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THE SOLUBILITY OF SEVERAL BARBITURIC
ACID DERIVATIVES IN
HYDROALCOHOLIC MIXTURES

BY

THOMAS L. BREON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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UNIVERSITY OF RHODE ISLAND

1969

TITLE ABSTRACT

SOLUBILITY OF SEVERAL BARBITURATES

-MASTER OF SCIENCE THESIS

OF

THOMAS L. BREON

Approved:

Thesis Committee:

Chairman _____

Dean of the Graduate School _____

UNIVERSITY OF RHODE ISLAND

1969

ABSTRACT

The solubility profiles for metharbital, butabarbital, thiopental and thiamylal were determined in ethanol-water solvent systems at 25°C, in an attempt to define an approximate correlation between therapeutic activity and various solubility relationships. The relative lipophilicity of a drug molecule is an important factor in the physical and chemical processes involved when the therapeutic agent is introduced into a biological system. Binary solvent mixtures aid in delineating the relative polarity, in terms of dielectric constants, of these drugs. Spectrophotometric and, where applicable, gravimetric analyses, were utilized to determine the concentration of drug, in mg./ml., in the 41 solvent systems ranging in composition from pure ethanol to pure water. The dielectric requirement (DR) of the barbiturates investigated, and those reported for barbital and vinbarbital, illustrated an approximate inverse relationship with the number of carbon atoms in the molecule. A similar correlation was found with the solubilities in pure water, and the ratios of the solubilities in ethanol and at the DR

to the solubility in water. The therapeutic indices of duration of action and the period of time involved between administration of the drug and the time when the activity is first manifested, increased as the relative polarity of these barbiturates declined. The duration of these pharmacological parameters were also found to increase with a corresponding reduction in the hydrophilic nature of this series of compounds.

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I. INTRODUCTION

The pharmacological action exerted by a drug molecule in contact with a biological system is the net result of the interactions and extent of interactions with the complex biological environment. The degree as well as the rate of interaction is governed by many parameters, many of which depend on the physical and chemical properties associated with the drug molecule.

To be physiologically active, a drug must be absorbed and distributed throughout the biological fluids. More specifically, it is noted that these actions occur on a molecular level and under these conditions it would be assumed that solution properties and characteristics are operative. Many biologically active substances are weak electrolytes and properties such as the pH of the medium, pKa of the drug, concentration gradients, surface tension and the aqueous and lipid solubilities of the various species contribute to the overall extent of activity.

The biologically active species, in order to initiate a response, would be presumed to have interacted with

cellular constituents and this process is involved with diffusion and permeability as well as those factors previously discussed.

Ordinarily, a uniquely implied or definitive relationship is not found between families or classes of drugs relative to the degree of action and a given physical property. Thus, this study is an initial investigation into the possible approximate correlation between solubility characteristics of several barbiturates and therapeutic activity.

It would be judicious if a homologous series of compounds, such as the barbituric acid derivatives, were studied with respect to the magnitudes of solubility in binary mixtures of ethanol and water of varying polarities.

These binary systems would serve as a physical model insofar as a smooth gradation of polarity is achieved and would aid in delineating and interpreting the lipophilicity of the drug moieties. The polarity of these binary mixtures has been measured and may be interpreted on a physical chemical basis by the dielectric constant. It may be possible that the relative magnitude, position and nature of the solubility curves would be an index to the degree of activity.

II. PAST WORK

Modification of basic chemical moieties showing therapeutic activity by molecular alteration such as the preparation of derivatives is a well established procedure.

This process of improving the activity of established drugs can be found with many classes of useful compounds such as the sympathomimetic amines (1), morphine analogs (2), and steroidal modifications (3). The existence of many useful and therapeutically efficient barbituric acid derivatives certainly attests to the success of this approach.

The barbiturates have been studied extensively relative to their physical properties (4, 5, 6, 7) and pharmacological actions. Tatum (8) discusses the structural activity relationship necessary to elicit a therapeutic response for these substances. He also devised a therapeutic scheme which classifies the numerous derivatives into four groups according to their duration of action: long, intermediate, short and ultrashort. The fact that the duration of action of a few members may fall on the boarder line be-

tween classes or that a compound may be included in two different classes by different investigators has also been considered.

Various studies, which may be found in the literature, present data on the induction time, duration, minimal lethal dose (MLD), 50% lethal dose (LD_{50}), 50% anesthetic dose (AD_{50}) and various other pharmacological parameters such as the effect of various routes of administration (9, 10, 11, 12, 13, 14). It is generally accepted that intravenous administration provides a rapid distribution of the drug to the sites of action as well as to other body depots. Conversely, intramuscular injection allows the drug to be slowly distributed due to the poor diffusion of blood through the muscle tissue. Oral administration provides some difficulties in quantitating therapeutic activity due to the numerous variables involved such as absorption and degradation rates, as well as the total amount absorbed.

A number of investigations have also been published relating physicochemical properties to the therapeutic action of narcotic agents. John Falk (15) points out that as early as 1899, Hans Meyer had postulated the efficiency of hypnotic agents to be "dependent upon their mechanical affinity for lipid substances on the one hand and for the remaining body

constituents, i.e., principally water, on the other hand." Thus, a correlation between partition coefficients and narcotic activity had been recognized. Among the works which support this theory are the works of Sabalitschka and Tiety (16). They observed the partition coefficients for a series of p-hydroxybenzoic acid esters to parallel the bactericidal activity. The activity was found to increase with an increase in lipid/water partition coefficient.

Although being one of the earliest reports recognizing the importance of physical parameters in drug action, Meyer's hypothesis suffered several shortcomings. This theory fails to explain how the depressant actually exerts its action nor why other substances which can also penetrate nerve cells do not exert a similar action. In addition it fails to account for certain hydrophilic substances which possess depressant properties, nor does it explain the varied depressant action of equally lipophilic compounds.

Later Ferguson (17) published his investigation dealing more extensively with the physical and chemical properties involved in narcosis. According to this theory, known as the Ferguson Principle, toxic action may be divided into two distinct classes: physical and chemical or as they have come to be known, structurally non-specific and struc-

turally specific. It has been observed that many diverse chemical compounds show narcotic action. This is indicative that mainly physical rather than chemical properties are involved.

Narcotic action is attained rapidly and remains at the same level as long as a reservoir or critical concentration of the drug is maintained, but rapidly disappears when the supply of drug is withdrawn. This suggests that an equilibrium exists between the external phase and the biophase, i.e., the phase at the site of action.

Rather than using concentrations, Ferguson chose chemical potentials as a parameter. Hence, if a true equilibrium exists between the phases involved, the chemical potential of the narcotic substance must be equal in both phases.

Ferguson further showed that the partition coefficients, vapor pressure of narcotics in solution, surface tension and various other solubility relationships of narcotics are all derivable in principle from the thermodynamic activity. Each of these relationships are dependent upon a distribution between dissimilar phases and consequently involve a coefficient of distribution. The log of this constant, according to Ferguson, is derived in part from the

difference in the partial molal free energies of the substance in its standard state in the dual phase systems.

Linus Pauling (18) has advanced a unique theory concerning the production of anesthesia by many diversified types of compounds. His concept is based on the formation of minute hydrated crystals which occlude anesthetic agents and proteins in the encephalic fluid of the brain. These crystals interfere with the normal electrical impulses by increasing the impedance of the neural network and restrict the electrical activity of the brain to that characteristic of anesthesia and unconsciousness. Hence, this theory not only encompasses lipophilic agents, such as Meyer's and Ferguson's, but also aids in explaining the narcotizing effect of many hydrophilic compounds as well as inert substances such as the rare gases.

Further considerations in the area of physical mechanisms involved in narcosis may be found in the excellent reviews by Mullins (19) and Daniels (20).

Evidence of the value of utilizing physical parameters in a discussion of drug action is found in the voluminous information published in the discipline of biopharmaceutics. Many excellent review articles concerned with this subject are readily available (21, 22, 23, 24).

When evaluating drug action from a biopharmaceutical stand point, one must consider a host of various processes. Drug dissolution, diffusion, absorption, transport, serum and tissue binding, distribution, adsorption onto and penetration into the cell, metabolism and excretion are all facets which govern the overall effect a drug produces on a biological system.

Factors such as the surface tension of the medium (25) and the relative polarity of the drug (26, 27) as well as the pH of the medium and the pKa of the drug (28), in the case of weak electrolytes, influence these processes. Many studies have been undertaken concerning these parameters (29, 30, 31, 32).

Studies of certain biopharmaceutical parameters utilizing barbituric acid derivatives have also been reported. Levy, et al. (33), have studied the absorption of secobarbital in goldfish. Inclusion of the surfactant polysorbate 80 into the aqueous environment of the fish enhanced the rate of absorption of the drug significantly. The effect of other surfactants on the rectal absorption in rabbits of several barbiturates has been studied by Fincher, et al. (34). In some bases the absorption was found to be enhanced while in others binding of the drug in the base was suspected.

Several reports included in a series by Kakemi, et al., on absorption and excretion of drugs are concerned with barbituric acid derivatives. In an investigation of the absorption of these compounds from the rat's stomach (35), the absorption rate was found to be influenced by the pH of the medium and was directly correlated with the partition coefficient of the drug. In a second study involving the rat's small intestine (36), the pH-partition hypothesis was found to be only partially operative and a correlation between absorption rates and partition coefficients was not as well defined as with the stomach. The actual mechanism of absorption was not elucidated but the authors speculated binding of the drug to the mucosal membrane to cause this aberrant behavior.

It is ordinarily inadvisable to consider the effect of a drug by examining only a few of these biopharmaceutical parameters and neglecting other possible contributory factors. The net result of the complex interaction among these processes dictates the pharmacological response. However, for the sake of simplicity, mathematical models of each are usually studied independently (37, 38, 39, 40) and the resulting data collated into a tentative quantitative conclusion.

Of interest is the work of Bischoff and Dedrick (41) on the pharmaco-kinetics of thiopental. These authors have developed a complex model which describes the distribution of thiopental in four types of body tissue. Such factors as lipid solubility, flow limitations, protein binding and metabolism were taken into account in the construction of the model. The calculated concentration of drug in certain tissues at a specified time after administration was found to be in close agreement with existing experimental data.

The importance of solubility studies has been implicitly noted in the previous discussion especially with regard to relative magnitudes in various solvent systems. The extent of interaction of a drug with a given semipolar environment, i.e., body fluid, cellular membranes or cellular fluids would be an index to pharmacological activity. Modification of a basic structure, such as barbituric acid, with various substituents studied in this fashion may aid in delineating a relative scale of activity.

The solubility of non-polar organic moieties in non-polar liquids has been quantified by Hildebrand and Scott (42). An analytic expression for the calculation of ideal solubility has been derived from various thermodynamic constants.

An index to the polarity of solvents was also considered by Hildebrand and Scotchard (42) in the concept of cohesive energy density or solubility parameters. A high value of the solubility parameters (δ) meant high polarity, i.e., δ of water = 24.3 whereas a low value indicated very low polarity, i.e., δ of hexane = 7.3.

Although ideal solubilities do not depend on solubility parameters, non-ideal perturbations depend strongly on solvent polarity. Thus, the non-ideal solubility found for drug moieties depends on the nature of the environment in the biological fluid.

Paruta, et al. (44), has recognized that an empirical relationship exists between the solubility parameters and the dielectric constant of many solvents. Utilization of binary solvents of various percentage strengths enables one to construct a system of solvents with dielectric constants ranging between the values of the pure solvents. By determining the solubility of a compound in mixtures, as well as in each of the pure solvents, a solubility profile is produced possessing a smooth function of solubility with incremental values of dielectric constants. Typical solutes exhibit one or more solubility peaks corresponding to the dielectric requirement (DR) (45) of the drug molecule.

This approach, utilizing binary solvent systems, not only allows the use of dielectric values as a polarity indicator, but it also provides an expanded scale.

