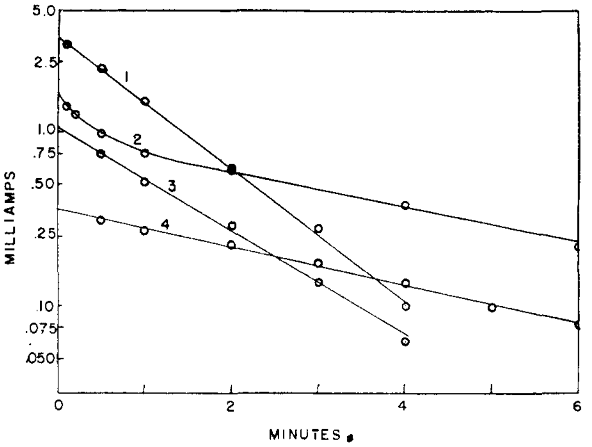

Fig. 6.—Current (log scale) as a function of time in constant potential stripping of amalgams: 1, 10^{-4} *M* cadmium amalgam decomposed at 0.0 volt *vs.* S.C.E.; 2, 10^{-4} *M* cadmium amalgam decomposed at −0.4 volt *vs.* S.C.E.; 3, 10^{-4} *M* thallium amalgam decomposed at 0.0 volt *vs.* S.C.E.; 4, 10^{-4} *M* thallium amalgam decomposed at −0.45 volt *vs.* S.C.E.

should be given by the expression $2.303\ \Delta \log I = Ak'/nFv'\ (\Delta t)$, where the symbols have the same meaning previously ascribed to them. This expression was used to calculate the value of k' which was used to calculate the theoretical curve in Fig. 3.

When attempts were made to decompose deposits of cobalt and nickel electrolytically, the results indicated that the metals could not be removed from the mercury at an appreciable rate at potentials negative enough to prevent the dissolution of mercury. This is in agreement with the findings of Coriou, Hure and Meunier.[3]

When a polarogram of nickel ion in 0.1 *M* potassium chloride was run at this electrode without stirring and a portion of the nickel was allowed to accumulate in the amalgam before rescanning the potential in the reverse direction, it was found that the current due to the dissolution of the nickel, if any, was obscured by the dissolution of mercury from the pool. However, when the medium was changed to 0.1 *M* potassium thiocyanate, an anodic polarogram showed some solution of nickel before the dissolution of mercury commenced. Attempts for normal decomposition were not successful. Preliminary experiments indicate that bismuth amalgam is similarly irreversible in a medium of 0.1 *M* sodium tartrate, 0.05 *M* sodium acetate and 0.05 *M* acetic acid.

Conclusion

The results of this work indicate that the electrolytic decomposition of dilute amalgams can be carried to completion in the case of polarographic reversible systems and that insight into the general phenomena may be obtained using conventional Ohm's law and Nernst equation calculations together with appropriate concentration polarization terms. In the case of the decomposition with a constant current, there is the necessity of postulating an intermediate, with indications that this intermediate is a film of mercurous chloride.

Any quantitative evaluation of results is, however, rendered hazardous by the fact that in a well agitated system there seems to be a significant departure from the ideal reversibility observed in polarographic studies.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF NORTH CAROLINA]

The Second Ionization of 5,5-Disubstituted Derivatives of Barbituric Acid[1]

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The apparent ionization exponents concerned with the second dissociation have been determined spectrophotometrically for a series of ten 5,5-disubstituted derivatives of barbituric acid at 38° and ionic strength of 0.1. The values of pK'_2 lie in the range of 11.77 to 12.67.

The 5,5-disubstituted derivatives of barbituric acid, a class comprising a number of compounds extensively used as hypnotic drugs, were recognized even in the earliest studies to behave as weak acids capable of forming salts. The constants for the first ionization have been measured by several investigators for numerous compounds of this type, the most comprehensive study of the problem being that of Krahl.[2] The question of a second ionization has received but little attention notwithstanding the obvious structural possibility of dissociation of protons from both of the nitrogen atoms and the evidence that has been available for some time indicating that such dissociation actually occurs. In one of the earliest synthetic investigations of this type of compound the di-silver salt of 5,5-dimethylbarbituric acid was prepared.[3] In recent years several studies of the ultraviolet absorption of 5,5-disubstituted barbituric acids in aqueous solu-

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[2] M. E. Krahl, *J. Phys. Chem.*, **44**, 449 (1940).

