A Rapid Ultraviolet Spectrophotometric Method for the Detection, Estimation and Identification of Barbiturates in Biological Material

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(*Received* 12 *October* 1955)

Ultraviolet spectrophotometric methods for the determination of 5:5′-disubstituted barbiturates in biological material are based on the fact that these exist in three forms in solution: (1) an un-ionized form in acid solution, with almost no selective light absorption in the range 230–270 mμ.; (2) first ionized form at pH 9·8–10·5 with an absorption maximum at 240 mμ. but no minimum in the range 230–270 mμ.; (3) a second ionized form at pH 13–14 with an absorption maximum at 252–255 mμ. and a minimum at 234–237 mμ.

Walker, Fisher & McHugh (1948), Lous (1950, 1954), Wright & Johns (1953) and others have used methods in which the light extinction was measured at 240 mμ. at pH 10 and in acid solution. Barbiturate was detected by the finding of an absorption maximum at this wavelength at pH 10, and its concentration was proportional to the difference, $E_{pH\,10} - E_{acid}$. Salicylic acid and sulphonamides also absorb in this region of the spectrum, and may therefore interfere with the detection and estimation of barbiturate by these methods. Walker *et al.* (1948) and Wright & Johns (1953) found that recovery of barbiturate, added to blood and determined by their methods, was low (55–70%), and applied correction for this. Born (1949) noted that blood extracts, prepared by the method of Walker *et al.* (1948), may become turbid after the addition of acid, and the present author has confirmed this.

Goldbaum (1948, 1952) developed a method for use with blood, urine and tissues, in which barbiturate was detected by the characteristic absorption spectra at 228–270 mμ. in solutions at pH 13 and at pH 10. The extinction difference, $E_{pH\,13} - E_{pH\,10}$, at 260 mμ., was proportional to barbiturate concentration, and in solutions containing no barbiturate $E_{pH\,13} = E_{pH\,10}$. This method appeared to be specific and gave good recoveries of added barbiturate, and has therefore been used as a basis for the work reported here.

Methods currently used for the identification of particular barbiturates extracted from biological material are usually slow and insensitive. Wolff (1951) developed a series of micro tests, some of which depended on qualitative differences in the extent of alkaline hydrolysis of different barbiturates under standard conditions. Goldbaum (1952) mentioned that attempts were being made to differentiate closely related barbiturates by their rates of decomposition in alkali, and described a method of distinguishing different barbiturates by comparing ratios of $E_{pH\,13} - E_{pH\,10}$ at different wavelengths.

This paper describes a critical examination of the variables in Goldbaum's (1952) method, and a modification is recommended for the detection and estimation of barbiturates in biological material. The influence of other materials on the absorption spectra obtained in this method has been investigated and the interpretation of these spectra is discussed. A method has been developed for the partial identification of barbiturates by measurement of the amount of alkaline hydrolysis under standard conditions.

EXPERIMENTAL

Materials. A.R. grade chemicals were used. The pure barbiturates used as standards are listed in Table 1, and were obtained commercially. These may be prescribed as either the acid or the sodium salt, and all concentrations reported in this paper are of the free dibasic acids.

Method

Barbiturate present in blood was extracted with $CHCl_3$, which was then extracted with 0·45N-NaOH. The absorption spectrum of the barbiturate in the clear alkaline solution was measured at pH 13·4 and at pH 10 (approx.) from 228–265 mμ. in 1 cm. silica cells, with a Unicam SP. 500 spectrophotometer.

Reagents. 0·6M-H_3BO_3–KCl; 0·5M-K Na phosphate buffer, pH 7·4; 0·45N-NaOH; $CHCl_3$; borate blank solution, prepared by mixing equal vols. of 0·45N-NaOH and 0·6M-H_3BO_3–KCl.

Extraction. Whole blood (5–10 ml.) is extracted three times with about 30 ml. of $CHCl_3$; the use of insufficient $CHCl_3$ tends to produce emulsions. The combined $CHCl_3$ extracts are filtered through an 11 cm. Whatman no. 31 filter paper into a separating funnel, and the paper is washed with a little $CHCl_3$. The $CHCl_3$ solution is extracted with 0·45N-NaOH (usually 5 ml., but 10 ml. if the rate of alkaline hydrolysis is to be measured) by vigorous shaking for 1 min. After separation of the phases the aqueous layer is clarified by centrifuging.