It has been reported that other factors in addition to dielectric constants affect solubility. Gorman and Hall, (46), in an attempt to correlate dielectric constants with solubility parameters, constructed a linear plot of the Hildebrand expression predicting the solubility of a solute in a solvent of specified solubility parameter. This was repeated substituting dielectric constants for solubility parameters and poor correlation resulted. When the solvents were restricted to those exhibiting similar bonding characteristics, such as a homologous chemical series, the correlation was greatly improved. Further restrictions on the types of chemical interaction involved were imposed by utilization of several blends of two solvents. The resulting plot illustrated a linear relationship.

Willis Moore (47, 48) has proposed the use of solvent blends to construct solvent systems of predetermined dielectric constant values. The basic premise of this author's work is that the solvent systems employed are ideal in behavior and the dielectric constant of the solvent blend is directly related to the concentration of the individual com-

ponents. However, most solvent systems of pharmaceutical interest exhibit the properties of non-ideal or regular solutions and this linear relationship would not be operative. Examination of the dielectric constants of various solvent blends determined by Akerlof (49) and Critchfield et al. (50), illustrate the linear correlation to exist only for certain solvent blends, e.g., acetone-water.

Moore also advocates the use of V/V or W/V percentage units in constructing binary solvent mixtures. Sorby et al. (51), however, has found better results in a comparison of experimental and theoretical dielectric constants if a W/W percent is used. Since a system based on weights, rather than volume, connotes number of molecules, this approach seems more applicable.

The dielectric concept has been utilized to construct solubility profiles for several pharmaceuticals in binary solvent systems (52, 53, 54, 55).

Reber and Pathamanon (56) and Paruta (57) have studied vinbarbital and barbital respectively in hydroalcoholic mixtures and their data will be discussed below.

Reports concerned with the identification of barbituric acid derivatives are abundant in the literature (58, 59, 60, 61). Many quantitative procedures for these compounds in the

pure state and in various physiological media have also been reported (62, 63, 64). The investigation most pertinent to this study is that by Stuckey (65). His work with barbital and phenobarbital points out that the ultraviolet absorption spectra of these compounds in acidic media is due to the relatively weak carbonyl chromophores. In a basic environment, however, enol tautomerism occurs producing an olefinic linkage with a correspondingly higher extinction coefficient.

III. EXPERIMENTAL

Materials.--The materials used in this study were as follows: barbituric acid¹, m.p. 252-55°C; metharbital (Gemonil)², m.p. 151-55°C; butabarbital³, m.p. 166-68°C; and thiamylal (Surital)⁴, m.p. 133-35°C. Thiopental was prepared from thiopental sodium (Pentothal Sodium)⁵. The sodium salt was dissolved in a quantity of distilled water and the free acid precipitated by the addition of 1.0 M hydrochloric acid⁶ solution. The slurry was filtered and washed with three portions of distilled water. The melting point range of the dried precipitate was 156-58°C. Melting points of pooled

¹Aldrich Chemical Company, Milwaukee, Wisconsin, lot 072281

²Abbott Laboratories, North Chicago, Illinois, lot 685-7608

³McNeil Laboratories, Fort Washington, Pennsylvania, lot 5086

⁴Parke, Davis and Company, Detroit, Michigan, lot 405838

⁵Abbott Laboratories, North Chicago, Illinois, lot 780-7657

⁶Mallinckrodt Chemical Works, New York, New York

and dried samples from the gravimetric procedure were also made and found not to vary more than $\pm 2^{\circ}\text{C}$ outside the range of the original material and none had a range in excess of 4°C . This was done to ascertain if any aberrant behavior such as hydrate formation or crystalline modification occurred in these binary solvent mixtures.

Hydroalcoholic solvents were prepared volumetrically by the use of burettes, previously determined densities for absolute ethyl alcohol¹ and distilled water at ambient room temperature. These mixtures ranged from 0.0 to 100.0% W/W distilled water in 2.5% increments and represent a polarity range in terms of dielectric constant values of 24.3 to 78.5.

A pH 10.7 buffer was prepared with seventy-one grams of anhydrous sodium dibasic phosphate² (reagent grade) dissolved in 1000 milliliters of distilled water and adjusted to pH 10.7 with 1.0 M sodium hydroxide³ solution.

Equipment.--A rotating apparatus was constructed which held forty-eight screw capped glass vials of twenty-one milliliter

¹U.S. Industrial Chemicals Company, New York, New York

²Fisher Scientific Company, Fair Lawn, New Jersey

³Mallinckrodt Chemical Works, New York, New York

volume and revolved at thirty-two revolutions per minute. The vials were rotated in such a way that the solute was caused to traverse the full length of the vial twice per revolution, thus causing sufficient agitation of the contents. No caking was observed in any of the samples. This apparatus was immersed in a ten gallon water bath maintained at $25.0 \pm 0.3^{\circ}\text{C}$ by a Tecan Tempunit¹.

A Cary Model 16 Spectrophotometer², Mettler type H6T³ analytical balance, a Leeds Northup Model 7401 pH meter⁴, and a Sorvall Model GLC-1⁵ centrifuge were utilized in the assay procedure. Computational treatment of the data was aided through utilization of an IBM System/360 Model 50 digital computer⁶.

Dissolution Procedures.--Hydroalcoholic solvents in ten to twenty milliliter volumes were placed in the vials along with an excess of drug. These were rotated in the water bath for twenty-four hours, a period found to be adequate for equi-

¹Techne (Cambridge) Limited, Cambridge, England

²Cary Instruments, Belmont, Massachusetts

³Mettler Instrument Corporation, Hightstown, New Jersey

⁴Leeds and Northup Company, Philadelphia, Pennsylvania

⁵Sorvall, Newton, Connecticut

⁶International Business Machines, Armonk, New York

libration. Sample aliquots were removed through pipets tipped with glass wool while the vials remained in the water bath to insure continued temperature equilibrium. Due to the very fine nature of the suspended material, it was necessary to centrifuge the thiopental and thiamylal solutions before the samples were withdrawn from the supernatant and a negligible change in temperature was found to occur during this operation.

Where the magnitude of solubility permitted, a spectrophotometric and a gravimetric assay was made. Where the solubility was expected to be of low order from the nature of the asymptotic portion of the solubility curve, dual spectrophotometric determinations were utilized. Each solubility curve represents the average values from at least three runs of the forty-one solvent systems covering the total variation in solvent composition.

Assay Procedure.--Ultraviolet spectra of each of the compounds used were determined with an aqueous dilution of the drug. All solutions in this and subsequent spectrophotometric analyses were buffered to a pH of 10.7. An appropriate peak in the spectra was chosen as the wavelength to use in the assay. These wavelengths are tabulated in Table I. Absorbancies of a minimum of seven dilutions for each drug

TABLE I.--A SUMMARY OF THE ULTRAVIOLET ABSORPTION
MAXIMA IN MILLIMICRONS (m μ), DETERMINED
SPECTROPHOTOMETRICALLY COMPARED
WITH LITERATURE VALUES

Derivative	Observed Maxima m μ	Literature Maxima m μ	Reference
Metharbital	245	244	(66)
Butabarbital	240	240	(5)
Thiopental	255	255	(66)
Thiamylal	256	-	-
Barbituric Acid	257	-	-

were determined and a plot of absorbance versus concentration constructed. A linear relationship was found to exist indicating the Beer-Lambert relationship operative in all cases, within the concentration ranges studied. Concentrations of all subsequent dilutions subjected to spectrophotometric analysis were maintained within the appropriate concentration limits. The absorptivities were calculated as the slopes of these lines by the method of least squares.

Gravimetric assays were accomplished by pipetting a specified volume of supernatant into a preweighed glass vial and drying to a constant weight.

IV. DISCUSSION

In the present study a series of barbituric acid derivatives were studied relative to their solubility behavior in ethanol-water mixtures. The derivatives studied represent a range of therapeutic action from ultrashort duration and onset of activity to those possessing a long duration and a long period of latency before the pharmacological activity is manifested. Table II illustrates the basic chemical moiety of the barbiturates and also lists the derivatives utilized in this study along with their substituent chemical groups corresponding to the R_1 , R_2 , R_3 and R_4 positions of the parent molecule.

It can be seen from this table that the side chains occupying the R_1 and R_2 positions show a general progressive increase in the number of carbon atoms as the series is descended. Hydrogen atoms occupy the R_3 position in every case with the exception of metharbital which has a methyl group bonded to the nitrogen atom. A sulfur atom is present at the R_4 position of the thiamylal and thiopental molecule and in the remainder of this series and oxygen atom occupies this position.

TABLE II.--A SUMMARY OF THE SUBSTITUENTS FOUND
IN THE NOTED POSITIONS FOR THE BARBITURIC
ACID DERIVATIVES USED IN THIS STUDY

Derivative	R ₁	R ₂	R ₃	R ₄
Barbituric Acid	H	H	H	O
Barbital	-CH ₂ CH ₃	-CH ₂ CH ₃	H	O
Metharbital	-CH ₂ CH ₃	-CH ₂ CH ₃	-CH ₃	O
Butabarbital	-CH ₂ CH ₃	-CHCH ₂ CH ₃ CH ₃	H	O
Vinbarbital	-CH ₂ CH ₃	-C=CHCH ₂ CH ₃ CH ₃	H	O
Thiopental	-CH ₂ CH ₃	-CHCH ₂ CH ₂ CH ₃ CH ₃	H	S
Thiamylal	-CH ₂ CH=CH ₂	-CHCH ₂ CH ₂ CH ₃ CH ₃	H	S

It is assumed that any change in the physical properties among these derivatives, such as the solubility in hydroalcoholic mixtures, is caused by the interaction of the various substituent groups with the molecular structure of the parent moiety.

The data obtained during this study proved to be voluminous. To facilitate its handling, a digital computer was employed in the majority of the calculations involved. A program was written utilizing the statistical method of least squares in calculating the Beer-Lambert relationship of spectrophotometric absorbance versus concentration (Appendix A). A second program was constructed which yielded the solubilities of the various derivatives in each of the solvents employed. Given the absorptivity of a particular compound, the absorbance obtained from a dilution of the solvent saturated with the drug and the appropriate dilution factors, the solubility in mg./ml. was calculated (Appendix B). Lastly, a program was utilized which calculated the average solubility and its standard deviation for a particular barbiturate in a specified solvent mixture given the solubilities obtained from the individual spectrophotometric and gravimetric assays (Appendix C).

In the foregoing discussion of the data obtained in

this study, the solubilities are presented in both tabulated and graphical form. For the purpose of illustration and clarity, the solubility axis of the plots has been expanded as much as possible. Therefore, when comparing the various curves, it is essential that the differences in the scales be noted. The various derivatives are discussed in the order of their pharmacological action, beginning with barbital, a long acting drug with a long onset of activity, and ending with thiamylal which possesses a relatively brief duration of action and a short period of onset.

The solubility of barbital in ethanol-water systems has been determined by Paruta (57) and is included in this discussion for comparative purposes. The solubility of this compound, as a function of W/W percent water and dielectric constant, is presented in Table III. Values for the dielectric constants of the ethanol-water mixtures, in this and all subsequent tables, are those determined by Paruta (57) at 25°C. The values compare favorably with those obtained by Akerlof (49).

