solid (0.17 g.); R_f 0.12; D, pink; E and N, negative. The base picrate crystallized from ethyl alcohol as orange-yellow needles, m.p. 242°, mixed melting point with authentic choline picrate (m.p. 242°) remained undepressed. Usual pharmacological testing (25) with a portion of the base also showed its identity with choline.

Fraction D—The reineckate salt, precipitated under acidic conditions, was treated in the same manner; a brown basic gum (82 mg.) was obtained. The gum showed three Dragendorff-positive spots on TLC, R_f 0.09, 0.12, and 0.28.

Betaine—An ethyl alcohol solution of the above base was treated with a solution of picric acid in the same solvent, and the mixture was concentrated. Betaine picrate separated as yellow needles, m.p. and mixed m.p. 188–190°.

The ethyl alcohol mother liquor, after separation of betaine picrate, was passed through a column of De-Acidite FF. The regenerated mixture of bases was separated by preparative TLC.

Quaternary Indole-3-alkylamines—The major component (R_f 0.09; D, orange, E, blue; N, violet) showed UV $\lambda_{max.}$ 218, 272 (sh), and 285 nm., characteristic of indole-3-alkylamines. The minor component, (R_f 0.12; D, orange; E, blue; N, dull violet) showed $\lambda_{max.}$ 224–226, 272, 294, and 305–310 (sh) nm., characteristic of 5-oxy-indole-3-alkylamines.

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Degradation of Urea in Concentrated Aqueous Solution

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Abstract □ The degradation of urea in 2.00, 4.00, 6.00, and 8.00 *M* aqueous solutions was studied at 25.0, 35.0, and 45.0°. Data were obtained by measuring the specific conductivity of the solutions at 6-hr. intervals over 3.5 days. The results show that the degree of degradation is extremely small and that the overall process conforms to a first- and second-order reversible reaction. Rate constants were determined for the forward and reverse reactions and compare favorably to values reported by other workers for the separate reactions.

Keyphrases □ Urea—degradation in concentrated aqueous solution, temperature control □ Conductivity—urea degradation monitoring □ Rate constants—aqueous urea degradation □ Specific conductivity—urea degradation monitoring

The hydrolysis of urea in various aqueous media has been studied extensively. An excellent review and bibliography on the subject are given by Frost and Pearson (1). However, these earlier investigations, including a more recent study by Lynn (2), were concerned with the nature of the hydrolysis reaction as it occurs in relatively dilute solutions of urea and, for the most part, at temperatures between 60 and 100°. As a consequence, little information exists relative to the degradation process under conditions of current interest. Thus, workers who are studying solubility (3–5) and denaturation (6, 7) phenomena in solutions ranging up to 9 *M* in urea, and at temperatures between 25 and 50°, are unable to appraise the untoward effects produced by this reaction. Instead, and because degradation is known to occur, investigators are compelled to initiate their studies with freshly prepared urea solutions in the hope that these effects will be minimized. Since solubility studies, for example, characteristically require days for equilibration, even this technique offers little in the way of reduced uncertainty.

Table I—Density and Viscosity of Urea Solutions

Temperature	[Urea], *M*	ρ, g. ml.$^{-1}$	η, cps.
25.0°	0.00	0.997[a]	0.89[a]
	2.00	1.032[b]	0.98
	4.00	1.062[b]	1.09
	6.00	1.090[b]	1.25
	8.00	1.112[b]	1.44
35.0°	0.00	0.994[a]	0.72[a]
	2.00	1.024	0.79
	4.00	1.054	0.89
	6.00	1.083	1.02
	8.00	1.112	1.11
45.0°	0.00	0.990[a]	0.60[a]
	2.00	1.020	0.66
	4.00	1.059	0.74
	6.00	1.078	0.84
	8.00	1.106	0.97

[a] From the "Handbook of Chemistry and Physics," 47th ed., Chemical Rubber Co., Cleveland, Ohio, 1966. [b] Derived from data of F. T. Gucker, Jr., F. W. Gage, and C. E. Moser, *J. Amer. Chem. Soc.*, **60**, 2528(1938).