[3] M. Conrad and M. Guthzeit, *Ber.*, **15**, 2844 (1882).

tion have shown that changes occur in the spectra as the *p*H is increased above the range in which the first ionization is essentially complete.[4-7] This spectral shift has been explicitly attributed by some workers to the dissociation of the second proton from nitrogen.[4,7] So far as we are aware, however, measurements of constants for the second ionization have not been reported heretofore.

Particularly with respect to those barbituric acid derivatives that are used as drugs, the second dissociation, while not occurring to a significant extent in the physiological range of *p*H, is nevertheless of practical as well as theoretical interest. Some of the ultraviolet spectrophotometric methods that have been used in the analytical determination of barbituric acid derivatives in biological materials utilize absorption measurements on solutions in which the drug is very largely in the form of the bivalent ion.[8-10] Quantitative knowledge concerning the second ionization might be expected to be helpful in the design of analytical procedures of optimal efficiency. Furthermore the second ionization constant, like the first, is of potential value in the characterization and identification of barbituric acid derivatives, inasmuch as it is a physical constant that can be measured accurately by spectrophotometric methods even if the compound is available only in the form of a very dilute solution of unknown concentration from which isolation of the pure substance would not be feasible.

The present report concerns the measurement of the second ionization exponents for a series of ten 5,5-disubstituted derivatives of barbituric acid. All of these compounds are, or have been, in medicinal use as hypnotic drugs.

Theoretical Basis of the Method.—This method entails the spectrophotometric estimation of the relative concentrations of the various ionization species of the compound in buffers of different *p*H values. If the species with and without the dissociable proton differ in their absorption spectra, the apparent ionization exponent at constant ionic strength may be calculated from the formula

$$pK' = pH - \log \frac{D_1 - D}{D - D_2} \qquad (1)$$

where D_1, D_2 and D are, respectively, the optical densities at a single wave length of solutions of the same total concentration at a value of *p*H at which only the proton-donor species exists, at a value of *p*H at which only the proton-acceptor species exists, and at an intermediate value of *p*H at which both species are present. Implicit in this formulation are the following assumptions: that the various ionic species existing simultaneously absorb independently and additively; that the absorption of each conforms with Beer's law; and that *p*H affects only the concentration of a particular species, not its inherent absorption characteristics. Evidence for the validity of these assumptions was furnished by a study of the absorption spectra of barbital over a wide range of *p*H values. Within the limits of error of the methods there was conformity to the theoretical relationships for a substance undergoing two dissociations, the spectra of the three ionization species being those shown in Fig. 1. The first ionization exponent as calculated from spectrophotometric measurements is in satisfactory agreement with values obtained by other methods.[11]

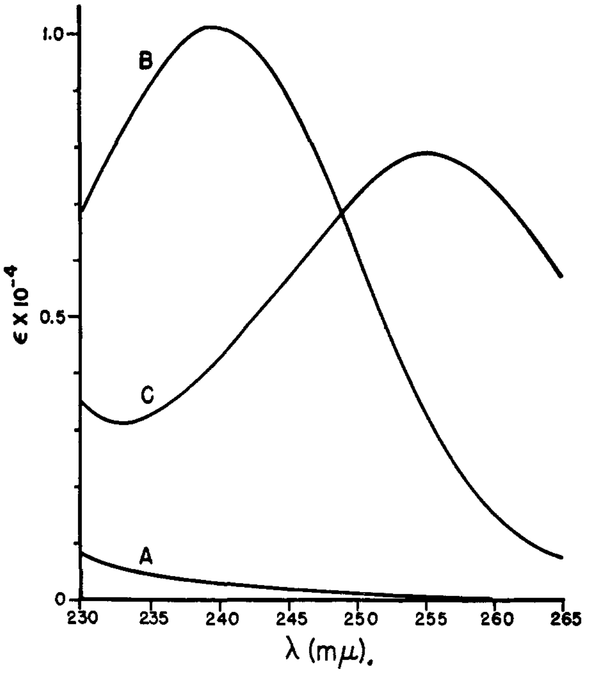

Fig. 1.—Absorption spectra of the three ionization species of 5,5-diethylbarbituric acid (barbital): A, undissociated form; B, univalent ion; C, bivalent ion.