A graphical illustration of this data is shown in Figure 1, in which the solubility is plotted in mg./ml. as a function of percent water by weight at 25°C. A smooth curve is obtained with a peak solubility at 15.0% W/W water

TABLE III.--A SUMMARY OF THE SOLUBILITY OF BARBITAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, AS A FUNCTION OF W/W PERCENT WATER AND DIELECTRIC CONSTANT(57)

W/W PERCENT WATER	DIELECTRIC CONSTANT(ϵ)	SOLUBILITY IN MG./ML.
0.0	24.3	92.3
2.5	25.5	98.6
5.0	26.5	103.1
7.5	27.6	110.0
10.0	29.0	113.3
12.5	29.7	118.3
15.0	30.6	120.7
17.5	31.5	117.2
20.0	32.7	112.5
22.5	33.8	107.7
25.0	34.7	100.1
27.5	36.4	94.3
30.0	37.5	90.2
32.5	38.6	85.1
35.0	39.8	80.8
37.5	41.3	75.6
40.0	42.8	70.0
42.5	44.2	66.3
45.0	45.7	60.2
47.5	47.4	56.5
50.0	49.0	51.6
52.5	50.5	47.7
55.0	52.0	43.1
57.5	53.6	39.2
60.0	55.4	34.1
62.5	57.0	30.6
65.0	58.4	28.3
67.5	60.0	24.1
70.0	61.7	20.9
72.5	63.3	17.1
75.0	64.5	15.6
77.5	66.1	14.2
80.0	67.5	13.3
82.5	68.9	12.5
85.0	70.2	11.1
87.5	71.7	10.1
90.0	73.2	9.0
92.5	74.5	8.0
95.0	75.7	7.5
97.5	77.1	7.4
100.0	78.5	7.3

Figure 1.--The solubility of Barbitol at 25°C in mg./ml. as a function of composition (w/w) for ethanol-water mixtures (57).

representing a dielectric requirement (DR) of about 30.6. It will also be seen that the solubility at the DR is quite high relative to the other barbituric acid derivatives. The solubility at this point on the curve is 120.7 mg./ml. A solubility of 7.3 mg./ml. was obtained in pure water which is also relatively large.

Further, it is interesting to note that a fair degree of linearity relative to the solubility and solvent composition exists over a range of 27.5 to 65.0% water by weight. One might assume that this is the partical operative pharmaceutical range of dielectric constants. The slope of this linear portion of the curve is calculated to be -1.8 mg./percent water by weight.

This behavior is notable in counterdistinction to the co-solvency effect noted over the general area of the solubility isotherm, wherein a portion of the solubility profile is a predictable value once the appropriate slope is calculated.

A tabulation of the data representing the solubility of metharbital in ethanol-water solvents is presented in Table IV and the solubility profile illustrated in Figure 2, in the manner previously described. It is noted from this figure that the addition of a methyl group to the R_3 position

TABLE IV.--A SUMMARY OF THE SOLUBILITY OF METHARBITAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, AS A FUNCTION OF W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ϵ)	SOLUBILITY IN MG./ML.	STD. DEVIATION IN MG./ML.
0.0	24.3	41.9	0.50
2.5	25.5	43.7	0.73
5.0	26.5	46.1	0.58
7.5	27.6	47.9	0.96
10.0	29.0	50.0	0.60
12.5	29.7	50.7	0.64
15.0	30.6	51.2	0.68
17.5	31.5	50.9	0.71
20.0	32.7	50.3	0.77
22.5	33.8	49.3	0.33
25.0	34.7	48.0	0.57
27.5	36.4	46.0	0.96
30.0	37.5	44.7	1.55
32.5	38.6	43.4	1.87
35.0	39.8	39.0	0.50
37.5	41.3	36.6	0.46
40.0	42.8	36.2	2.63
42.5	44.2	33.4	2.16
45.0	45.7	28.9	0.63
47.5	47.4	26.2	0.38
50.0	49.0	23.5	0.73
52.5	50.5	21.2	0.54
55.0	52.0	18.7	0.47
57.5	53.6	16.2	0.43
60.0	55.4	14.0	0.29
62.5	57.0	12.1	0.31
65.0	58.4	10.2	0.40
67.5	60.0	8.61	0.219
70.0	61.7	7.39	0.151
72.5	63.3	6.28	0.191
75.0	64.5	5.29	0.216
77.5	66.1	4.50	0.220
80.0	67.5	3.95	0.165
82.5	68.9	3.51	0.173
85.0	70.2	3.20	0.167
87.5	71.7	2.87	0.161
90.0	73.2	2.68	0.115
92.5	74.5	2.48	0.105
95.0	75.7	2.32	0.139
97.5	77.1	2.17	0.127
100.0	78.5	2.00	0.084

Figure 2.--The solubility of Metharbital at 25°C in mg./ml. as a function of composition (w/w) for ethanol-water mixtures.

of the molecule dramatically reduces the maximum solubility as compared to barbital with a hydrogen at this position. A DR is observed at 30.6 and a solubility at this point of 51.2 mg./ml. It is also seen that a shouldering effect, unique to this derivative, is observed in the range of 41.3 to 44.2 in terms of dielectric constants. Excluding this plateau, an area of approximate linearity exists in the range of solvent composition of 30.0 to 62.5% water by weight, representing a rate of change in solubility of -1.0 mg./percent W/W water. The pharmacological activity of this derivative is also unusual in that it not only produces sedation, but possesses anticonvulsant properties.

A brief consideration of the chemical structure of the metharbital molecule will also yield some unusual characteristics relative to the non-methylated analogs. It has been reported (65) that the barbituric acid derivatives undergo enol-keto tautomerism. Molecules devoid of alkyl substituents on the nitrogen atoms can provide a maximum of two (N-H) hydrogen atoms which would be available for contribution to mono- and dienolized structures with the three neighboring carbonyl groups. Figure 3a illustrates the three possible dienolized structures as well as the keto form. It is noted from this illustration that the carbonyl group at carbon 2

3a.--Derivatives devoid of N-alkyl substituents showing only the dienol combinations

3b.--N-alkyl derivatives showing all possible combinations of enol species

Figure 3.--Enol-keto tautomerism of the barbituric acid derivatives

has twice the number of chances of becoming enolized as those at carbons 4 or 6, due to its vicinal position to both nitrogen atoms. Metharbital, on the other hand, has only one (N-H) hydrogen available. With the methyl group on the nitrogen at position 3, only two possible monoenolized species can form as shown in Figure 3b. Other effects being equal, the chances of the carbonyl group at position 2 or 6 being converted to the enol form are equal.

Tautomerism is not a static situation where tautomeric species of molecules exist in only keto or in enol forms, but rather a dynamic equilibrium where active hydrogen atoms are rapidly interchanging between the various species.

It may be possible that the limitations imposed on the tautomerism of the metharbital molecule by the N-methyl group could cause the shouldering effect on the solubility profile. A second possibility for this unusual behavior for a barbiturate derivative which may be proposed is that the polarity of the various tautomeric forms is different. The assay procedure, not being specific for any particular species of this molecule, would detect the cumulative solubility of all the various species present.

Neither of these proposals are to be interpreted as factual evidence of the particular events occurring, but

are included here only as interesting possibilities which may aid in elucidating the underlying cause of the unusual solubility profile for metharbital.

The solubility data for butabarbital is found in Table V and Figure 4 represents the isothermal data graphically as solubility in mg./ml. as a function of solvent composition. This molecule is identical to barbital with the exception of the ethyl group on the R_2 position which is replaced with a sec-butyl group. The addition of these two carbon atoms decreased the solubility over the entire range of the solvent composition. A maximum solubility of 90.6 mg./ml. is noted to occur at 12.5% W/W water representing a DR of 29.7. Again a relatively linear section of the curve is observed, this in the range of 27.5 to 55.0% water by weight. The slope calculated for this portion of the plot is -1.9 mg./percent W/W water. It is also seen that the solubility in pure water is 0.86 mg./ml., less than one half of metharbital and almost one tenth that of barbital.

Reber and Pathamanon (56) have determined the solubility of vinbarbital in ethanol-water mixtures. A tabulation of their data is found in Table VI and plotted in Figure 5, as described previously. This particular derivative has a fifth carbon atom and an olefinic bond added to the substituent at the R_2 position of the butabarbital molecule.

TABLE V.--A SUMMARY OF THE SOLUBILITY OF BUTABARBITAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C. AS A FUNCTION OF W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	SOLUBILITY IN MG./ML.	STD. DEVIATION IN MG./ML.
0.0	24.3	84.0	0.76
2.5	25.5	85.9	0.39
5.0	26.5	87.9	0.60
7.5	27.6	89.3	1.11
10.0	29.0	90.1	1.54
12.5	29.7	90.6	1.37
15.0	30.6	89.6	1.19
17.5	31.5	88.5	0.47
20.0	32.7	85.9	0.87
22.5	33.8	82.6	1.17
25.0	34.7	79.2	0.82
27.5	36.4	73.4	0.78
30.0	37.5	68.6	0.65
32.5	38.6	63.6	0.65
35.0	39.8	58.6	0.90
37.5	41.3	53.7	0.49
40.0	42.8	48.2	0.70
42.5	44.2	43.0	0.75
45.0	45.7	38.4	0.56
47.5	47.4	33.4	0.41
50.0	49.0	29.4	0.24
52.5	50.5	24.8	1.10
55.0	52.0	21.1	0.26
57.5	53.6	17.7	0.20
60.0	55.4	14.5	0.15
62.5	57.0	11.9	0.15
65.0	58.4	9.59	0.170
67.5	60.0	7.50	0.099
70.0	61.7	6.43	0.147
72.5	63.3	4.78	0.136
75.0	64.5	3.74	0.093
77.5	66.1	2.91	0.095
80.0	67.5	2.40	0.027
82.5	68.9	2.00	0.084
85.0	70.2	1.70	0.094
87.5	71.7	1.50	0.094
90.0	73.2	1.35	0.084
92.5	74.5	1.20	0.088
95.0	75.7	1.08	0.050
97.5	77.1	0.97	0.065
100.0	78.5	0.86	0.057

Figure 4.--The solubility of Butabarbital at 25°C in mg./ml. in the binary solvent systems studied.

TABLE VI.--A SUMMARY OF THE SOLUBILITY
OF VINBARBITAL IN ETHANOL-WATER
MIXTURES IN MG./ML. AT 25°C,
AS A FUNCTION OF W/W
PERCENT WATER (56)

W/W PERCENT WATER	SOLUBILITY IN MG./ML.
0.16	62.3
7.56	63.3
20.96	55.8
28.77	46.6
38.62	32.8
48.85	20.20
58.26	11.38
67.62	5.03
79.87	1.76
90.20	.96
100.00	.71

Figure 5.--The solubility of Vinbarbital at 25°C in mg./ml. versus percent water by weight in the binary solvents studied (56).

The DR of this molecule is observed to be about 27.6 with a solubility in this solvent composition of 63.3 mg./ml. A solubility of 0.70 mg./ml. at the most polar region of the curve, i.e., pure water, is slightly less than that of butabarbital. A portion of this isotherm is also seen to be approximately linear. Between the ranges of 20.0 to 60.0% water by weight, the rate of change of solubility with solvent composition is -1.2 mg./percent W/W water.

The general shape of this curve is very similar to those of butabarbital and metharbital. In each of these solubility profiles the maxima has been flattened and made somewhat broader than that of barbital.

The following two compounds to be discussed differ from the previously mentioned derivatives in that the oxygen at the R_4 position has been replaced with a sulfur atom. The effect of this substitution may decrease the polarity of the molecule from its oxy analog. On the electronegativity scale, the value for the oxygen atom is one unit higher than those for the sulfur and carbon atoms which are approximately equal. Thus, the chemical bond between the oxygen at the R_4 position and the adjacent carbon atom may be more polar in character than the similar situation with the sulfur atom.

Aside from the above mentioned substitution, the chemical structure of thiopental varies from barbital by a 1-methylbutenyl group replacing an ethyl substituent on the R_2 position.

Table VII and Figure 6 tabulate and graphically illustrate the solubility data for thiopental, in the manner previously described. From this plot it is noted that the DR is well defined at about 27.6. This peak is relatively sharp in contrast to those previously observed. The solubility is reduced sharply as the polarity of the solvent systems increase and asymptotically approaches the solvent composition axis. The solubility at the DR is observed to be 97.1 mg./ml. and in pure water it is reduced to 0.08 mg./ml., approximately a hundred fold decrease from the solubility of barbital at this particular solvent composition. Further, a relatively linear section of the plot is observed between the limits of 10.0 to 30.0% W/W water. The slope of this section represents a change in solubility with respect to solvent composition of -3.1 mg./percent by weight of water.

