another palaemonid, the large, river shrimp, *Macrobrachium ohione* (Smith), was described originally from specimens collected in the Ohio River near the Indiana shore.

A unique member of Kentucky's crustacean fauna is the blind, transluscent atyid, *Palaemonias ganteri* Hay. In 1902 Hay described this species from Mammoth Cave, and it has not been reported from any other locality.

Our knowledge of the crayfishes of Kentucky was summarized by Rhoades (1944), who added six new species and four new subspecies to the state list. His paper is an excellent bibliographic source for anyone interested in the existing literature on that group of decapods from Kentucky. Unfortunately, Rhoades presented no taxonomic keys to the 27 species and subspecies which were included in an annotated listing. Rhoades' list includes a great many subspecies; for example, *Cambarus bartoni* is represented by no less than five. This suggests the presence of very effective isolating mechanisms between drainage systems in Kentucky. On the other hand, a taxonomic "lumper" might have presented the picture of a somewhat more impoverished decapod fauna for Kentucky than Rhoades did in his compilation.

SUMMARY

Past reports of crustaceans from Kentucky are discussed, and new records are presented. A list of apparently valid species and subspecies includes 116 names, of which 23 are reported for the first time.

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LIST OF CRUSTACEANS REPORTED FROM KENTUCKY

Does not include recorded species which are now unrecognizable, nor the various synonyms which have appeared in earlier reports.

- BRANCHIOPODA
  - Anostraca
    - *Eubranchipus vernalis* (Verrill)
    - *E. neglectus* Garman
    - *Streptocephalus seali* Ryder
  - Conchostraca
    - *Cyzicus mexicanus* (Claus)
  - Cladocera
    - *Alona guttata* Sars
    - *A. rectangula* Sars
    - *Alonella rostrata* (Koch)[1]
    - *Bosmina longirostris* (O.F.M.)
    - *B. coregoni* Baird[1]
    - *Bosminopsis deitersi* Richard[1]
    - *Ceriodaphnia lacustris* Birge
    - *C. pulchella* Sars[1]
    - *C. quadrangula* O.F.M.[1]
    - *C. reticulata* (Jurine)
    - *Chydorus sphaericus* (O.F.M.)
    - *Daphnia* sp.[2]
    - *D. pulex* (de Geer)[3]
    - *Diaphanosoma brachyurum* (Liéven)
    - *Ilyocryptus sordidus* (Liéven)
    - *Leydigia acanthocercoides* (Fischer)[1]
    - *L. quadrangularis* (Leydig)
    - *Macrothrix laticornis* (Jurine)
    - *Moina affinis* Birge[1]
    - *Pleuroxus denticulatus* Birge
    - *Scapholeberis mucronata* (O.F.M.)
    - *Sida crystallina* (O.F.M.)[4]
    - *Simocephalus serrulatus* (Koch)
    - *S. vetulus* (O.F.M.
- COPEPODA
  - Calanoida
    - *Diaptomus pallidus* Herrick
    - *D. sanguineus* Forbes
    - *D. stagnalis* Forbes
    - *Osphranticum labronectum* Forbes
  - Cyclopoida
    - *Cyclops bicuspidatus thomasi* Forbes
    - *C. latipes* Lowndes[1]
    - *C. vernalis* Fischer
    - *Eucyclops agilis* (Koch)
    - *Macrocyclops albidus* (Jurine)
    - *M. ater* (Herrick)[1]
    - *M. fuscus* (Jurine)
    - *Mesocyclops edax* (Forbes)
    - *Microcyclops varicans rubellus* (Lilljeborg)
    - *Orthocyclops modestus* (Herrick)
    - *Paracyclops fimbriatus* (Fischer)
    - *P. finitimus* Kiefer[4]
    - *Tropocyclops prasinus* (Fischer)
  - Harpacticoida
    - *Attheyella pilosa* Chappuis
    - *Bryocamptus morrisoni elegans* (Chappuis)
  - Parasitic copepods
    - *Argulus* sp.[1]
    - *Cauloxenus stygius* Cope
    - *Lernaea* sp.[1]
- OSTRACODA
    - *Candona jeanneli* Klie[4]
    - *Candona* sp.[1]
    - *Chlamydotheca unispinosa* (Baird)[1]
    - *Cypria turneri* Hoff[1]
    - *Cypricercus reticulatus* (Zaddach)
    - *Cypridopsis vidua* (O.F.M.)
    - *Cyprinotus* sp.[1]
    - *Darwinula stevensoni* (Brady and Robertson)[1]
    - *Limnocythere* sp.
    - *Physocypria pustulosa* Sharpe
- MALACOSTRACA
  - Isopoda (Asellota)
    - *Asellus brevicaudus* Forbes
    - *A. intermedius* Forbes
    - *A. militaris* Hay
    - *A. stygius* (Packard)
    - *Asellus* sp.
    - *Lirceus fontinalis* Rafinesque
  - Isopoda (Oniscoidea)
    - *Armadillidium nasatum* Budde-Lund[1]