With urine, gastric contents and other liquid samples it is often advisable to take larger volumes (i.e. 10–25 ml.). The sample is acidified with H_2SO_4 and extracted as described above and the combined $CHCl_3$ extracts are washed twice with 5 ml. of phosphate buffer before filtering. Blood extracts suspected of containing salicylic acid or sulphonamides should also be washed twice with phosphate.

Tissues may be extracted by homogenizing a weighed sample (1–5 g.) with $CHCl_3$ and then proceeding as described for urine.

Hydrolysis. The clear 0·45N-NaOH extract (5 ml., preferably containing more than 100 μg. of barbiturate) is heated in a tube (calibrated at 5 ml.) in a boiling-water bath for exactly 15 min., and cooled rapidly in cold water and made up to 5 ml. with distilled water. The solution may froth slightly at the beginning of the heating owing to volatilization of traces of $CHCl_3$.

Measurement. Portions (2 ml.) of the 0·45N-NaOH extract are added to 2 ml. of 0·45N-NaOH and to 2 ml. of 0·6M-H_3BO_3–KCl. The extinctions, E_N and E_B, of these two solutions are measured from 227 to 265 mμ., with 0·45N-NaOH as a blank for the former and the borate-blank solution for the latter. Particular attention should be paid to the wavelengths at which maxima, minima and isosbestic points occur (Fig. 1). It is advisable to measure the extinction of the borate solution first; if necessary this solution must be diluted with the borate-blank solution until the extinction at 240 mμ. is, preferably, 1·0–1·5. Measurement of the extinction at other wavelengths and reference to Fig. 1 will often enable this dilution to be found. The NaOH solution must be diluted with 0·45N-NaOH by the same amount before measurement.

The same procedure is used with the solution after hydrolysis.

Calculation and interpretation of results. The criteria for the spectrophotometric detection of barbiturates are as follows (see Fig. 1): maximum at 238–240 mμ. in borate; maximum at 252–255 mμ. and a minimum at 234–237 mμ. in NaOH; isosbestic points at 227–230 mμ. and at 247–250 mμ. The greatest differences between E_N and E_B are at 260 mμ. (positive) and 236 mμ. (negative).

If all these criteria are obtained barbiturate may be said to be present. Dilute solutions (Fig. 4), however, may not give all these criteria and other compounds (see Figs. 5 and 6) may give some of them. Proof of the presence of barbiturates in such solutions must therefore depend on the absorption spectrum obtained; the mere finding of an extinction difference at 260 mμ. is not such a proof.

If c is the concentration of barbiturate (μg./ml.) in the solution in the spectrophotometer cell, and K_N and K_B are the specific extinction coefficients of the barbiturate in NaOH and borate solution respectively, then $E_N = K_N c + x_N$ and $E_B = K_B c + x_B$, where x_N and x_B represent absorptions due to materials other than barbiturate. At 260 mμ. $x_N \simeq x_B$ (Fig. 2), so that at this wavelength $E_N - E_B = c(K_N - K_B)$ or $c = F(E_N - E_B)$, where $F = 1/(K_N - K_B)$, and is independent of concentration (Table 1).

The barbiturate may be partially identified by measuring the percentage of barbiturate remaining, R, after alkaline hydrolysis, and using values of R given in Table 1. A complete analysis, including alkaline hydrolysis and calculation of results, may be made within an hour.

RESULTS

Detection and estimation of barbiturate

Fig. 1 shows the absorption spectra at two pH values of a barbitone solution. The solution in 0·45N-NaOH (pH 13·4, Ingles & Israel, 1948) has a maximum at 255 mμ. and a minimum at 234 mμ., and the borate solution (pH approximately 10) has a maximum at 239 mμ. The two curves cross at 227 mμ. and at 249 mμ. Absorption spectra at intermediate pH values (not reproduced) also pass through these points, which are therefore isosbestic points. The maximum positive difference between the extinctions of the NaOH and borate solutions of barbiturate is at 260 mμ. All other 5:5′-disubstituted barbiturates tested gave spectra with these maxima, minima and isosbestic points.

Absorption spectra at these two pH values of a blood extract containing no barbiturate is given in Fig. 2. Extracts from other biological materials gave similar spectra, and in each case $E_N \simeq E_B$ at 260 mμ. This wavelength appeared to be suitable for the estimation of barbiturates.