Bull *et al.* (8), presumably measuring the extent of the reaction:

$$CO(NH_2)_2 \xrightarrow{k_1} NH_4^+ + CNO^- \qquad \text{(Eq. 1)}$$

observed that the specific conductivity of concentrated urea solutions increased linearly with time. Further, they noted that the rate of increase in specific conductivity was proportional to the product *relative viscosity* × *urea concentration*. It appears, then, that urea degradation follows first-order kinetics in both dilute (9) and concentrated solutions. Schwartz and Nelson (10), on the other hand, suggested that the degradation in concentrated solutions is more accurately described as:

$$CO(NH_2)_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} NH_4^+ + CNO^- \qquad \text{(Eq. 2)}$$

The present study was undertaken to: (*a*) test the validity of the suggestion made by Schwartz and Nelson (10), and (*b*) obtain more extensive, quantitative information relative to the degradation of urea in the temperature and concentration ranges frequently cited in the current literature. Because of the relative ease with which conductivity data may be obtained, and in view of the problems certain to arise in a chemical analysis for ammonium cyanate, it was decided to monitor the degradation process by observing the change in the specific conductance of the urea solutions with time.

EXPERIMENTAL

Solutions—Reagent grade urea, without further purification, was dissolved in distilled water having a specific conductance of 1.1 μmho cm.$^{-1}$, to make solutions containing 2.00, 4.00, 6.00, and 8.00 moles/l. of solute, respectively. A fresh set of solutions was prepared for the study at each of the three temperatures used. Each set was prepared in 500-ml. volumetric flasks and at the particular temperature of interest.

Conductivity Measurements—At 6-hr. intervals, 10-ml. samples were withdrawn from the flasks and transferred to a fill-type conductivity cell suspended in the same bath. The cell had been previously calibrated against standard potassium chloride solutions. The conductance was measured using a Beckman model RC-19 bridge, and the observed values were converted to specific conductances using standard procedures. These measurements, at each of the three temperatures, were continued into the 4th day.

Thermostating—All density, viscosity, and conductance measurements were made in a thermostated bath. Temperatures were adjusted to 25.0, 35.0, and 45.0° with the aid of a quartz thermometer. The same instrument indicated a maximum variation of ± 0.01° at all three temperatures.

Densities—The density of each solution was determined at 35.0 and 45.0° using a 50-ml. pycnometer.

Viscosities—Kinematic viscosities of freshly prepared solutions were measured at the three temperatures using an Ostwald viscometer. Viscosities were then calculated relative to the density and flow time of distilled water at the corresponding temperature.

RESULTS

The density and viscosity data obtained in the present investigation are shown in Table I. The densities are in accord with values given by Stokes (11). The viscosity data agree with literature values at 25 (12) and 35° (13). Since they were obtained in the same fashion, it is assumed that the data at 45° are equally valid, at least for the purposes of this work.

The specific conductivity *versus* time data were subjected to a polynomial regression analysis using an IBM 360/60 O.S. computer. The results of the analysis, given in Table II, are of the form:

$$K = a + bt + ct^2 + dt^3 \qquad \text{(Eq. 3)}$$

where K is the specific conductivity, and t is the elapsed time in hours. In view of the corresponding uncertainties in the concentrations, temperatures, densities, and viscosities, the observed conductances were rounded to four significant figures. The data for the 8.00 *M* urea solutions are illustrated in Fig. 1. Note the departure from linearity as the temperature increases and the apparent equilibrium condition approached at 45°. Data for the other solutions describe a similar, though proportionately less pronounced, departure from linearity. In the time allowed, only the 8.00 *M* at 45° reached the equilibrium state.