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[1] New record for Kentucky.

[2] Reported incorrectly as *D. longispina* (O.F.M.). Probably applies to one or more different species.

[3] Probably includes additional species.

[4] Questionable Kentucky record.

- *A. vulgare* (Latreille)
- *Haplophthalmus danicus* Budde-Lund
- *Ligidium longicaudatum* Stoller[1]
- *Porcellio scaber* Latreille[1]
- *P. laevis* Latreille[1]
- *Porcellionides pruinosus* (Brandt)[1]
- *Trachelipus rathkei* (Brandt)[1]

Amphipoda
- *Crangonyx anomalus* Hubricht
- *C. gracilis* Smith[3]
- *C. obliquus* (Hubricht and Mackin)
- *C. packardi* Smith
- *C. setodactylus* Bousfield
- *C. shoemakeri* (Hubricht and Mackin)[4]
- *Gammarus minus* Say
- *Gammarus* sp.[1]
- *Hyalella azteca* (Saussure)
- *Stygobromus vitreus* Cope
- *S. exilis* Hubricht
- *Synurella dentata* Hubricht

Decapoda (other than crayfishes)
- *Palaemonetes kadiakensis* Rathbun
- *Palaemonias ganteri* Hay
- *Macrobrachium ohione* (Smith)

Decapoda (crayfishes)[5]
- *Cambarus bartoni bartoni* (Fabricius)
- *C. b. laevis* Faxon
- *C. b. ornatus* Rhoades
- *C. b. striatus* (Hay)
- *C. b. tenebrosus* Hay
- *C. cornutus* Faxon
- *C. diogenes* Girard
- *C. distans* Rhoades
- *C. extraneous* Hagen
- *C. ortmanni* Williamson
- *C. rusticiformis* Rhoades
- *Orconectes bisectus* Rhoades
- *O. compressus* (Faxon)
- *O. immunis immunis* (Hagen)
- *O. juvenilis* (Hagen)
- *O. kentuckiensis* Rhoades
- *O. pellucidus pellucidus* (Tellkampf)
- *O. p. packardi* Rhoades
- *O. propinquus jeffersoni* Rhoades
- *O. p. sanborni* (Faxon)
- *O. rafinesquei* Rhoades
- *O. rusticus rusticus* (Girard)
- *O. r. barrenensis* Rhoades
- *O. r. placidus* (Hagen)
- *O. tricuspis* Rhoades
- *Procambarus blandingi acutus* (Girard)
- *P. clarki* (Girard)

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[5] List compiled from Rhoades (1944).

ACADEMY AFFAIRS

THE 1959 SPRING MEETING

The Kentucky Academy of Science held its 1959 spring meeting at Lake Cumberland on May 8 and 9.

The Friday after dinner program consisted of a panel discussion on "Preprofessional Training Related to the Sciences." The fields represented and the speakers were as follows:

Medicine—Dr. Wm. J. Hockaday, Director of Admissions, University of Louisville, School of Medicine.

Dentistry—Dr. Raymond E. Myers, Dean, School of Dentistry, University of Louisville.

Medical Technology—Dr. Malcolm Barnes, Norton's Infirmary, Louisville.

Pharmacy—Dr. Charles A. Walton, Head, Department of Materia Medica, and Professor of Pharmacology, College of Pharmacy, University of Kentucky.