Effect of NaOH *concentration on* E_B. At 260 mμ. phenobarbitone gave constant values of E_N in

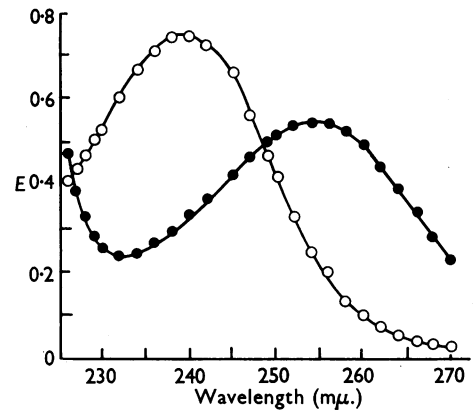

Fig. 1. Absorption spectra of a barbitone solution (13·5 μg./ml.). ●, In 0·45N-NaOH; ○, in borate.

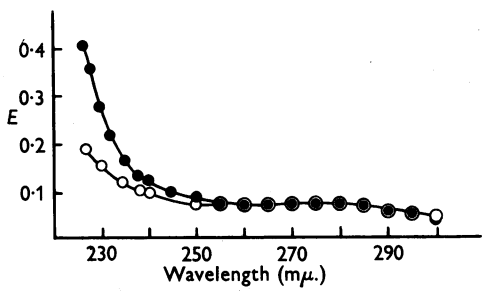

Fig. 2. Absorption spectra of a normal blood extract. ●, In 0·45N-NaOH; ○, in borate.

NaOH solutions stronger than 0·42N, but E_N of quinalbarbitone solutions had not reached a maximum in 0·54N-NaOH. Other barbiturates tested were intermediate between these two. It was considered that 0·45N-NaOH was a suitable solvent for the measurement of E_N, although it was recognized that the dissociation of quinalbarbitone from first to second ionized forms, indicated by the increase of E_N at 260 mμ., was not complete in this solution.

*Effect of boric acid–*KCl *concentration on* E_B. Boric acid–KCl solutions stronger than 0·8M tended to crystallize at room temperature. When solutions weaker than 0·5M were mixed with equal volumes of barbiturate in 0·45N-NaOH the extinctions, E_B, of the mixtures at 260 mμ. were higher than those obtained with the more concentrated boric acid–KCl solutions, indicating that the barbiturate had not been completely converted into the first ionized form because of insufficient decrease in pH. Suitable readings of E_B were given with 0·6M boric acid–KCl, and the pH of the mixture measured with the glass electrode was 9·9.

Effect of NaOH *concentration on* E_B. When equal volumes of 0·6M boric acid–KCl were mixed with barbiturate solutions of varying NaOH concentration the extinction E_B was constant for 0·34–0·56N-NaOH, but increased with higher concentrations of NaOH. Stable, buffered solutions, giving reproducible extinctions, could be obtained with 0·45N-NaOH and 0·6M boric acid–KCl, and these were therefore used in subsequent experiments.

Effect of wavelength on $E_N - E_B$. Results given in Fig. 3 indicate that $E_N - E_B$ reaches a flat maximum at about 260 mμ., and that minor errors in wavelength setting or calibration will not materially affect the measurement of the required value.

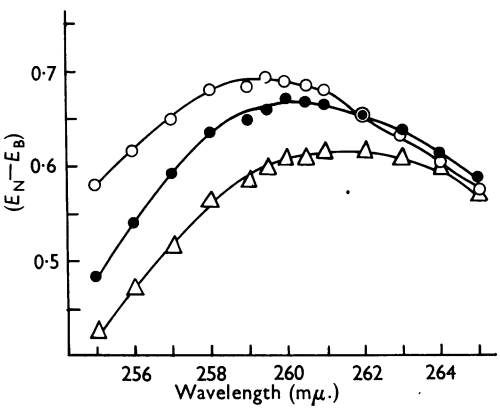

Fig. 3. Effect of wavelength on the extinction difference $(E_N - E_B)$ of barbiturates (30 μg./ml.). ●, Phenobarbitone; ○, amylobarbitone; △, quinalbarbitone.

Calibration. E_N at 260 mμ. was measured for solutions of pure barbiturates in 0·45N-NaOH. These were then mixed with equal volumes of 0·6M boric acid–KCl and E_B was measured. A linear relationship between $E_N - E_B$ and barbiturate concentration was obtained, and values of K_N, K_B and F were calculated for the different barbiturates (Table 1). It should be noted that standard solutions of barbiturate in alkali are not stable.