Further indication that the spectral shift at high *p*H values is associated with the loss of the second proton from nitrogen was afforded through the study of the spectra of 1-methyl-5,5-diethylbarbituric acid and 1-methyl-5-ethyl-5-phenylbarbituric acid, compounds in which substitution of a methyl group on one nitrogen atom leaves only one dissociable proton in the molecule. In these compounds the spectral changes associated with change of *p*H conform to the theoretical relationship for a single dissociation. There is no further change in absorption as the *p*H is raised above the values at which one ionization is complete.

The spectra of other barbituric acids in which the two substituents in the 5-position are alkyl or alkenyl groups are almost identical to those of barbital. The spectra of phenobarbital are modified to a small extent by the additional absorption contributed by the aromatic ring. As can be seen in Fig. 1 there are two spectral regions (above and below the isosbestic point at about 249mμ) that can be utilized in the estimation of the second ionization constant. One advantage of the spectropho-

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[4] J. T. Walker, R. S. Fisher and J. J. McHugh, *Am. J. Clin. Pathol.*, **18**, 451 (1948).

[5] T. C. Gould and C. H. Hine, *J. Lab. Clin. Med.*, **34**, 1462 (1949).

[6] P. Lous, *Acta Pharmacol. Toxicol.*, **6**, 227 (1950).

[7] T. C. Butler, *J. Pharmacol. Exp. Therap.*, **106**, 235 (1952).

[8] L. R. Goldbaum, *ibid.*, **94**, 68 (1948).

[9] L. R. Goldbaum, *Anal. Chem.*, **24**, 1604 (1952).

[10] A. Giotti and E. W. Maynert, *J. Pharmacol. Exp. Therap.*, **101**, 296 (1951).

[11] G. G. Manov, K. E. Schuette and F. S. Kirk, *J. Research Natl. Bur. Standards*, **48**, 84 (1952).

tometric method over other methods of measuring ionization constants is that the presence of impurities in the sample of compound will impair accuracy only if the impurities absorb at the wave lengths used and only if the absorption of the impurities changes with pH in the range used in the measurements.

Experimental

The compounds were obtained from the laboratories of the manufacturers or purchased in their commercial forms. All of the samples were the acid forms except vinbarbital and secobarbital, which were in the form of the sodium salts.

Measurements of pH were made with a Beckman Type "E" glass electrode (for high pH) in conjunction with a Cambridge Instrument Co. Model R pH meter. The solution to be studied together with the electrodes were contained in a vessel which was immersed in an electrically shielded bath maintained at a constant temperature of 38°. A stream of carbon dioxide-free nitrogen was blown continually over the surface of the solution. The primary standard of pH was a 0.05 M solution of trisodium phosphate. It was prepared from a special buffer grade of disodium hydrogen phosphate (Fisher Scientific Co.) and a carbonate-free solution of sodium hydroxide. In accordance with the measurements of Bates, *et al.*,[12] it was assigned a pH value of 11.74 at 38°.

Spectrophotometric measurements were made with a Beckman ultraviolet spectrophotometer with the cell compartment maintained at a constant temperature of 38°. Fused silica absorption cells with ground glass stoppers were used.