Graduate Study in Zoology—Dr. Robert Kuehne, Instructor in Zoology, University of Kentucky.

Graduate Study in Physics—Dr. Francis L. Yost, Head, Department of Physics, University of Kentucky.

Saturday morning was devoted to field trips. These included nature hikes and a tour of the power plant at the dam.

THE 1959 FALL MEETING

The Kentucky Academy held the forty-fifth annual fall meeting on October 23-24 at Western Kentucky State College in Bowling Green.

After the dinner at the Paul L. Garrett Student Center the group adjourned to Snell Hall where Dr. Raymond Cravens, Dean of Western Kentucky State College, greeted the Academy. Dr. Roger Barbour gave the after dinner address in which he discussed his experiences in Indonesia.

At the business session on Saturday morning the following officers were elected for the coming year:

President elect	H. H. LaFuze
Vice President	Charles Whittle
Secretary	Gerrit Levey
Treasurer	R. A. Chapman
Transactions Editor	Roger Barbour
Representative to AAAS	Mary Wharton
Board of Directors	Hazel Nollau William Clay
Counselor to Junior Academy	R. M. Boyer

Dr. Pete Panzera of Murray State College will serve as President for the coming year.

Resolutions adopted are:

Whereas, Dr. Fenton T. West of Morehead State College passed away on Nov. 9, 1958 and whereas Dr. West was a loyal member of the Kentucky Academy of Science and a great biologist and teacher,

Therefore be it resolved that the Academy by this resolution express its appreciation of his work and its sense of loss at his passing, and that a copy of this resolution be sent to his family and spread upon the minutes of this meeting.

Whereas, Dr. Gerald A. Cole of the University of Louisville has so efficiently served the Kentucky Academy of Science as Editor of the Transactions of the Academy for two years, and

Whereas, Dr. Cole has now left Kentucky and thereby terminated his efforts in our behalf,

Therefore be it resolved that the Academy express to him its sincere appreciation and gratitude by sending to him a copy of this resolution and by spreading it upon the minutes of this 1959 meeting as a record thereof.

Whereas, Western State College has seen fit to invite the Kentucky Academy of Science to hold its Fall meeting of 1959 on its campus, and

Whereas, the College has extended its hospitality to the Academy in the form of use of its facilities and services of its faculty, and

Whereas, the gentlemen of the Western State College faculty forming the local Committee on arrangements have so efficiently performed their functions and contributed in such high degree to the success of this 1959 meeting,

Therefore be it resolved that the Academy express its sincere gratitude to the local committee and to Western State College, and that this resolution be spread upon the minutes of the meeting as a record thereof, and copy be sent to President Kelley Thompson of Western State College.

Whereas the National Science Foundation has instituted programs for the improvement of science teachers and science teaching and

Whereas, Kentucky has benefited by several of these programs in the form of summer institutes and in-service institutes since 1957 and

Whereas the institutes have given teachers the opportunity of improving their subject matter knowledge and the ability to discover and to encourage scientific talent,

Therefore be it resolved that the Kentucky Academy of Science express its hearty endorsement and appreciation of these programs and that a copy of this resolution be sent to Dr. Alan Waterman, Director of National Science Foundation and the Kentucky senators and congressmen and that it be spread on the minutes of this meeting.

Whereas the Kentucky State Board of Education acting on the advice of the Council on Higher Education has improved the curriculum for elementary and high school certification by requiring 12 credit hours in science and mathematics, half of which must be in laboratory sciences,

Therefore be it resolved that the Kentucky Academy of Science endorse this move toward better preparation and education of our teachers and that copies of this resolution be sent to the Presidents of colleges represented on the Council of Higher Education and spread upon the minutes of this meeting.

The Sectional programs were as follows:

BACTERIOLOGY AND MEDICAL TECHNOLOGY SECTION

Cherry Hall, Room 203, 10:00 A.M.

Ralph Wiseman, Chairman; O. F. Edwards, Secretary

1. Preliminary studies of the morphology of the aerobic *Actinomycetes*. O. F. Edwards and M. Hotchkiss, Dept. of Bacteriology and the Keeneland Foundation Electron Microscope Laboratory, University of Kentucky.
2. The utilization of uric acid by a rumen coliform Bacterium. C. Thornsberry and R. Wiseman, Dept. of Bacteriology, University of Kentucky.