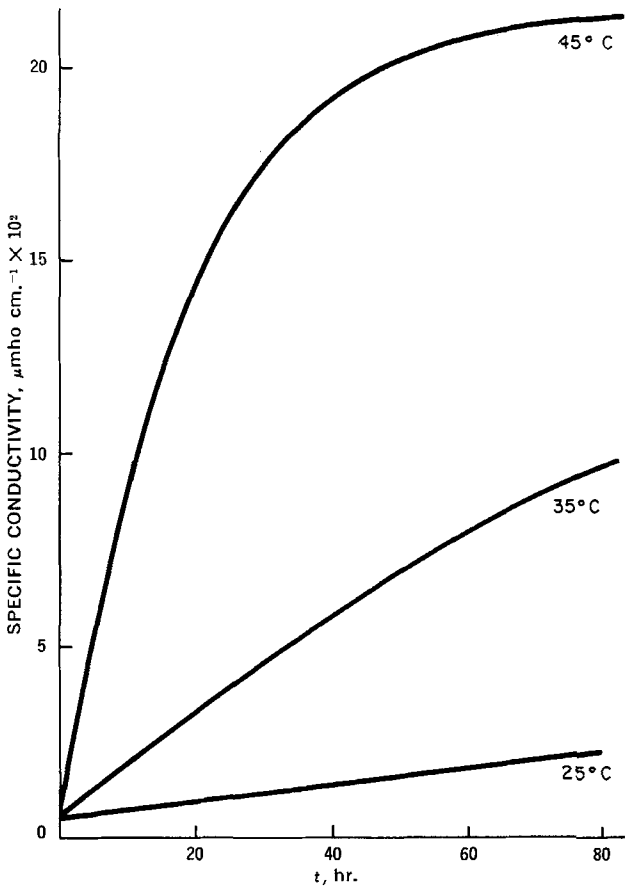

Figure 1—*Specific conductivity of 8.00* M *urea solutions as a function of time at 25, 35, and 45°.*

Table II—Constants for Eq. 3: $K = a + bt + ct^2 + dt^3$

Temperature	[Urea], M	a	b	10^2c	10^4d	Average Deviation, Observed–Calculated, μmho
25.0°	2.00	17.23	0.759			±3.50
	4.00	29.47	1.367			±5.69
	6.00	40.64	1.805			±7.16
	8.00	51.88	2.037			±7.66
35.0°	2.00	22.61	5.111	−0.6416		±1.63
	4.00	17.07	9.781	−1.944		±1.85
	6.00	16.68	14.01	−4.311		±8.88
	8.00	35.48	15.43	−4.794		+4.69
45.0°	2.00	16.19	31.77	−16.76	0.0440	±3.58
	4.00	31.51	61.47	−62.44	21.41	±6.49
	6.00	40.57	78.21	−99.70	44.62	±5.99
	8.00	71.17	94.58	−153.4	85.52	±6.84

DISCUSSION

The data in Table II describe kinetic systems of greater complexity than was indicated by Bull *et al.* (8). At 35 and 45°, for example, the rate of degradation is a decreasing function with time. This suggests that the reverse reaction is a factor in the degradation of concentrated urea solutions, in accordance with Eq. 2. In this event, the net rate expression is given by:

$$dC/dt = k_1M - k_2C^2 \qquad \text{(Eq. 4)}$$

where dC/dt is the rate of appearance of ammonium cyanate, k_1 is the rate constant for the degradation of urea, M is the concentration of urea, k_2 is the rate constant for the formation of urea from ammonium cyanate, and C is the concentration of ammonium cyanate.