Biological extracts containing no barbiturate

The determination of barbiturate depends on the fact that, at 260 mμ., $E_N = E_B$ for extracts containing no barbiturate (Fig. 2). In practice, however, small differences were obtained, and analysis of twenty-seven normal urines gave a mean value of $E_N - E_B = +0·003$, S.D. ±0·031. This would be equivalent to an apparent barbiturate concentration in the original urine of ±0·25 mg./100 ml. (±2 S.D.). Normal blood gave similar results.

Biological extracts containing barbiturates

Absorption spectra of extracts from urines containing varying amounts of added barbitone are given in Fig. 4. At small concentrations (0·48 mg./100 ml.) no maxima were obtained (Fig. 4*a*). This spectrum could not be said to prove the presence of barbiturate, but the crossing of the two curves is suggestive when compared with that of a normal extract (Fig. 2). At higher concentrations (0·96 mg./100 ml.) there was a maximum or shoulder at 238–240 mμ. in borate (Fig. 4*b*). Urine containing 6·5 mg. of barbitone/100 ml. gave the typical barbiturate spectrum (Fig. 4*c*): i.e. two isosbestic points, in borate a maximum at 240 mμ., and in NaOH a minimum at 235 mμ. and a maximum at 252 mμ. The precise concentration at which this typical spectrum is obtained cannot be stated, since the amount of general background absorption varies with different extracts and is usually greater for urine than for blood. Increasing the volume of sample extracted will increase the level of this absorption. Characteristic spectra have, however, been obtained with extracts from the following materials: blood containing 0·8 mg. of barbiturate/100 ml.; stomach washings, 0·2 mg./100 ml.; urine and stomach contents, 1·8 mg./100 ml. Spectra similar to Fig. 4*c* have been obtained with extracts of blood, urine, tissues and other pathological material from patients with barbiturate poisoning.

When the seven barbiturates listed in Table 1 were added to blood and urine and determined by the method given, recoveries were good for all except barbitone, for which recoveries were low (mean 90%). It appeared that this material was not completely extracted from $CHCl_3$ by 0·45N-NaOH. Accordingly, the method has been calibrated for barbitone by extracting known amounts, whereas

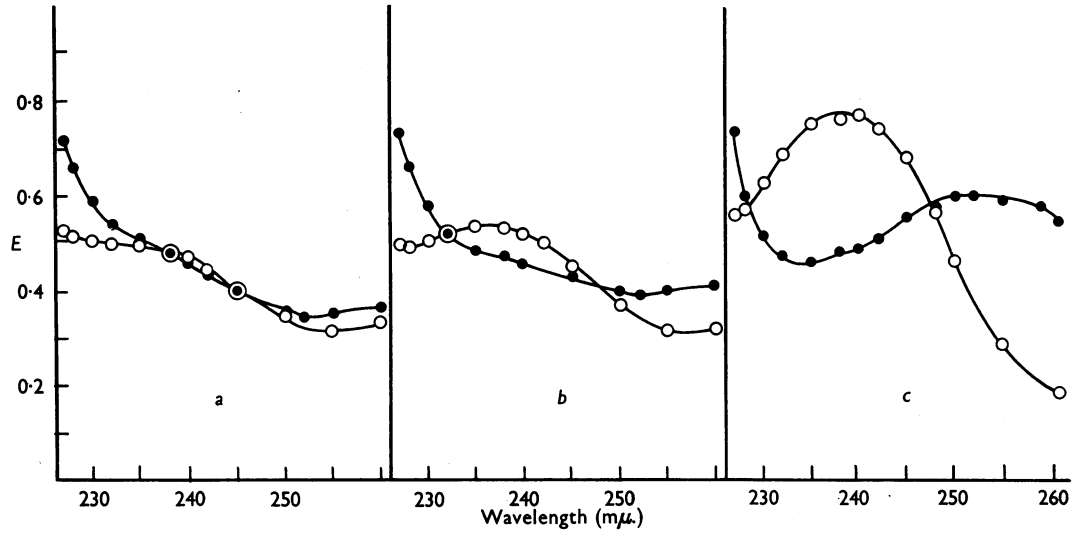

Fig. 4. Absorption spectra of extracts of urines containing added barbitone. *a*, 0·48 mg./100 ml.; *b*, 0·96 mg./100 ml.; *c*, 6·5 mg./100 ml. ●, In 0·45N-NaOH; ○, in borate.

other barbiturates were calibrated directly. Results were then satisfactory, as shown by the following example: barbitone was added to blood to give a concentration of 5·0 mg./100 ml. Eleven separate determinations were made, and 10 ml. of blood was extracted by the recommended method. The mean concentration found was 4·82 mg./100 ml., S.D. ± 0·21 mg./100 ml. The mean ± twice the standard deviation is therefore equivalent to a recovery of 88–105%. The accuracy of the method will obviously depend on the actual value of the extinctions measured.