The ultrashort acting barbiturate thiamylal is similar in chemical structure to thiopental with the exception of an allyl substituent replacing an ethyl group in the R_1

TABLE VII.--A SUMMARY OF THE SOLUBILITY OF THIOPENTAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, AS A FUNCTION OF W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	SOLUBILITY IN MG./ML.	STD. DEVIATION IN MG./ML.
0.0	24.3	56.3	2.54
2.5	25.5	62.3	8.32
5.0	26.5	74.2	8.69
7.5	27.6	97.1	4.31
10.0	29.0	94.9	2.75
12.5	29.7	86.6	1.68
15.0	30.6	79.9	2.00
17.5	31.5	71.6	1.57
20.0	32.7	63.7	1.73
22.5	33.8	55.4	0.31
25.0	34.7	50.4	0.91
27.5	36.4	41.1	0.87
30.0	37.5	36.3	0.58
32.5	38.6	31.0	0.70
35.0	39.8	28.0	0.26
37.5	41.3	23.5	0.49
40.0	42.8	18.8	0.39
42.5	44.2	16.3	0.44
45.0	45.7	14.0	0.35
47.5	47.4	11.2	0.22
50.0	49.0	9.13	0.228
52.5	50.5	7.31	0.171
55.0	52.0	5.40	0.488
57.5	53.6	4.52	0.705
60.0	55.4	3.15	0.116
62.5	57.0	2.41	0.087
65.0	58.4	1.96	0.500
67.5	60.0	1.13	0.028
70.0	61.7	0.91	0.022
72.5	63.3	0.68	0.025
75.0	64.5	0.47	0.023
77.5	66.1	0.30	0.089
80.0	67.5	0.28	0.013
82.5	68.9	0.23	0.013
85.0	70.2	0.19	0.014
87.5	71.7	0.15	0.007
90.0	73.2	0.15	0.010
92.5	74.5	0.12	0.005
95.0	75.7	0.11	0.020
97.5	77.1	0.09	0.005
100.0	78.5	0.08	0.006

Figure 6.--The solubility of Thiopental at 25°C in mg./ml. as a function of composition (w/w) for ethanol-water mixtures.

position. The data for this derivative is found in Table VIII. A plot of this data, in the usual fashion, is represented in Figure 7. This curve is unique in that no maxima is observed, but the solubility profile is rising sharply towards pure ethanol having a dielectric constant of about 24.3. This would indicate that a DR of less than 24.3 would exist for this compound. The magnitude of solubility in pure ethanol is observed to be 160.8 mg./ml. and is high relative to the other derivatives. The remainder of the curve is similar to that of thiopental in that a sharp decline in solubility occurs between the solvents comprised of pure ethanol and 30% by weight of water. The rate of change in solubility, with respect to the solvent composition, is calculated as -4.1 mg./percent W/W water. The asymptotic nature of the curve, relative to the axis denoting the concentration of water in the solvents, is noted, and a low solubility of 0.05 mg./ml. is found in distilled water.

The remaining solubility profile to be discussed is that of barbituric acid, the parent chemical moiety of the derivatives studied. This particular compound is devoid of any therapeutic activity. Chemically, the molecule has hydrogen atoms occupying the R_1 , R_2 and R_3 position and an oxygen atom at position R_4 . The solubility data for this

TABLE VIII.--A SUMMARY OF THE SOLUBILITY OF THIAMYLAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, AS A FUNCTION OF W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ϵ)	SOLUBILITY IN MG./ML.	STD. DEVIATION IN MG./ML.
0.0	24.3	160.8	1.19
2.5	25.5	149.9	1.66
5.0	26.5	135.4	3.33
7.5	27.6	124.6	2.01
10.0	29.0	112.7	1.88
12.5	29.7	102.0	2.09
15.0	30.6	93.2	0.73
17.5	31.5	82.3	1.80
20.0	32.7	71.8	1.34
22.5	33.8	61.9	0.82
25.0	34.7	54.9	0.84
27.5	36.4	43.3	0.81
30.0	37.5	37.7	1.10
32.5	38.6	32.3	0.81
35.0	39.8	28.7	0.59
37.5	41.3	23.1	0.61
40.0	42.8	18.1	1.27
42.5	44.2	15.4	0.70
45.0	45.7	13.0	0.42
47.5	47.4	10.3	0.28
50.0	49.0	8.21	0.236
52.5	50.5	6.46	0.232
55.0	52.0	4.74	0.132
57.5	53.6	3.41	0.152
60.0	55.4	2.52	0.094
62.5	57.0	1.96	0.128
65.0	58.4	1.41	0.092
67.5	60.0	.94	0.101
70.0	61.7	.73	0.085
72.5	63.3	.51	0.036
75.0	64.5	.35	0.023
77.5	66.1	.23	0.018
80.0	67.5	.19	0.011
82.5	68.9	.15	0.009
85.0	70.2	.12	0.005
87.5	71.7	.10	0.004
90.0	73.2	.09	0.006
92.5	74.5	.07	0.005
95.0	75.7	.06	0.012
97.5	77.1	.06	0.004
100.0	78.5	.05	0.006

Figure 7.--The solubility of Thiamylal at 25°C in mg./ml. as a function of percent w/w water.

compound is tabulated in Table IX. A plot of the data, in the manner described above, may be found in Figure 8. A sharp but minor deflection in solubility occurs at a concentration of 22.5% W/W water or a dielectric constant of 33.8. A broad shoulder is also noticed in the range of dielectric constant values from 55 to 70. Two major dissimilarities may be observed between the solubility curves of this compound and those of its derivatives. Most noticeable is the increase in solubility as the more polar end of the scale is approached. This may be contrasted with the decreasing solubility in this solvent range for the barbituric acid derivatives. The magnitude of solubility is also drastically reduced over almost the entire range of solvent composition. The maximum solubility exhibited by this molecule is 10.9 mg./ml. in pure water. Increasing the scale of this plot to correspond with the previous figures would reduce the solubility profile of barbituric acid to a rather straight line with minor deviations relative to the profiles of its derivatives.

It was noted in the case of all the barbituric acid derivatives that some portion of the solubility isotherm possessed a fair degree of linearity relative to the solvent composition. The rates calculated as the slopes of the

TABLE IX.--A SUMMARY OF THE SOLUBILITY OF BARBITURIC ACID
IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, AS A FUNCTION
OF W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ϵ)	SOLUBILITY IN MG./ML.	STD. DEVIATION IN MG./ML.
0.0	24.2	2.34	0.092
2.5	25.5	2.71	0.079
5.0	26.5	3.25	0.105
7.5	27.6	3.85	0.095
10.0	29.0	4.67	0.117
12.5	29.7	5.28	0.112
15.0	30.6	6.04	0.136
17.5	31.5	6.85	0.178
20.0	32.7	7.69	0.190
22.5	33.8	8.62	0.144
25.0	34.7	7.59	0.110
27.5	36.4	7.73	0.097
30.0	37.5	7.87	0.204
32.5	38.6	8.12	0.178
35.0	39.8	8.31	0.200
37.5	41.3	8.56	0.185
40.0	42.8	8.73	0.988
42.5	44.2	8.92	0.130
45.0	45.7	9.14	0.038
47.5	47.4	9.25	0.120
50.0	49.0	9.29	0.184
52.5	50.5	9.47	0.046
55.0	52.0	9.52	0.085
57.5	53.6	9.68	0.063
60.0	55.4	9.71	0.053
62.5	57.0	9.70	0.083
65.0	58.4	9.61	0.151
67.5	60.0	9.67	0.070
70.0	61.7	9.66	0.056
72.5	63.3	9.55	0.093
75.0	64.5	9.57	0.037
77.5	66.1	9.57	0.092
80.0	67.5	9.58	0.097
82.5	68.9	9.64	0.183
85.0	70.2	9.65	0.094
87.5	71.7	9.89	0.080
90.0	73.2	10.0	0.04
92.5	74.5	10.2	0.09
95.0	75.7	10.4	0.04
97.5	77.1	10.6	0.16
100.0	78.5	10.9	0.17

Figure 8.--The solubility of Barbituric Acid at 25°C in mg./ml. as a function of composition (w/w) for ethanol-water mixtures.

straight line best representing these approximately linear sections are summarized in Table X along with the ranges in solvent composition in which this relationship is valid. It is seen from this tabulation that the range of linearity is very limited in the case of thiopental. It is also noted that the rates of change in solubility relative to the solvent composition are rather constant with the exception of thiopental and thiamylal. In these latter cases, the rates are about twice those of the remaining derivatives.

The limits of solvent composition, within which these rates are operative, lie well within the range of pharmaceutical interest. It might be assumed then, there would be some pharmaceutical formulations advantages in this information.

In a study such as this, where the effect on the DR is being assessed by varying the substituent groups on the parent molecule, it would prove valuable to plot the difference in solubility between a standard barbiturate and each of the remaining derivatives. On a chemical basis, a logical choice of a standard would rest upon the barbituric acid molecule due to the fact it is the basic chemical structure for the derivatives. However, due to the relatively low solubility of this compound and the fact that it possesses no therapeutic activity another choice was sought.

TABLE X.--A SUMMARY OF THE RATES OF CHANGE IN
SOLUBILITY IN MG./PERCENT W/W WATER,
CALCULATED FROM THE LINEAR RANGE
OF THE SOLUBILITY PROFILES

Derivative	Range in percent W/W water	Rate in mg./percent W/W water
Barbital	27.5 - 65.0	-1.8
Metharbital	30.0 - 62.5	-1.0
Butabarbital	27.0 - 55.0	-1.9
Vinbarbital	20.0 - 60.0	-1.2
Thiopental	10.0 - 30.0	-3.1
Thiamylal	0.0 - 30.0	-4.1

The barbital molecule, which has two relatively short chemical groups attached to the R_1 and R_2 positions, provides a second standard with which to compare the solubility profiles. The fact that this molecule also exhibits a pharmacological response and that its action is typical of a long acting barbiturate also make it suitable as a standard.

Again the digital computer was employed to make the necessary computations. A program was constructed which computed the difference in solubilities of two derivatives in a particular solvent composition (Appendix D). Given two sets of solubility data a third was compiled which contained these differences or delta values.

The difference in solubility between metharbital and barbital are tabulated in Table XI, along with the corresponding solvent composition and dielectric constant. These values represent the change in solubility effected by the replacement of a hydrogen atom with a methyl group on the R_3 position of the barbital molecule. A plot of this data, expressed in mg./ml. as a function of percent W/W water, may be found in Figure 9. As is the case of all the derivatives studied, with the exception of thiamylal, the solubility of metharbital was reduced below that of barbital over the entire range of solvent composition. This phenomenon

TABLE XI.--A SUMMARY OF THE DIFFERENCES IN THE SOLUBILITY OF METHARBITAL AND BARBITAL IN MG./ML. AT 25⁰ C, AS A FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	DIFFERENCE IN MG./ML.
0.0	24.3	-50.4
2.5	25.5	-54.9
5.0	26.5	-56.9
7.5	27.6	-62.1
10.0	29.0	-63.3
12.5	29.7	-67.6
15.0	30.6	-69.5
17.5	31.5	-66.3
20.0	32.7	-62.2
22.5	33.8	-58.4
25.0	34.7	-52.1
27.5	36.4	-48.3
30.0	37.5	-45.5
32.5	38.6	-41.7
35.0	39.8	-41.8
37.5	41.3	-39.0
40.0	42.8	-33.8
42.5	44.2	-32.9
45.0	45.7	-31.3
47.5	47.4	-30.3
50.0	49.0	-28.1
52.5	50.5	-26.5
55.0	52.0	-24.4
57.5	53.6	-23.0
60.0	55.4	-20.1
62.5	57.0	-18.5
65.0	58.4	-18.1
67.5	60.0	-15.5
70.0	61.7	-13.5
72.5	63.3	-10.8
75.0	64.5	-10.3
77.5	66.1	-9.7
80.0	67.5	-9.4
82.5	68.9	-9.0
85.0	70.2	-7.9
87.5	71.7	-7.2
90.0	73.2	-6.3
92.5	74.5	-5.5
95.0	75.7	-5.2
97.5	77.1	-5.2
100.0	78.5	-5.3

Figure 9.--A plot of the difference of the magnitude of solubility for Metharbital and Barbitol in mg./ml. for the binary systems studied.

might be expected due to the decrease in polarity as the series is descended, produced by the increasing number of carbon atoms in the chemical structure.