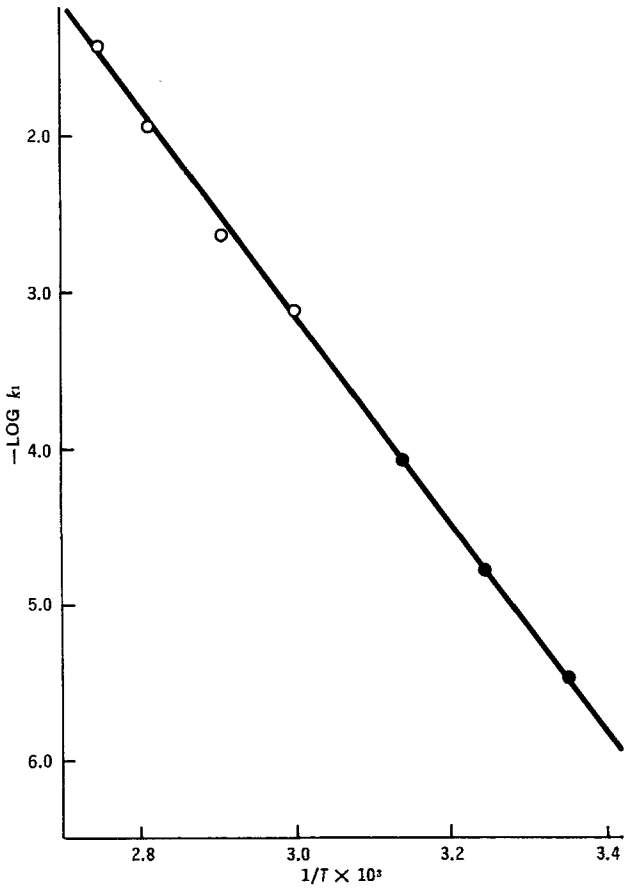

Figure 2—*Arrhenius plot for decomposition of urea in water. Points obtained in the present investigation* (●) *are compared with those reported by Shaw and Bordeaux* (9) (○). *The slope of the line corresponds to* $E_a = 31.6$ *kcal.*

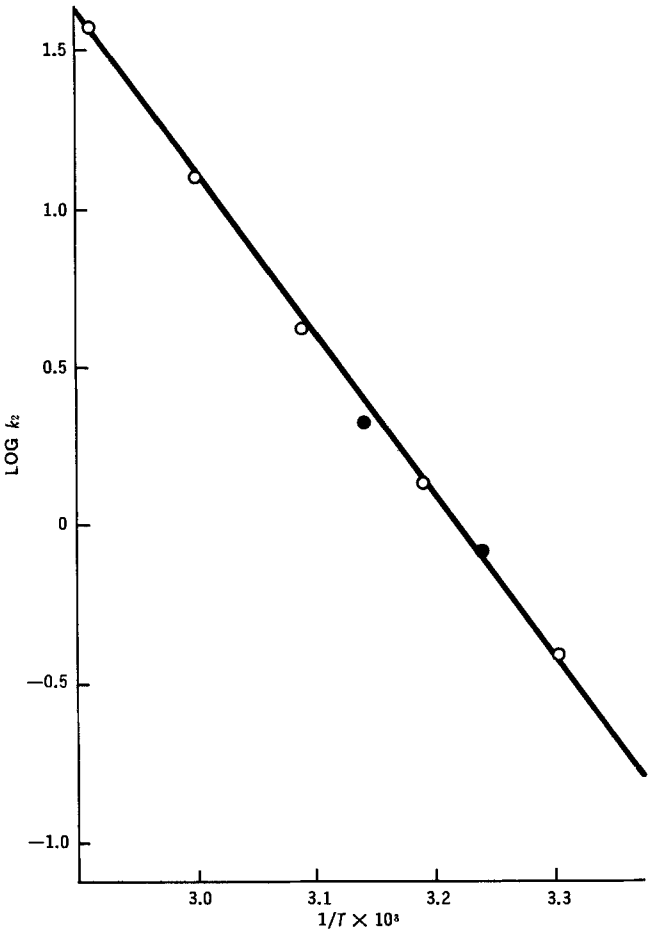

Figure 3—*Arrhenius plot for formation of urea from ammonium cyanate in water. Points obtained in the present investigation* (●) *are compared with those reported by Svirbely and Warner* (18) (○). *The slope of the line corresponds to* $E_a = 23.6$ *kcal.*

Qualitatively, Eq. 4 satisfies the two most obvious criteria established by the experimental observations. First, it can account for the decrease in the overall rate of urea degradation with time at 35 and 45°. Second, it can become a linear function at 25°, where, presumably, the concentration of ammonium cyanate is not great enough to cause a noticeable reverse reaction.