There was no significant difference between recoveries of barbiturate added to blood and urine, and washing of the extract with phosphate buffer did not result in significant loss of barbiturate.

Interfering materials

The absorption spectra of salicylic acid and sulphanilamide in 0·45N-NaOH, borate and acid solutions are given in Figs. 5*a* and 6*a*. At 260 mμ. E_N is not equal to E_B, and these substances may therefore interfere in the determination of barbiturate unless previously removed.

Extracts of blood containing added salicylate and barbitone gave the absorption spectra given in Fig. 5*b*, with an apparent recovery of 140% of the barbiturate. When the $CHCl_3$ extract of this blood was washed with phosphate buffer the typical barbiturate spectrum was obtained with a satisfactory recovery (85%) of barbiturate. This treatment is therefore effective in removing salicylate. Blood and urine from a patient taking large quantities of salicylate by mouth (blood-plasma salicylate 35 mg./100 ml.) gave values of $E_N - E_B$ within the normal range when extracts were washed with phosphate buffer, and it is concluded that salicylate and its metabolites do not interfere in the determination of barbiturate by this method.

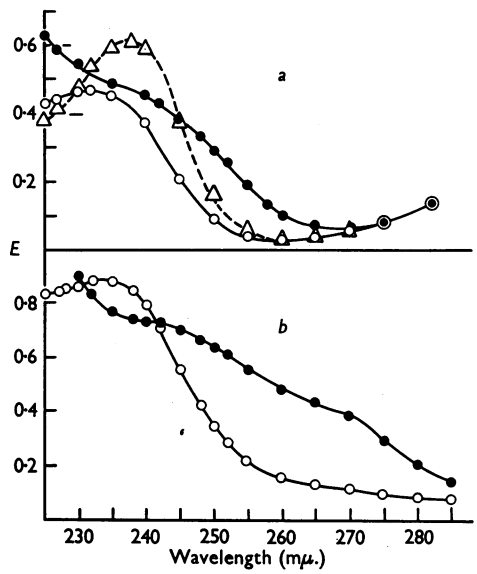

Fig. 5. Absorption spectra of *a*, 0·01 mg. of salicylic acid/ml., and *b*, an extract of blood containing 50 mg. of salicylic acid and 3 mg. of barbitone/100 ml. ●, In 0·45N-NaOH; ○, in borate; △ (broken line), in 0·1N-H_2SO_4.

Fig. 6*b* shows the absorption spectra of an extract of blood containing added sulphanilamide and butobarbitone. The sulphanilamide spectrum distorts that of the barbiturate and results in

apparently low recovery of the latter. When sulphanilamide was added *in vitro* this interference could be reduced by washing with phosphate buffer, but absorption spectra of extracts of urines from patients treated with sulphonamides still showed this interference. It was concluded that sulphonamides and their metabolic products will probably interfere with the determination of barbiturate, but that this may usually be recognized by the distortion of the barbiturate spectrum in the 240–260 mμ. range owing to absorption by sulphonamides at 250–260 mμ. in NaOH and borate.

Megimide (β-ethyl-β-methylglutarimide) has recently been recommended (Shulman, Shaw, Cass & Whyte, 1955) in the treatment of cases of acute barbiturate poisoning, and since the structure of this compound is similar to that of barbiturates, it was thought possible that it might interfere with the analysis of barbiturates. Megimide in 0·45N-NaOH has an absorption maximum at 230 mμ., and E_N is not equal to E_B at 260 mμ. In solutions containing less than 4 mg./100 ml. this extinction difference is not large enough to be measured as barbiturate, and the absorption maximum at 230 mμ. in NaOH is apparent at this concentration, even in the presence of barbiturate. Megimide decomposes rapidly in 0·45N-NaOH and its presence in extracts may therefore be confirmed by repeating extinction measurements on the NaOH solution 30 min. later. Megimide will react with cobaltous salts in a manner similar to that of barbiturates (Koppanyi, Dille, Murphy & Krop, 1934), and positive results have been obtained with this test when applied to extracts of urines containing more than 10 mg. of Megimide/100 ml.