It will be noted from this figure that the largest decrease in solubility occurred at 15.0% water by weight, this corresponding to the DR of both metharbital and barbital. The addition of the N-methyl group substantially decreased the sharpness of the solubility peak. A shoulder at 37.5 to 40.0% W/W water corresponds to the plateau observed on the solubility profile for metharbital.

The delta values for butabarbital and barbital may be found in Table XII and a graphical representation, expressed in the manner previously described, in Figure 10. In this case the figures represent the change in solubility produced by a sec-butyl group replacing an ethyl substituent on the barbital molecule. Again the maximum change in solubility is found in the region of the DR. As was the case with metharbital, the solubility maxima became rounded with the addition of the sec-butyl group. The inflection in the curve, in the range of solvent composition of 30.0 to 60.0% by weight of water, demonstrates that the solubility of metharbital is decreasing at a higher rate than barbital as the water content is increased over the range.

TABLE XII.--A SUMMARY OF THE DIFFERENCES IN THE SOLUBILITY OF BUTABARBITAL AND BARBITAL IN MG./ML. AT 25° C, AS A FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT(ϵ)	DIFFERENCE IN MG./ML.
0.0	24.3	-8.3
2.5	25.5	-12.6
5.0	26.5	-15.1
7.5	27.6	-20.7
10.0	29.0	-23.2
12.5	29.7	-27.7
15.0	30.6	-31.1
17.5	31.5	-28.7
20.0	32.7	-26.6
22.5	33.8	-25.1
25.0	34.7	-20.9
27.5	36.4	-20.9
30.0	37.5	-21.6
32.5	38.6	-21.5
35.0	39.8	-22.2
37.5	41.3	-21.9
40.0	42.8	-21.8
42.5	44.2	-23.3
45.0	45.7	-21.8
47.5	47.4	-23.1
50.0	49.0	-22.2
52.5	50.5	-22.9
55.0	52.0	-22.0
57.5	53.6	-21.5
60.0	55.4	-19.6
62.5	57.0	-18.7
65.0	58.4	-18.7
67.5	60.0	-16.6
70.0	61.7	-14.5
72.5	63.3	-12.3
75.0	64.5	-11.8
77.5	66.1	-11.3
80.0	67.5	-10.9
82.5	68.9	-10.5
85.0	70.2	-9.4
87.5	71.7	-8.6
90.0	73.2	-7.6
92.5	74.5	-6.8
95.0	75.7	-6.4
97.5	77.1	-6.4
100.0	78.5	-6.4

Figure 10.--A plot of the difference in the magnitude of solubility for Butabarbital and Barbitital in mg./ml. as a function of composition (w/w) for ethanol-water mixtures.

For the purpose of comparing the solubility profiles for vinbarbital and barbital, special treatment of the data presented by Reber and Pathamanon (56) was necessary. Their profile consisted of eleven pieces of data, only one of which corresponded to an exact solvent composition used in this study. It was necessary, therefore, to analyze their data and determine the apparent solubility of vinbarbital in each of the 41 solvent systems employed for the remaining compounds. Rather than arbitrarily picking points off a plot of their data, the data itself was subjected to a polynomial regression. A previously compiled and published digital computer program (67) was employed in double precision (Appendix E). This program is based on a mathematical method presented by Ostle (68). The coefficients of an eight degree polynomial, representing the best fit of a curve to the data of Reber and Pathamanon, were computed. From this equation, the apparent solubilities of vinbarbital in hydroalcoholic solvents of identical composition to those used in this study were calculated. It is suggested by Ostle that this particular method is not valid for data which is presented in uneven increments of the independent variable. However, it is felt that the published solubility data for vinbarbital closely approximates even increments of solvent

composition and, therefore, a close approximation of the true equation describing the data should be rendered by this method. A comparison of the original data in Table VI may be made with the values computed by this method which are found in Table XIII.

A table and a graphical illustration, expressed as described above, of the values representing the difference in solubility between vinbarbital and barbital are found in Table XIV and Figure 11, respectively. This data illustrates the effect on the solubility profile produced by substituting a 1-methylbutenyl group for an ethyl substituent on the R_2 position of the barbital molecule with a corresponding increase of three carbon atoms and the introduction of an olefinic bond. As was the case with butabarbital and metharbital, the largest decrease in solubility is observed in the less polar region of solvent composition, tending to reduce the sharpness of the solubility peak. The DR for vinbarbital was found at 27.6 and the maximum difference in solubility from barbital at a dielectric constant of 30.6. An approximately linear region is observed on this curve in a range of solvent composition of 30.0 to 70.0% by weight of water.

The striking similarity between the curves represent-

TABLE XIII.--SUMMARY OF THE SOLUBILITY OF VINBARBITAL IN ETHANOL-WATER MIXTURES IN MG./ML. AT 25°C, COMPUTED FROM A POLYNOMIAL, VERSUS W/W PERCENT WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ϵ)	SOLUBILITY IN MG./ML.
0.0	24.3	62.3
2.5	25.5	62.7
5.0	26.5	63.1
7.5	27.6	63.3
10.0	29.0	63.0
12.5	29.7	62.2
15.0	30.6	61.0
17.5	31.5	59.2
20.0	32.7	56.9
22.5	33.8	54.2
25.0	34.7	51.3
27.5	36.4	48.1
30.0	37.5	44.8
32.5	38.6	41.3
35.0	39.8	37.9
37.5	41.3	34.4
40.0	42.8	31.1
42.5	44.2	27.8
45.0	45.7	24.7
47.5	47.4	21.8
50.0	49.0	19.0
52.5	50.5	16.4
55.0	52.0	14.0
57.5	53.6	11.8
60.0	55.4	9.84
62.5	57.0	8.09
65.0	58.4	6.56
67.5	60.0	5.25
70.0	61.7	4.16
72.5	63.3	3.27
75.0	64.5	2.57
77.5	66.1	2.05
80.0	67.5	1.67
82.5	68.9	1.41
85.0	70.2	1.23
87.5	71.7	1.10
90.0	73.2	0.99
92.5	74.5	0.88
95.0	75.7	0.77
97.5	77.1	0.68
100.0	78.5	0.70

TABLE XIV.--A SUMMARY OF THE DIFFERENCES IN THE SOLUBILITY OF VINBARBITAL AND BARBITAL IN MG./ML. AT 25°C, AS A FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	DIFFERENCE IN MG./ML.
0.0	24.3	-30.0
2.5	25.5	-35.9
5.0	26.5	-40.0
7.5	27.6	-46.7
10.0	29.0	-50.3
12.5	29.7	-56.0
15.0	30.6	-59.8
17.5	31.5	-58.0
20.0	32.7	-55.6
22.5	33.8	-53.5
25.0	34.7	-48.8
27.5	36.4	-46.2
30.0	37.5	-45.4
32.5	38.6	-43.8
35.0	39.8	-42.9
37.5	41.3	-41.2
40.0	42.8	-38.9
42.5	44.2	-38.4
45.0	45.7	-35.5
47.5	47.4	-34.7
50.0	49.0	-32.6
52.5	50.5	-31.3
55.0	52.0	-29.1
57.5	53.6	-27.4
60.0	55.4	-24.2
62.5	57.0	-22.5
65.0	58.4	-21.7
67.5	60.0	-18.8
70.0	61.7	-16.7
72.5	63.3	-13.8
75.0	64.5	-13.0
77.5	66.1	-12.2
80.0	67.5	-11.6
82.5	68.9	-11.1
85.0	70.2	-9.9
87.5	71.7	-9.0
90.0	73.2	-8.0
92.5	74.5	-7.1
95.0	75.7	-6.7
97.5	77.1	-6.7
100.0	78.5	-6.6

Figure 11.--The difference in the magnitude of solubility for Vinbarbital and Barbital expressed in mg./ml. plotted as a function of percent water by weight.

ing the differences in the solubilities between barbital and butabarbital, metharbital and vinbarbital should be noted. In each case a very sharp decrease in solubility is found in the less polar region at a point approximating the DR of the derivative being compared with barbital. The curves then show a rapid decrease in the magnitude of the delta values up to about 70% W/W water, at which point the change becomes more gradual. Approaching the solvent composed of pure water, the change in solubility is noted to be between 5.0 and 7.0 mg./ml. for each of these three curves, the largest difference being observed for the compound possessing the largest number of carbon atoms.

In a comparison of the chemical structure of thiopental with barbital, a change in the substituents at the R_2 and R_4 position is found. At the R_4 position is a sulfur atom replacing the oxygen of barbital and a 1-methylbutyl group in place of the ethyl substituent on the R_2 position. The tabulated data for this compound is found in Table XV and plotted in Figure 12, in a manner similar to the previous illustrations.

The general shape for this curve deviates from that of the previous three in that the largest change in solubility is found at a solvent composition removed from that of the

TABLE XV.--A SUMMARY OF THE DIFFERENCES IN THE SOLUBILITY OF
THIOPENTAL AND BARBITAL IN MG./ML. AT 25°C, AS A
FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	DIFFERENCE IN MG./ML.
0.0	24.3	-36.0
2.5	25.5	-36.3
5.0	26.5	-28.9
7.5	27.6	-12.9
10.0	29.0	-18.4
12.5	29.7	-31.7
15.0	30.6	-40.8
17.5	31.5	-45.6
20.0	32.7	-48.8
22.5	33.8	-52.3
25.0	34.7	-49.7
27.5	36.4	-53.2
30.0	37.5	-53.9
32.5	38.6	-54.1
35.0	39.8	-52.7
37.5	41.3	-52.1
40.0	42.8	-51.2
42.5	44.2	-50.0
45.0	45.7	-46.2
47.5	47.4	-45.3
50.0	49.0	-42.5
52.5	50.5	-40.4
55.0	52.0	-37.7
57.5	53.6	-34.7
60.0	55.4	-31.0
62.5	57.0	-28.2
65.0	58.4	-26.3
67.5	60.0	-23.0
70.0	61.7	-20.0
72.5	63.3	-16.4
75.0	64.5	-15.1
77.5	66.1	-13.9
80.0	67.5	-13.0
82.5	68.9	-12.3
85.0	70.2	-10.9
87.5	71.7	-9.9
90.0	73.2	-8.8
92.5	74.5	-7.9
95.0	75.7	-7.4
97.5	77.1	-7.3
100.0	78.5	-7.2

Figure 12.--A plot of the difference in the magnitude of solubility for Thiopental and Barbitol in mg./ml. for the binary systems investigated.

solubility maxima of the derivative being compared with barbital. This illustrates the rather sharp decline in the solubility of thiopental in the range of 20.0 to 40.0% by weight of water relative to that of barbital. A well defined but small change is noted at 7.5% W/W water or a dielectric constant value of 27.6, corresponding to the DR for thiopental.

The data representing the difference in the solubilities of thiamylal and barbital are presented in Table XVI and plotted in Figure 13, in a manner previously described. As well as having a sulfur atom replacing the oxygen atom on the R_4 position and a 1-methylbutyl in place of an ethyl group on the R_2 position as in thiopental, thiamylal also has an allyl group which replaces the ethyl substituent on the R_1 position of barbital.