The solutions of various pH values on which the absorption measurements were made were prepared by dilution of more concentrated stock solutions of each compound. The final concentrations of all of the barbituric acids in all of the solutions were 10^{-4} M. The absorption of the univalent ionic species was studied in a solution of 0.025 M sodium carbonate plus 0.025 M sodium bicarbonate. The pH of this buffer as measured by Bates, *et al.*,[12] is 9.91 at 38°. As the values of pK_1' for the compounds studied lie in the range of 7.2 to 8.0, there will not be a sufficient proportion of the undissociated form of any compound in a solution of this pH to cause significant error. Likewise, as may be calculated from the values of pK'_2 in Table I, there is no significant proportion of bivalent ions. There is sufficient separation between the values of pK'_1 and pK'_2 that the pH of the buffer used for study of the pure univalent ionic species is not a critical value. The absorption of the bivalent ionic species was measured in a 4.6 M sodium hydroxide solution. That the second ionization in this solution is sufficiently near completion for the purposes of the present measurements is indicated by the fact that no significant changes in the absorption spectra are detectable when the concentration of sodium hydroxide is further increased. Measurements were also made in three other solutions of intermediate pH in which both univalent and bivalent ions are present in different proportions. These solutions were all of equal ionic strength (0.1). The compositions (in addition to the barbituric acid) together with the measured values of pH were as follows: 0.050 M sodium hydroxide plus 0.050 M potassium chloride (pH 12.17), 0.075 M sodium hydroxide plus 0.025 M potassium chloride (pH 12.29), and 0.100 M sodium hydroxide (pH 12.40). The concentration of the barbituric acid is too low to have appreciable influence on the pH of these solutions.

TABLE I

THE APPARENT IONIZATION EXPONENT CONCERNED WITH THE SECOND DISSOCIATION IN A SERIES OF 5,5-DISUBSTITUTED DERIVATIVES OF BARBITURIC ACID AT 38° AND IONIC STRENGTH OF 0.1

The names in parentheses are the non-proprietary designations of the U. S. Pharmacopoeia, New and Nonofficial Remedies, and National Formulary.

Barbituric acid derivative	pK'_2
5,5-Diethyl (barbital)	12.31
5-Ethyl-5-isopropyl (probarbital)	12.59
5-Ethyl-5-*sec*-butyl (butabarbital)	12.62
5-Ethyl-5-isopentyl (amobarbital)	12.42
5-Ethyl-5-(1-methylbutyl) (pentobarbital)	12.67
5-Ethyl-5-(1-methyl-1-butenyl) (vinbarbital)	12.06
5-Ethyl-5-phenyl (phenobarbital)	11.77
5-Allyl-5-isopropyl (aprobarbital)	12.52
5-Allyl-5-isobutyl (allylbarbituric acid)	12.36
5-Allyl-5-(1-methylbutyl) (secobarbital)	12.60

During the time required for the measurements there was no indication from decrease in absorption that any of the compounds except phenobarbital were undergoing hydrolytic opening of the barbituric acid ring. The absorption of phenobarbital in the more strongly alkaline solutions changed to a significant extent but so slowly that satisfactory corrections could be applied by extrapolation to the time the stock solution was diluted.

Absorption measurements were made at 239, 255 and 260 mμ. With three density measurements at each of three values of pH, there are nine combinations in which pH and D can be substituted in equation 1 for the calculation of pK'. The arithmetic mean of these nine calculated values is the figure shown in Table I. The generally close agreement among the nine values for each compound can be taken as an indication of lack of contamination with impurities that would interfere with the method. The error in the value of pK'_2 as determined by this method is estimated to be of the order of ±0.05 unit.

Results and Discussion

The calculated values of the apparent ionization exponents are shown in Table I. They lie in a comparatively narrow range, as do the exponents for the first ionization. Eight of these compounds are included in the series studied by Krahl.[2] While the two exponents are not related in any simple, regular manner, there is a statistically significant level of positive correlation between Krahl's values for pK_1 and the present values for pK'_2. It is noteworthy that the values of both exponents for phenobarbital are lower than the corresponding values for the compounds containing only alkyl or alkenyl substituents. The acid-strengthening inductive effect of the phenyl group is conspicuous in the second dissociation as well as in the first.

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[12] R. G. Bates, G. D. Pinching and E. R. Smith, *J. Research Natl. Bur. Standards*, **45**, 418 (1950).