3. Technics of fluorescent microscopy. Z. S. Gierlach, Central Baptist Hospital, Lexington.
4. Control method for assured daily accuracy of clinical chemistry. B. Turpin, The Lexington Clinic, Lexington.
5. Lethel congenital neutropenia in two siblings. J. P. Andrews, The Lexington Clinic, Lexington.
6. The specificity of leukocytic hypersensitivity. M. Scherago, Dept. of Bacteriology, University of Kentucky.

Papers limited to approximately 10 minutes to allow five minutes of discussion following each.

BIOLOGY SECTION

Cherry Hall, Room 227, 9:00 A.M.

Frank Gailey, Chairman; Lloyd Alexander, Secretary

1. Response of peripheral leucocytes to nonspecific stress in rodents. William G. Downs, Jr. and G. B. Pennebaker, Tennessee Polytechnic Institute.
2. Some factors which influence the greening of dark grown seedlings. Frank Gailey, Berea College.
3. Studies on cold hardiness in *Drosophila* species. Wallace D. Dawson and John M. Carpenter, University of Kentucky.
4. Evidences of stream capture between the Cumberland and Kentucky rivers. Robert A. Kuehne, University of Kentucky.
5. Paper Chromatography as a possible tool in the identification of species. H. P. Riley and T. R. Bryant, University of Kentucky.
6. Reports on the International Botanical Congress meeting which was held at Montreal from August 19-29.

CHEMISTRY SECTION

Cherry Hall, Room 125, 10:30 A.M.

J. Paul Ray, Chairman; Walter T. Smith, Jr., Secretary

1. Identification and characterization of organic compounds through micro-fusion techniques. Ward C. Sumpter and Wendell Oden.
2. Dielectric constants of solutions of sulfanilamides in selected non-aqueous media. Paul G. Sears.
3. Solvates of Acetylacetonates. Francis Clarke, J. F. Steinbach and W. F. Wagner.
4. Preparation and carcinogenic action of some quinoline azo dyes. Ellis Brown.
5. Analysis and determination of constitutents of samples in the Na2So4-S03-H2O system. Paul G. Sears.

PSYCHOLOGY SECTION

Cherry Hall, Room 221, 9:00 A.M.

John Donahoe, Chairman; Clara Cooper, Secretary

1. ''Mama''—from babble to symbol. Ray H. Bixler, University of Louisville.
2. Effects of verbal reinforcement on rote serial learning. James Leonard, University of Kentucky.
3. Problem-solving performance as a function of variations in feedback. John Donahoe, University of Kentucky.
4. A report on research in progress in this country and abroad. Hans Rahn, Transylvania College.
5. A binomial measure of degree of discrimination. James Calvin, University of Kentucky.

6. Materials, techniques and methods in teaching psychology in thirty-four secondary schools. Paul R. McNeely, Asbury College.
7. Is there some aspect of religion that psychology cannot reach? W. Gordon Ross, Berea College.
8. A comparative study of delinquents and non-delinquents. Clara Chassell Cooper, Berea College.

PHYSICS SECTION

Cherry Hall, Room 229

Charles E. Whittle, Chairman

ENGINEERING SECTION

Karl O. Lange, Chairman; Oliver W. Gard, Secretary

REPORT OF THE TREASURER FOR 1958-59

Balance, October 1, 1958			$ 109.65
Income, October 1, 1958 to October 1, 1959			
Regular members, 1958-1959 dues	$ 492.00		
Regular members, 1957-1959 dues	64.00		
Regular members, 1959-1960 dues	4.00		
Sustaining members, 1958-1959 dues	340.00		
Industrial members, 1958-1959 dues	500.00		
Subscription for ''Kentucky Engineer''	1.00		
Postage reimbursement	.04		
Total from members	$1401.04	$1401.04	
Sales of Transactions of the Kentucky Academy of Science Volumes and subscriptions to non-members	$ 26.00		
200 copies, Volumes 19 and 20 to the University of Louisville for exchange	400.00		
	$ 426.00	$ 426.00	
AAAS research grant	100.00	100.00	
Total income		$1927.04	$1927.04
			$2036.69
Expenditures, October 1, 1958 to October 1, 1959			
1958 fall meeting	$ 22.43		
Secretary, postage, printing, etc.	94.76		
Treasurer, postage, printing, etc.	13.85		
Junior Academy, councilor travel, prize fund, printing, postage, etc.	539.50		
Volume 19 (3, 4) Transactions	413.77		
AAAS research grant	100.00		
Academy Conference dues	5.40		
Subscription to ''Kentucky Engineer''	1.00		
State tax (checking account)	.84		
Total	$1191.55		$1191.55
Balance, October 1, 1959			$ 845.14