It is seen in this figure that the combination of these substitutions increases the solubility of thiamylal over that of barbital in the range of solvent composition from pure ethanol to 10.0% W/W water or a range of dielectric constant values of 24.3 to 29.0. Thiopental, with the same chemical structure as thiamylal excepting the allyl substitution, did not demonstrate this characteristic. It may be surmised then, that the increase in solubility in this range is attributed to the addition of the allyl group.

TABLE XVI.--A SUMMARY OF THE DIFFERENCES IN THE SOLUBILITY OF THIAMYLAL AND BARBITAL IN MG./ML. AT 25°C, AS A FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	DIFFERENCE IN MG./ML.
0.0	24.3	68.6
2.5	25.5	51.3
5.0	26.5	32.3
7.5	27.6	14.6
10.0	29.0	-0.6
12.5	29.7	-16.3
15.0	30.6	-27.5
17.5	31.5	-34.9
20.0	32.7	-40.7
22.5	33.8	-45.8
25.0	34.7	-45.2
27.5	36.4	-51.0
30.0	37.5	-52.5
32.5	38.6	-52.8
35.0	39.8	-52.1
37.5	41.3	-52.5
40.0	42.8	-51.9
42.5	44.2	-50.8
45.0	45.7	-47.1
47.5	47.4	-46.2
50.0	49.0	-43.4
52.5	50.5	-41.2
55.0	52.0	-38.4
57.5	53.6	-35.8
60.0	55.4	-31.6
62.5	57.0	-28.6
65.0	58.4	-26.9
67.5	60.0	-23.2
70.0	61.7	-20.2
72.5	63.3	-16.6
75.0	64.5	-15.2
77.5	66.1	-14.0
80.0	67.5	-13.1
82.5	68.9	-12.3
85.0	70.2	-11.0
87.5	71.7	-10.0
90.0	73.2	-8.9
92.5	74.5	-7.9
95.0	75.7	-7.4
97.5	77.1	-7.3
100.0	78.5	-7.2

Figure 13.--A plot of the difference of the magnitude of solubility for Thiamylal and Barbitol expressed in mg./ml. as a function of solvent composition.

A general similarity exists between the curves representing the differences in the solubilities of barbital and those of thiopental and thiamylal. In both cases the maximum difference in solubility is observed at a concentration of 30.0 to 40.0% water by weight. It can be supposed that this effect is due in part to the addition of the sulfur atom on the R_4 position, since the oxy- derivatives did not show this effect. It is also noticed, by considering the change in scale of the axis connotating the difference in solubilities and excepting the initial portion of the curves, Figures 12 and 13 are approximately identical in magnitude as well as shape.

In the previous discussion the concentrations of the saturated solutions consisting of several barbiturates in ethanol-water systems have been considered. The magnitudes of the differences in the solubilities between each of the derivatives and the standard, barbital, has also been discussed. It would be pertinent then to consider the changes in solubility produced by altering the substituent groups on the barbital molecule in a relative manner, rather than in absolute values. As in the case of the delta values, barbital is used as the standard of comparison.

A computer program was written which computed these

ratios (Appendix F). Given the solubility data corresponding to barbital and one other derivative in each of the 41 solvents employed, this program computed the ratio of the solubility of the derivative to the solubility of the standard for each of the separate solvents.

The ratios computed in this manner for metharbital are tabulated with the solvent composition and dielectric constant in Table XVII. A plot of this data, as a function of W/W percent water, may be found in Figure 14. It can be seen in this illustration that the magnitude of solubility of metharbital is substantially lower than that of barbital over the entire range of solvent composition. The peak observed between 30.0 and 40.0% water by weight corresponds to the shoulder on the solubility profile. An inflection on this curve may be observed at 15% W/W water which corresponds to the maximum solubility of both metharbital and barbital.

The ratios calculated between barbital and butabarbital are arranged in Table XVIII and graphically illustrated in Figure 15, in a manner previously described. The characteristics of this curve are quite different from that of the previous figure for metharbital. It can be seen from this figure that the solubility of butabarbital approaches that of barbital in the less polar region of the curve, i.e. low water concentrations, but is only about 10% that of

TABLE XVII.--A SUMMARY OF THE RATIOS OF THE SOLUBILITY OF
METHARBITAL TO BARBITAL AT 25°C, AS A FUNCTION OF
PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (C)	RATIO
0.0	24.3	0.454
2.5	25.5	0.443
5.0	26.5	0.447
7.5	27.6	0.435
10.0	29.0	0.442
12.5	29.7	0.428
15.0	30.6	0.424
17.5	31.5	0.435
20.0	32.7	0.447
22.5	33.8	0.458
25.0	34.7	0.480
27.5	36.4	0.488
30.0	37.5	0.495
32.5	38.6	0.510
35.0	39.8	0.482
37.5	41.3	0.483
40.0	42.8	0.517
42.5	44.2	0.503
45.0	45.7	0.481
47.5	47.4	0.463
50.0	49.0	0.455
52.5	50.5	0.444
55.0	52.0	0.434
57.5	53.6	0.413
60.0	55.4	0.411
62.5	57.0	0.395
65.0	58.4	0.360
67.5	60.0	0.357
70.0	61.7	0.354
72.5	63.3	0.367
75.0	64.5	0.339
77.5	66.1	0.317
80.0	67.5	0.297
82.5	68.9	0.281
85.0	70.2	0.289
87.5	71.7	0.284
90.0	73.2	0.30
92.5	74.5	0.31
95.0	75.7	0.31
97.5	77.1	0.29
100.0	78.5	0.27

Figure 14.--The solubility of Metharbital relative to the solubility of Barbital versus percent by weight water in the solvent systems studied.

TABLE XVIII.--A SUMMARY OF THE RATIOS OF THE SOLUBILITY
OF BUTABARBITAL TO BARBITAL AT 25°C, AS A FUNCTION OF
PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT(ϵ)	RATIO
0.0	24.3	0.910
2.5	25.5	0.872
5.0	26.5	0.853
7.5	27.6	0.812
10.0	29.0	0.795
12.5	29.7	0.766
15.0	30.6	0.742
17.5	31.5	0.755
20.0	32.7	0.764
22.5	33.8	0.767
25.0	34.7	0.792
27.5	36.4	0.778
30.0	37.5	0.761
32.5	38.6	0.748
35.0	39.8	0.726
37.5	41.3	0.710
40.0	42.8	0.688
42.5	44.2	0.648
45.0	45.7	0.637
47.5	47.4	0.592
50.0	49.0	0.570
52.5	50.5	0.519
55.0	52.0	0.490
57.5	53.6	0.452
60.0	55.4	0.426
62.5	57.0	0.388
65.0	58.4	0.339
67.5	60.0	0.311
70.0	61.7	0.308
72.5	63.3	0.280
75.0	64.5	0.240
77.5	66.1	0.205
80.0	67.5	0.181
82.5	68.9	0.160
85.0	70.2	0.154
87.5	71.7	0.148
90.0	73.2	0.15
92.5	74.5	0.15
95.0	75.7	0.14
97.5	77.1	0.13
100.0	78.5	0.12

Figure 15.--The ratio of the solubility of Butabarbital to the solubility of Barbitol as a function of composition (w/w) for ethanol-water systems.

barbital in pure water. The direction of change of this ratio is reversed from the overall trend in the solvents containing 15.0 to 25.0% W/W water indicating the solubility profile of barbital decreasing at a more rapid rate than butabarbital in this region.

The ratios of the solubilities of vinbarbital to those of barbital in each of the 41 solvent systems are found in Table XIX. Solubility values used for vinbarbital are those determined by the polynomial and utilized previously in calculating the difference in the magnitudes of solubilities of these compounds. This data is plotted in Figure 16, in a manner similar to that used for metharbital.

The similarity between the shape of this plot and the previous one should be noted although the magnitude of the ratios are somewhat reduced in the case at hand. Again the solubility of this compound approaches that of barbital in the solvents containing a high concentration of ethanol, but is substantially less in the higher polarity range. A plateau is found at 15.0 to 25.0% water by weight or a dielectric constant range of 30.6 to 34.7. This corresponds to a slightly larger peak found in this same solvent range for the ratios of the solubilities of butabarbital to those of barbital. The behavior exhibited in this limited range indicates the rate of decrease in the solubility of barbital

TABLE XIX.--A SUMMARY OF THE RATIOS OF THE SOLUBILITY OF
VINBARBITAL TO BARBITAL AT 25°C, AS A FUNCTION OF
PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ϵ)	RATIO
0.0	24.3	0.675
2.5	25.5	0.636
5.0	26.5	0.612
7.5	27.6	0.575
10.0	29.0	0.556
12.5	29.7	0.526
15.0	30.6	0.505
17.5	31.5	0.505
20.0	32.7	0.506
22.5	33.8	0.504
25.0	34.7	0.512
27.5	36.4	0.510
30.0	37.5	0.496
32.5	38.6	0.486
35.0	39.8	0.469
37.5	41.3	0.556
40.0	42.8	0.444
42.5	44.2	0.420
45.0	45.7	0.411
47.5	47.4	0.385
50.0	49.0	0.368
52.5	50.5	0.344
55.0	52.0	0.325
57.5	53.6	0.301
60.0	55.4	0.289
62.5	57.0	0.264
65.0	58.4	0.232
67.5	60.0	0.218
70.0	61.7	0.199
72.5	63.3	0.191
75.0	64.5	0.165
77.5	66.1	0.144
80.0	67.5	0.126
82.5	68.9	0.113
85.0	70.2	0.111
87.5	71.7	0.109
90.0	73.2	0.11
92.5	74.5	0.11
95.0	75.7	0.10
97.5	77.1	0.09
100.0	78.5	0.10

Figure 16.--A plot of the solubility of Vin-barbital relative to the solubility of Bar-bital for each of the binary systems studied.

in solvents of increasing polarity to be greater than that of vinbarbital.

Data representing the solubility ratios of thiopental to barbital are listed in Table XX. A graphical illustration may be found in Figure 17, presented in a manner consistent with the previous illustrations.

It is seen that this plot is very similar to the solubility profile for thiopental with a maximum ratio at a dielectric constant value of 27.6. The sharpness of this peak is an indication of the wide contrast between the shape of the maxima on the individual solubility isotherms of thiopental and barbital. The asymptotic character of the solubility profile is also reproduced on this plot.

Ratios calculated from the solubilities of thiamylal and barbital are found in Table XXI and plotted as described previously in Figure 18. As in the previous case, the curve also simulates the solubility profile of the barbiturate being compared with barbital. A smooth curve is noted exhibiting no maximum or shouldering effects. In solvent concentrations up to 10% by weight of water, the solubility of thiamylal is greater than that of barbital. This characteristic is unique for thiamylal. The extremely low solubility of this compound in solvents of high water content, relative

TABLE XX.--A SUMMARY OF THE RATIOS OF THE SOLUBILITY OF THIOPENTAL TO BARBITAL AT 25°C, AS A FUNCTION OF PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	RATIO
0.0	24.3	0.610
2.5	25.5	0.632
5.0	26.5	0.720
7.5	27.6	0.882
10.0	29.0	0.838
12.5	29.7	0.732
15.0	30.6	0.662
17.5	31.5	0.611
20.0	32.7	0.566
22.5	33.8	0.514
25.0	34.7	0.504
27.5	36.4	0.436
30.0	37.5	0.402
32.5	38.6	0.365
35.0	39.8	0.347
37.5	41.3	0.311
40.0	42.8	0.269
42.5	44.2	0.246
45.0	45.7	0.233
47.5	47.4	0.198
50.0	49.0	0.177
52.5	50.5	0.153
55.0	52.0	0.125
57.5	53.6	0.115
60.0	55.4	0.092
62.5	57.0	0.079
65.0	58.4	0.069
67.5	60.0	0.047
70.0	61.7	0.044
72.5	63.3	0.040
75.0	64.5	0.030
77.5	66.1	0.021
80.0	67.5	0.021
82.5	68.9	0.018
85.0	70.2	0.017
87.5	71.7	0.015
90.0	73.2	0.016
92.5	74.5	0.015
95.0	75.7	0.015
97.5	77.1	0.013
100.0	78.5	0.012

Figure 17.--The solubility of Thiopental relative to the solubility of Barbitol plotted as a function of solvent composition.