An interpretation of Eq. 4, using the present data, must rely on the relationship:

$$C = 1000K/\Lambda \qquad \text{(Eq. 5)}$$

where C, as before, is the concentration of ammonium cyanate, K is the specific conductance of the solution, and Λ is the equivalent conductance for the conditions that pertain at the time of the measurement. Since Λ will vary among the systems, the solutions to Eq. 5 are not straightforward. Nevertheless, realistic estimates of C can be obtained if two simplifying assumptions are made regarding Λ. These are: (*a*) that Walden's conductance–viscosity rule is applicable, and (*b*) that the equivalent conductance is essentially constant over the range of electrolyte concentrations encountered in this study.

The assumption relative to Walden's rule requires acceptance of a condition in which the movement of ions is a function of viscosity only. However, in view of the variety of solvent systems traditionally used to illustrate this theorem (14), it seems reasonable that the rule will provide good estimates for the purpose at hand. Accordingly, limiting equivalent conductances, Λ_0, for each urea solution at 25, 35, and 45°, were calculated using the relationship:

$$\Lambda_0\eta = \Lambda_0'\eta' \qquad \text{(Eq. 6)}$$

Table III—Limiting Equivalent Conductance of Ammonium Cyanate in Various Solutions of Urea[a]

Temperature	[Urea], M				
	Λ_0, ohm^{-1} cm.^{2}				
	0.00	2.00	4.00	6.00	8.00
25.0°	138[b]	127	113	99	86
35.0°	170	158	139	120	102
45.0°	206	187	166	146	127

[a] Calculated according to Walden's rule. [b] Value obtained from *Reference 15*.

where Λ_0 and η are the limiting equivalent conductance and viscosity, respectively, in one solvent system, and Λ_0' and η' are the corresponding values in a second system. The calculations were based on the viscosities given in Table I and the limiting equivalent conductance of ammonium cyanate in water at 25° (15). Thus, the limiting equivalent conductance of ammonium cyanate in a 2 M urea solution at 25° is given by:

$$\Lambda_0(2\ M\ \text{urea})_{25°} = \frac{\eta(\text{water})_{25°}}{\eta(2\ M\ \text{urea})_{25°}}\ \Lambda_0(\text{water})_{25°} \qquad \text{(Eq. 7)}$$

Table III is a compilation of the Λ_0 values derived in this manner. The values at 25°, when plotted as Λ_0 *versus* M, yield a line with a slope of 6.5 Λ_0/M. This value compares favorably with the 7.0 Λ_0/M value obtained by plotting the experimental data of Bull *et al.* (8) for ammonium chloride at 30° in solutions of the same urea concentration.

The second assumption implies that $\Lambda \approx \Lambda_0$ when, in fact, the two conductances are related by the expression:

$$\Lambda = \Lambda_0 - SC^{1/2} \qquad \text{(Eq. 8)}$$

where S is a function of viscosity, temperature, and dielectric constant; and C is the concentration of the conducting species (16). It is possible to evaluate the corrective term on the right by estimating: (*a*) S using the viscosity data from the present study and dielectric constants for urea solutions (17), and (*b*) C using the specific conductance data in Table II and the corresponding limiting equivalent conductance values in Table III. In so doing, it is found that the slope, S, decreases as temperature and urea concentration increase. However, this is offset by the proportionately higher values of C under the same conditions. The net result is that the maximum error in assuming $\Lambda \approx \Lambda_0$ occurs at the limit of the study involving the 8 M urea solution at 45°. Since the error at that point was calculated to be less than 4%, and because the uncertainty in Λ is lower as the time interval, temperature, and urea concentration are decreased, the assumption was thought to be reasonable. The alternative was to treat Λ as a function of time. However, it was felt that neither the conditions nor the objectives of the investigation warranted this step.