Respectfully submitted,

Richard A. Chapman, *Treasurer*

Approved by Dr. Weaver and Committee

NEWS AND NOTES

Dr. J. G. Rodriguez, UK associate professor of agricultural entomology, has been awarded a $29,325 National Health Institute grant for a three year study of the possibility of using mites to control the housefly.

Index to Volume 20

INSTRUCTIONS FOR CONTRIBUTORS

The TRANSACTIONS OF THE KENTUCKY ACADEMY OF SCIENCE is a medium of publication for original investigations in science. Also as the official organ of the Kentucky Academy of Science, news and announcements of interest to the membership are published therein. These include programs of meetings, titles, abstracts of papers presented at meetings, and condensations of reports by the Academy's officers and committees.

Papers may be submitted at any time to the editor or one of the associate editors. Each manuscript will be reviewed by one or more persons qualified in the field in addition to the editors before it is accepted for publication, and an attempt will be made to publish papers in the order of their acceptance. Papers are accepted for publication with the understanding that they are not to be submitted for original publication elsewhere, and that any additional printing shall be at a later date and shall be designated in an appropriate credit line as a reprint from the TRANSACTIONS OF THE KENTUCKY ACADEMY OF SCIENCE.

Manuscripts should be typed, double-spaced, with wide margins, on paper of good stock. The original and one carbon copy should be submitted, and the author should retain one additional carbon copy. It is desirable that the author have his colleagues read the manuscript for clarity of expression and typographical or other errors.

Titles must be clear and concise, and provide for precise cataloging. Textual material should be in clear, brief, condensed form. Footnotes should be avoided. Tables and illustrations are expensive and should be included only to give effective presentation of the data. Articles with an excessive number of tables or illustrations, or with poorly executed tables or illustrations, may be returned to the author for modification.

Line drawings will appear as *text-figures,* while half-tones or other processes requiring paper inserts will appear as *plates.* Text-figures should be jet-black on white; shading may be indicated by stippling or lines but not by half-tone washes. Drafting should be carefully done (hand lettering generally is not satisfactory). Photographs for plates should have good contrast and be printed on glossy paper. Plates and text-figures are to be numbered consecutively and independently; on the back of each its number and the author's name should be written lightly in pencil. Each plate and text-figure must be referred to specifically in the text and must be provided also with a legend, the latter to be supplied as typed copy separate from the figures. Figures should be arranged into groups whenever possible and the legend for each group written as a separate paragraph. The amount of reduction desired should be indicated and should be consistent with the page dimensions of this journal. Indications of magnification should apply to the reduced figure.

The aim of the paper should be made clear in the introductory portion. If the paper is of more than a few pages it should contain a brief ''Summary,'' which should be lucid without recourse to the rest of the article. In the interest of bibliographic uniformity, arrange all references under a ''Literature Cited'' heading, in either of two sequences: (a) alphabetically by author and date, unnumbered, with textual citation by parenthetic insertion of author and date, as (Jones, 1940), or (b) numbered in the sequence in which textual citation by numbers is made, as (1), (2), etc. Use initials for given names. Titles of books must be included, but those of articles in a serial may be omitted at the author's discretion. Abbreviate names of journals, using the form employed by Chemical Abstracts or Biological Abstracts. Separate the volume number from page numbers by a colon. References to books should include also the place of publication and the publisher.

The author is responsible for correcting the galley proof. Extensive alterations from the original are expensive and must be avoided or paid for by the author Galley proofs must be returned promptly. Blanks for reprint orders will be supplied with the galley proof.