TABLE XXI.--A SUMMARY OF THE RATIOS OF THE SOLUBILITY OF
THIAMYLAL TO BARBITAL AT 25° C, AS A FUNCTION OF
PERCENT W/W WATER AND DIELECTRIC CONSTANT

W/W PERCENT WATER	DIELECTRIC CONSTANT (ε)	RATIO
0.0	24.3	1.74
2.5	25.5	1.52
5.0	26.5	1.31
7.5	27.6	1.13
10.0	29.0	0.994
12.5	29.7	0.862
15.0	30.6	0.772
17.5	31.5	0.702
20.0	32.7	0.638
22.5	33.8	0.575
25.0	34.7	0.548
27.5	36.4	0.459
30.0	37.5	0.418
32.5	38.6	0.379
35.0	39.8	0.355
37.5	41.3	0.305
40.0	42.8	0.258
42.5	44.2	0.233
45.0	45.7	0.217
47.5	47.4	0.182
50.0	49.0	0.159
52.5	50.5	0.135
55.0	52.0	0.110
57.5	53.6	0.087
60.0	55.4	0.074
62.5	57.0	0.064
65.0	58.4	0.050
67.5	60.0	0.039
70.0	61.7	0.035
72.5	63.3	0.030
75.0	64.5	0.022
77.5	66.1	0.016
80.0	67.5	0.014
82.5	68.9	0.012
85.0	70.2	0.010
87.5	71.7	0.010
90.0	73.2	0.010
92.5	74.5	0.009
95.0	75.7	0.008
97.5	77.1	0.007
100.0	78.5	0.006

Figure 18.--The solubility of Thiamylal relative to the solubility of Barbital versus percent water by weight.

to that of barbital, may be seen from the asymptotic portion of the curve.

The various solubility relationships which have been discussed for this series of compounds generally show a displacement of the DR to regions of lower polarity, as the total number of carbon atoms in the molecule is increased. It may be surmised that a corresponding reduction in the polar character of these compounds accompanies this systematic change in molecular structure.

Upon introduction of drugs into a biological system, the therapeutic action can be elicited only to a degree corresponding to the concentration of drug reaching the site of action. Consequently, the ability of the compound to be transported through the biological fluids and membranes of varying polarity may become the limiting step upon which the elicitation of therapeutic action is dependent. Within a chemical series, such as the barbituric acid derivatives being studied, an approximate correlation should exist between the extent of therapeutic activity and the relative affinity of these molecules for semi-polar solvents.

A summary of some of the more pertinent solubility data is presented in Table XXII, along with an index to the duration of activity and the period of latency exhibited by these compounds. For each derivative, the DR, solubility in

TABLE XXII.--A SUMMARY OF THE DIELECTRIC REQUIREMENT (DR), THE SOLUBILITIES IN ABSOLUTE ETHANOL, WATER, AND AT THE DIELECTRIC REQUIREMENT IN MG./ML., AS A FUNCTION OF THE DURATION AND ONSET OF ACTION

Derivative	Duration of Action		Onset of Action	DR	Sol. in Ethanol mg./ml.	Sol. at DR mg./ml.	Sol. in Water mg./ml.
	Ref. (69)	Ref. (70)	Ref. (69)				
Barbital	long	long	30-60 min. ^a	30.6	92.3	120.7	7.3
Metharbital	long	long	30-60 min. ^a	30.6	41.9	51.2	2.00
Butabarbital	intermediate	intermediate to short	20-30 min. ^a	29.7	84.0	90.6	0.86
Vinbarbital	intermediate	intermediate to short	20-30 min. ^a	27.6	62.3	63.3	0.70
Thiopental	ultrashort	ultrashort	30 sec. ^b	27.6	56.3	97.1	0.08
Thiamylal	ultrashort	ultrashort	20-60 sec. ^b	<24.3	160.8	>160.8	0.05

^a Oral administration

^b Intravenous administration

pure water and pure ethanol and the solubility in the solvent corresponding in composition to that at which the DR is observed, are listed.

Two references have been included to attest to the duration of action. It is seen that there is considerable overlap in the duration of sedative effect of these materials. The period of time between administration and the time at which the therapeutic effect is first noticed is also quite variable among the compounds.

As the duration of action is decreased in this series, a corresponding reduction is noticed in the DR indicating a greater affinity for the less polar solvents. As is expected, a clear distinction among the three pairs of derivatives exhibiting similar durations of action is not found on the scale of DR's. A general trend is seen to exist though, and it can be stated that although vinbarbital is classified as having an intermediate duration of action, it is closer to ultrashort acting thiopental than it is to long acting metharbital.

The relative hydrophilic nature of these derivatives is demonstrated by their aqueous solubilities. These values are seen to have a range of approximately two orders of magnitude. Barbital, being the most hydrophilic, has a solubility of 7.3 mg./ml. in water. The solubilities of the

remaining barbiturates in this solvent decrease along with the duration of action to thiamylal with the lowest solubility which is 0.05 mg./ml.

The solubility of these compounds in pure ethanol and in the solvent corresponding in composition to that at which the DR is observed do not show a definite trend in the magnitudes of solubility. Due to the variations in chemical structure, the ability of these derivatives to be solvated by the various ethanol-water mixtures may be limited in some cases. It is possible that steric hinderance produced by the substituent groups on the parent moiety may contribute to this effect.

In any discussion of the biopharmaceutical parameters involved in drug action, the partitioning of the drug between the various biological fluids and membranes must be considered. These phases range from relatively high polarity found in the blood and gastric contents to a low value for adipose tissue. It would be instructive then to calculate for each of the derivatives, the ratios of the solubilities in pure ethanol and at the DR, to that found in pure water. A summary of these ratios is found in Table XXIII.

It is seen that the ratios of the solubility in absolute ethanol to that in pure water are inversely related to the duration of action of this series. A similar relationship

TABLE XXIII.--A SUMMARY OF THE THERAPEUTIC ACTION, AND THE RATIOS OF THE SOLUBILITY IN ETHANOL AND AT THE DIELECTRIC REQUIREMENT (DR) TO THE SOLUBILITY IN WATER

Derivative	Duration Ref. (69)	Onset Ref. (69)	<u>Sol. in ethanol</u> <u>Sol. in water</u>	<u>Sol. at DR</u> <u>Sol. in water</u>
Barbital	long	30-60 min. ^a	12	16
Metharbital	long	30-60 min. ^a	21	25
Butabarbital	intermediate	20-30 min. ^a	34	100
Vinbarbital	intermediate	20-30 min. ^a	40	90
Thiopental	ultrashort	30 sec. ^b	670	1200
Thiamylal	ultrashort	20-60 sec. ^b	2300	>2300

^aOral administration

^bIntravenous administration

is found with the ratios of the solubility at the DR to that in pure water. The only exception is in the latter case where the ratio for vinbarbital is less than that of butabarbital. However, the difference between these two values represents a deviation of only about 10% and the general trend can still be observed. In the latter case a distinction may be seen in the ratios for the three pairs of compounds possessing similar durations of actions. The values for the ultrashort compounds are 10 to 20 times those of the intermediate group which in turn are increased over the long acting derivatives, by a factor of about 5.

This correlation is rather good in view of the nature of the solvents. Such a relationship might be expected with pure water and ethanol which anchor the ends of the spectrum of solvent composition. Between these endpoints, however, non-ideal solvents are involved and this cosolvency phenomenon produces solubilities which deviate from that which may be expected of ideal solutions.

It may be conjectured that the magnitude of these ratios are an indication of the extent to which these compounds become concentrated in the less polar biological fluids. Thus, the derivatives possessing a higher ratio, i.e. thiopental and thiamylal, become concentrated to a high-

er degree in the body lipids than do barbital or metharbital and might be expected to be ultrashort acting.

In considering this solubility data in total, an approximate correlation has been observed between the lipophilic nature of the various barbiturate analogs and their therapeutic action. One must view this study with proper perspective in relation to the numerous other physical and chemical properties as well as the various biopharmaceutical parameters which all contribute to the variation in the final therapeutic activity possessed by the members of this series. It is the net result of the complex interaction of these and other factors which determine the type and degree of the pharmacological activity which is involved.

V. SUMMARY

1. A dielectric requirement (DR), was determined for each of the barbiturates studied. In the case of thiamylal a solubility peak was not observed, but due to the rapid decrease in the initial portion of the curve, the DR was assumed to exist below 24.2, i.e. the dielectric constant of ethanol.

2. As the chemical series was descended, the DR's shifted to lower magnitudes corresponding to a general increase in the number of carbon atoms in the molecule. This trend was interpreted as an indication to greater lipophilicity of the molecules due to the increase in carbon atoms.

3. An approximate correlation existed among the duration and onset of pharmacological activity and the DR. Those derivatives possessing higher DR's show a longer duration and onset of activity.

4. The magnitudes of the solubility of these barbiturates in pure water also demonstrated a decrease with a corresponding general increase in carbon atoms, covering

two orders of magnitude. The solubility in ethanol and at the DR did not illustrate this relationship.

5. Ratios of the solubility of these compounds in pure ethanol and at the DR, to that found in water, showed this same inverse relationship. Differentiation in the therapeutic activity was reflected in the magnitude of the ratios of the solubility at the DR to that in pure water.

6. Each of the individual solubility profiles exhibited a limited region in which the solubility was approximately a linear function of solvent composition. The rates of change of solubility in these portions of the curves were computed.

7. A brief consideration of the tautomeric structures of these derivatives was discussed. It was noted that limitations in the number of tautomeric species possible for the N-methyl derivative occurred relative to those conceivable for the non-methylated compounds.

VI. REFERENCES

- (1) T.C. Daniels and E.C. Jorgensen, Textbook of Organic Medicinal and Pharmaceutical Chemistry, 4th ed., ed. by C.O. Wilson and O. Gisvold, (Philadelphia: J.P. Lippincott Co., 1962), p. 377.
- (2) T.O. Soine and R.E. Willette, ibid., p. 557.
- (3) R.H. Travis and G. Sayers, The Pharmacological Basis of Therapeutics, 3rd ed., ed. by L.S. Goodman and A. Gilman, (New York: Macmillan Co., 1968), p. 1608.
- (4) F.J. Millero, J.C. Ahluwalia and L.G. Hepler, J. Chem. Eng. Data, 10, 199 (1965).
- (5) J.P. Leyda, D.J. Lamb and L.E. Harris, J. Am. Pharm. Assoc., Sci. Ed., 49, 581 (1960).
- (6) D.J. Lamb and L.E. Harris, ibid., 49, 538 (1960).
- (7) R.J. Mesley and R.L. Clements, J. Pharm. Pharmacol., 20, 341 (1968).
- (8) A.L. Tatum, Physiol. Rev., 19, 472 (1939).
- (9) E.E. Swanson, W.E. Fry and O.W. Regan, J. Am. Pharm. Assoc., Sci. Ed., 34, 183 (1945).
- (10) W.H. Hunt, R.J. Fosbinder and O.W. Barlow, ibid., 35, 231 (1946).
- (11) Ibid., 35, 244 (1946).
- (12) A.W. Dox and A.M. Hjort, J. Pharmacol. Exp. Therap., 31, 455 (1927).
- (13) N.B. Eddy, ibid., 33, 43 (1928).