On the basis of these two assumptions, therefore, for a solution of given urea concentration and temperature,

$$dC/dt = (1000/\Lambda_0)\ (dK/dt) \qquad \text{(Eq. 9)}$$

where Λ_0 is the appropriate limiting equivalent conductance given in Table III, and dK/dt is the first derivative of Eq. 2.

Combining Eqs. 4, 5, and 9 yields the expression:

$$dK/dt = \frac{k_1\Lambda_0 M}{1000} - \frac{k_2 1000}{\Lambda_0} K^2 \qquad \text{(Eq. 10)}$$

which predicts a plot of dK/dt *versus* K^2 to be linear, with a slope equal to $-k_2 1000/\Lambda_0$, and an intercept of $k_1 \Lambda_0 M/1000$. While Λ_0 and M will vary from one urea solution to the next, k_1 and k_2 should be constant at any one temperature. Seven values for dK/dt and K^2 were determined at corresponding time intervals for each system studied, using Eq. 3 and the appropriate coefficients in Table II. Each set of dK/dt and K^2 values was then subjected to a linear regression analysis to evaluate the respective slopes and intercepts predicted by Eq. 10. Subsequently, k_1 and k_2 values were determined using the appropriate M and Λ_0. The results are shown in Table IV.

Actual plots of dK/dt *versus* K^2 displayed some scatter. The average deviation between the calculated dK/dt and those predicted by the equations representing the regression analyses ranged from 1 to 4% of the corresponding dK/dt. Because the points were scattered, in contrast to a smooth deviation from linearity, a treatment of Λ as a function of time would seem to offer little in the way of enhanced precision. As might be expected, however, the percent average deviation was observed to increase with urea concentration and temperature.

Table IV—Rate Constants for the Reaction: $(NH_2)_2CO \underset{k_2}{\overset{k_1}{\rightleftharpoons}} NH_4^+ + CNO^-$

Temperature	[Urea], M		$k_1 \times 10^5$, hr.$^{-1}$	k_2, 1 mole^{-1} hr.$^{-1}$
25.0°	2.00		0.298	—
	4.00		0.302	—
	6.00		0.303	—
	8.00		0.292	—
		Av.	0.299 ± 0.004	(0.200)[a]
35.0°	2.00		1.53	0.71
	4.00		1.53	0.73
	6.00		1.83	1.02
	8.00		1.81	0.83
		Av.	1.68 ± 0.14	0.82 ± 0.10
45.0°	2.00		7.92	1.98
	4.00		8.88	1.94
	6.00		7.37	1.92
	8.00		8.00	2.32
		Av.	8.04 ± 0.42	2.04 ± 0.14

[a] Estimated from Fig. 3.

In general, the precision in k_1 is considered to be less as the temperature increases, *i.e.*, as the plots of K *versus* t become more curvilinear. The opposite is true for k_2, since the reverse reaction is more pronounced at the higher temperatures. In the latter regard, a plot of dK/dt *versus* K^2 for the data at 25° has no noticeable slope. The value for k_2 given in Table IV was estimated from Fig. 3.

Figures 2 and 3 compare the rate constants obtained in this investigation with those observed by other workers who studied the forward (9) and the reverse (18) reactions separately and in relatively dilute solutions. In view of the approximations used in the present study, the agreement is excellent, suggesting that the mechanisms involved in the respective reactions are independent of the urea concentration.

CONCLUSIONS

The results of this investigation appear to substantiate the proposal by Schwartz and Nelson (10) that the degradation of urea in concentrated solutions is a reversible reaction. Accordingly, it is to be expected that the overall process will reach an equilibrium at a point in time that is dependent upon the temperature and urea concentration. The indications are that the amount of contaminant produced, presumably in the form of ammonium cyanate, is negligible for many current applications of concentrated urea solutions. Furthermore, unless the experiment demands, and unless the experiment is of relatively short duration, it is pointless to strive for ultrapure urea to be used in preparing concentrated solutions. The observations made in this study show that at 25, 35, and 45°, it would take but 23, 2.5, and 1 hr., respectively, for a solution of ultrapure urea to reach the same specific conductance level as the solutions prepared from reagent grade unrecrystallized urea.