- (14) A.M. Hjort and A.W. Dox, ibid., 35, 155 (1929).
- (15) J.E. Falk, Australian J. Sci., 7, 48 (1944).
- (16) T.C. Daniels and E.C. Jorgensen, Textbook of Organic Medicinal and Pharmaceutical Chemistry, 4th ed., ed. by C.O. Wilson and O. Gisvold, (Philadelphia: J.P. Lippincott Co., 1962), p. 15.
- (17) J. Ferguson, Proc. Roy. Soc. (London), ser. B, 127, 387 (1939).
- (18) L. Pauling, Science, 134, 15 (1961).
- (19) L.J. Mullins, Chem. Rev., 54, 289 (1954).
- (20) T.C. Daniels, Ann. Rev. Biochem., 12, 447 (1943).
- (21) B.E. Ballard, J. Pharm. Sci., 57, 357 (1968).
- (22) L.J. Ravin and P.D. Bernardo, ibid., 57, 1075 (1968).
- (23) J.G. Wagner, ibid., 50, 359 (1961).
- (24) L.S. Schanker, J. Med. Pharm. Chem., 2, 343 (1960).
- (25) P. Finholt and S. Solvang, J. Pharm. Sci., 57, 1322 (1968).
- (26) A. Goldstein and L. Aronow, J. Pharmacol. Exp. Therap., 128, 1 (1960).
- (27) L.C. Mark, E. Bernstein and B. Brodie, Federation Proc., 9, 300 (1950).
- (28) E. Garrett and P. Chemburkar, J. Pharm. Sci., 57, 949 (1968).
- (29) L.S. Schanker, Pharmacol. Rev., 14, 501 (1962).
- (30) J.G. Wagner, Drug Intelligence, 2, 181 (1968).
- (31) C. Adrian, M. Hogben, D.J. Tocco, B.B. Brodie and L.S. Schanker, J. Pharmacol. Exp. Therap., 125, 275 (1959).

- (32) J.G. Wagner, Drug Intelligence, 2, 244 (1968).
- (33) G. Levy and J. Anello, J. Pharm. Sci., 57, 101 (1968).
- (34) J.H. Fincher, D.N. Entrekin and C.W. Hartman, ibid., 55, 23 (1966).
- (35) K. Kakemi, T. Arita, R. Hori and R. Konishi, Chem. Pharm. Bull. (Tokyo), 15, 1534 (1967).
- (36) Ibid., 15, 1883 (1967).
- (37) S. Riegelman, J.C. Loo and M. Rowland, J. Pharm. Sci., 57, 117 (1968).
- (38) A.H. Goldberg, W.I. Higuchi, N.F.H. Ho and G. Zografii, ibid., 56, 1432 (1967).
- (39) R.G. Stehle and W. Higuchi, ibid., 56, 1367 (1967).
- (40) L.C. Mark, J.J. Burns, L. Brand, C.I. Campomanes, N. Trousof, E.M. Papper and B.B. Brodie, J. Pharmacol. Exp. Therap., 123, 70 (1958).
- (41) K.B. Bischoff and R.L. Dedrick, J. Pharm. Sci., 57, 1346 (1968).
- (42) J. Hildebrand and R. Scott, Regular Solutions, (Englewood Cliffs, N.J.: Prentice-Hall, Inc., 1962) p. 15.
- (43) A.N. Martin, Physical Pharmacy, (Philadelphia: Lea and Febiger, 1966), p. 356.
- (44) A.N. Paruta, B.J. Sciarrone, N.G. Lordi, J. Pharm. Sci., 51, 704 (1962).
- (45) N.G. Lordi, B.J. Sciarrone, T.J. Ambrosio and A. Paruta, ibid., 53, 463 (1964).
- (46) W. Gorman and G. Hall, J. Pharm. Sci., 53, 1017 (1964).
- (47) W.E. Moore, J. Am. Pharm. Assoc., Sci. Ed., 47, 855 (1958).

- (48) W.E. Moore, J. Pharm. Sci., 51, 391 (1962).
- (49) G. Akerlof, J. Am. Chem. Soc., 54, 4125 (1932).
- (50) F.E. Critchfield, J.A. Gibson, Jr., and J. Hall, ibid., 75, 1991 (1953).
- (51) D.L. Sorby, R.G. Bitter and J.G. Webb, J. Pharm. Sci., 52, 1149 (1963).
- (52) A.N. Paruta, B.J. Sciarrone and N.G. Lordi, ibid., 53, 1349 (1964).
- (53) Ibid., 54, 1325 (1965).
- (54) A.N. Paruta, ibid., 58, 204 (1969).
- (55) Ibid., 58, 216 (1969).
- (56) L. Reber and C. Pathamanon, Am. J. Pharm., 139, 234 (1967).
- (57) A.N. Paruta, personal communication.
- (58) R.T. Coutts and R.A. Locock, J. Pharm. Sci., 57, 2096 (1968).
- (59) Z.F. Ahmed, Z.I. El-Darawy, M.N. Aboul-Enein, M.A. Abu El-Naga and S.A. El-Leithy, ibid., 55, 433 (1966).
- (60) E. Brochmann-Hanssen and A. Baerheim Svendsen, ibid., 50, 804 (1961).
- (61) Ibid., 51, 494 (1962).
- (62) M. Elefant, L. Chafety and J. Talmage, ibid., 56, 1181 (1967).
- (63) T.L. Brown, ibid., 52, 274 (1963).
- (64) D.M. Stokes, W.J.R. Camp and E. Kirch, ibid., 51, 379 (1962).
- (65) R.E. Stuckey, Quart. J. Pharm. Pharmacol., 14, 217 (1941).

- (66) U.S. Food and Drug Administration, Ultraviolet Absorption Data of Pharmaceuticals, by C.N. Andres and S.W. Snow, Washington, D.C.
- (67) System/360 Scientific Subroutine Package (360A-CM-03X) Version III Programers Manual, (White Plains, N.Y.: International Business Machines Corp., 1968), p. 408.
- (68) B. Ostle, Statistics in Research, (Ames, Iowa: Iowa State College Press, 1954), chapter 6.
- (69) T.C. Daniels and E.C. Jorgensen, Textbook of Organic Medicinal and Pharmaceutical Chemistry, 4th ed., ed. by C.O. Wilson and O. Gisvold, (Philadelphia: J.P. Lippincott Co., 1962), pp. 334-38.
- (70) S.K. Sharpless, The Pharmacological Basis of Therapeutics, 3rd ed., ed. by L.S. Goodman and A. Gilman, (New York: Macmillan Co., 1968), p. 107.

VII. APPENDIX

VII. APPENDIX

A. Program for the Method of Least Squares Written in FORTRAN IV Computer Language

```
DIMENSION X(20),Y(20),XD(20),YD(20)
DIMENSION XDS(20),YDS(20),XY(20)
READ(5,2)N
2 FORMAT(I2)
DO 4 I=1,N
  READ(5,3)X(I),Y(I)
3 FORMAT(2F10.0)
4 CONTINUE
  XSM=0
  YSM=0
  DO 5 I=1,N
    XSM=XSM+X(I)
    YSM=YSM+Y(I)
5 CONTINUE
  XA=XSM/N
  YA=YSM/N
  YYS=0
  XDSS=0
  YDSS=0
  DO 6 I=1,N
    XD(I)=X(I)-XA
    YD(I)=Y(I)-YA
    XDS(I)=XD(I)**2
    YDS(I)=YD(I)**2
    XY(I)=XD(I)*YD(I)
    YYS=YYS+XY(I)
    XDSS=XDSS+XDS(I)
    YDSS=YDSS+YDS(I)
6 CONTINUE
  SLOPE=YYS/XDSS
  R=YYS/SQRT(XDSS*YDSS)
  WRITE(6,12)R,SLOPE
12 FORMAT(//,T30,'R= ',10X,F10.5,/,T30,'SLOPE = ',6X,F10.5)
  B=YA-(SLOPE*XA)
```

```

WRITE(6,13)B
13 FORMAT(/,T30,'Y INTERCEPT = ',F10.5//)
STOP
END

```

Input	Output
N = Number of sets of observations	Correlation coefficient
X = X values	Slope
Y = Y values	Intercept

B. Program Calculating Concentration from Absorbance (A),
Written in FORTRAN IV Computer Language

```

WRITE(6,3)
3 FORMAT(10X,'SAMPLE NO.',6X,'MG/ML',/)
READ(5,4)N
4 FORMAT(12)
READ(5,5)Z
5 FORMAT(F10.0)
DO 6 I=1,N
  READ(5,7)C,D,E,F,A
7 FORMAT(4F5.0,F10.0)
  IF(E) 101,102,101
102 E=1.
  F=1.
101 AG2=A/Z
  AG=((AG2*D*F)/(C*E))/1000.
  WRITE(6,8)I,AG
8 FORMAT(13X,I2,F17.5)
6 CONTINUE
STOP
END

```

Input	Output
N = Number of Samples	Sample number
Z = Absorptivity, A/mcg. per ml.	Concentration, mg./ml.
C,D,E,F = Dilutions, mls.	
A = Absorbance	

C. Program Calculating Average and Standard Deviation,
Written in FORTRAN IV Computer Language

```

      DIMENSION Y(12)
      WRITE(6,9)
9  FORMAT(12X,'SAMPLE',2IX,'STANDARD',/,14X,'NO.',
110X,'MEAN',7X,'DEVIATION',/)
      READ(5,10)M
10  FORMAT(I2)
      DO 3 L=1,M
      READ(5,5)N,(Y(I),I=1,N)
5  FORMAT(I2,12F6.0)
      YSUM=0.
      DO 6 I=1,N
      YSUM=YSUM+Y(I)
6  CONTINUE
      YAVE=YSUM/N
      YDSQSM=0
      DO 7 I=1,N
      YDEVSQ=(Y(I)-YAVE)**2
      YDSQSM=YDSQSM+YDEVSQ
7  CONTINUE
      S=SQRT(YDSQSM/(N-1))
      WRITE(6,8)L,YAVE,S
8  FORMAT(13X,I3,F16.5,F14.5)
3  CONTINUE
      STOP
      END

```

Input

M = Number of sets of data
 N = Number of observations
 in each set
 Y = Observations

Output

Sample number
 Average
 Standard deviation

D. Program Subtracting Corresponding Observations in Two
Sets of Data, Written in FORTRAN IV Computer Language

```

      DIMENSION X(100),A(100),B(100),C(100)
      READ(5,1)N
1  FORMAT(I3)
      READ(5,2)(X(I),A(I),I=1,N)
2  FORMAT(2F6.0)
      READ(5,3)(B(I),I=1,N)
3  FORMAT(I7,F6.0)

```

```

DO 4 I=1,N
C(I)=A(I)-B(I)
4 CONTINUE
WRITE(6,5) (X(I),C(I),I=1,N)
STOP
END

```

Input	Output
N = Number of observations per set	Solvent composition Difference
X = Solvent composition	
A = First set of data	
B = Second set of data	

E. Program Calculating a Polynomial Equation Describing a Set of Data

Polynomials of successively increasing degrees are calculated for a given set of x and y values. The residual sum of squares for each higher degree polynomial is compared with the value for the previous equation. When the residual sum of squares shows no reduction, the program terminates.

The output consists of the intercept and the coefficients for each of the polynomials computed. An analysis of variance is also calculated and displayed for each polynomial. A table is printed of the residuals calculated from the input data and that estimated by the second highest degree polynomial calculated. The input and estimated data are then simultaneously reproduced in a form similar to a graph.

A further description and a display of this program are found in Reference (67). The program was modified slightly to perform all calculations in double precision and to accommodate in the table of residuals the percent difference between the observed and estimated values.

F. Program Calculating the Ratios between Corresponding Observations in Two Sets of Data, Written in FORTRAN IV Computer Language

```

      DIMENSION X(100),A(100),B(100),C(100)
      READ(5,1)N
1  FORMAT(I3)
      READ(5,2)(X(I),A(I),I=1,N)
2  FORMAT(2F6.0)
      READ(5,3)(B(I),I=1,N)
3  FORMAT(T7,F6.0)
      DO 4 I=1,N
        C(I)=A(I)/B(I)
4  CONTINUE
      WRITE(6,5)(X(I),C(I),I=1,N)
      STOP
      END

```

Input

N = Number of observations
 per set
 X = Solvent composition
 A = First set of data
 B = Second set of data

Output

Solvent composition
 Ratio