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Combined GLC and High-Resolution Mass Spectroscopic Analysis of Diphenylhydantoin

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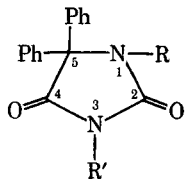
Abstract □ A sensitive GLC method for the analysis of diphenylhydantoin in biological material is described. This method involves the conversion of diphenylhydantoin to its methyl derivative. The identity of the methyl derivative was determined by combined GLC–mass spectroscopy. The high-resolution mass spectra of diphenylhydantoin methyl derivatives indicate that the methyl groups were introduced at the nitrogen atoms of the hydantoin ring to produce 3-methyl- and 1,3-dimethyldiphenylhydantoin.

Keyphrases □ Diphenylhydantoin and methyl derivatives, analysis in biological material—GLC and mass spectroscopy □ GLC—analysis, diphenylhydantoin and methyl derivatives □ Mass spectroscopy—analysis, diphenylhydantoin and methyl derivatives

Several spectrophotometric, colorimetric, and UV methods to determine levels of diphenylhydantoin (I) in biological material were published (1–5). However, most of these methods are nonspecific and time consuming, and they require many selective extractions to avoid interference of other drugs, *e.g.*, barbiturates. Recently, a GLC method was reported (6) which involved the conversion of diphenylhydantoin to its methoxy derivative by treatment with diazomethane. However, the structure of the methylated derivative has not been determined. In a recent report (7), a direct GLC method was described for the determination of the drug at therapeutic levels in blood. Another recent report (8) described a GLC method for the determination of diphenylhydantoin in which the drug was methylated with tetramethylammonium hydroxide. The corresponding peak of the methylated drug was identified by NMR and mass spectroscopy. However, no discussion of the mass spectra was included.

This report describes a more sensitive GLC method which measures even subtherapeutic levels of the drug in biological material. However, when using GLC for qualitative determination of drugs in biological material, it is necessary to have parameters other than the retention time of the drug in order to make a more certain identification of the drug. This is especially true if derivatives of the drugs are being analyzed when more than one reactive center is available in the drug molecule. Mass spectroscopy, therefore, was utilized as a tool for identification of diphenylhydantoin derivatives.



I: R = R′ = H; diphenylhydantoin
II: R = H, R′ = CH_3; 3-methyldiphenylhydantoin
III: R = R′ = CH_3; 1,3-dimethyldiphenylhydantoin

EXPERIMENTAL

Reagents—Diphenylhydantoin[1] and dimethyl sulfate[2] were used. Heptane and chloroform were redistilled before use. Acetate buffer (0.2 *M*, pH 5.6) was made by mixing 4.8 ml. of acetic acid solution (0.2 *M*, 11.55 ml. in 1000 ml. water) and 45.2 ml. of sodium acetate solution (0.2 *M*, 16.4 g. in 1000 ml. water), and the mixture was diluted with water to 100 ml. Methanolic potassium carbonate solution was made by mixing 1 ml. of 2–5% aqueous solution of potassium carbonate and 9 ml. of methanol (analytical reagent).

Preparation of Methyl Derivatives of Diphenylhydantoin—*Monomethyl Derivative*—A solution of 1.0 g. diphenylhydantoin in 30 ml. methanolic potassium carbonate was placed in a three-necked, 150-ml. flask fitted with a reflux condenser and a magnetic stirrer. Twenty milliliters of dimethyl sulfate was added, and the reaction was allowed to proceed for 15 min. at 70°. The reaction mixture was then cooled, and methanol was removed under reduced pres-

[1] Parke-Davis and Co., Detroit, Mich.
[2] Matheson Coleman and Bell, Norwood, Ohio.