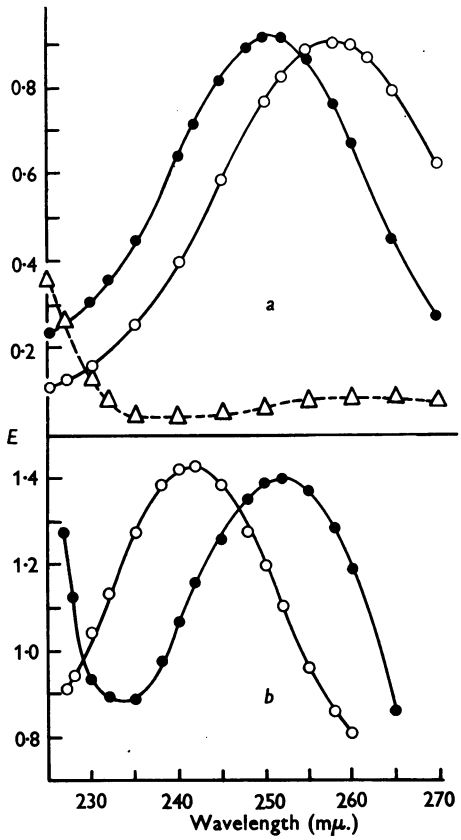

Fig. 6. Absorption spectra of *a*, 0·01 mg. of sulphanilamide/ml., and *b*, an extract of blood containing 10 mg. of sulphanilamide and 4 mg. of butobarbitone/100 ml. ●, In 0·45N-NaOH; ○, in borate; △ (broken line), in 0·1N-H_2SO_4.

Identification of particular barbiturates

Absorption spectra of the seven barbiturates tested were very similar, and no function of E_N and E_B could be found that would enable barbiturates to be identified. The decomposition of barbiturates in alkali was, however, successfully used in their partial identification. Solutions in 0·45N-NaOH were heated in a boiling-water bath and the percentage, R, of barbiturate remaining after a given time found by measuring $E_N - E_B$ at 260 mμ. before and after hydrolysis. After 15 min. hydrolysis R varied from 31·8% for phenobarbitone to 98·2% for pentobarbitone. With the more rapidly hydrolysed barbiturates the time of heating was critical, an extra minute producing up to 3% more destruction. The NaOH concentration at 0·45N was not critical, and R was independent of the concentration of barbiturate in the original solution. The validity of this method depends upon the fact that the products of the hydrolysis have $E_N = E_B$ at 260 mμ. After hydrolysis of a phenobarbitone solution containing 46·6 μg./ml. no barbiturate could be detected spectrophotometrically, and $E_N \simeq E_B$ at 260 mμ.

Values of R obtained after hydrolysis of pure barbiturate solutions for 15 min. are given in Table 1. This method has been applied to materials from patients with barbiturate poisoning and to normal bloods and urines containing added barbiturate; no significant differences were found between the results in these two types of sample. More than seventy specimens have been analysed by this method and the differences between the value of R found with biological extracts and the 'true' value (Table 1), obtained with pure solutions, had a standard deviation of $\pm 5\%$. Thus this method will not identify all the barbiturates listed, but it should at least distinguish the short, intermediate and long-acting ones. For example, in eleven separate analyses on blood containing added barbitone (5·0 mg./100 ml.) the mean value of R found was 46·5, S.D. $\pm$ 2·3. This result would not

Table 1. *Calibration data for 5:5'-disubstituted barbiturates*

K_N and K_B are specific extinction coefficients of barbiturate in NaOH and borate solutions at 260 mμ. F, calibration constant $[1/(K_N - K_B)]$. R, percentage barbiturate remaining after alkaline hydrolysis for 15 min. Recoveries of barbitone after $CHCl_3$ extraction were low (90%), and the values of K_N, K_B and F given for this substance were determined by extracting known amounts of barbitone.

Barbiturate	Substituent groups	Duration of pharmacological action*	K_N	K_B	F	R (%)
Phenobarbitone	Phenyl Ethyl	Long	0·0315	0·0086	43·7	31·8
Barbitone	Ethyl Ethyl	Long	0·0329	0·0062	37·5	42·5
Butobarbitone	*n*-Butyl Ethyl	Intermediate	0·0314	0·0056	38·8	49·6
Amylobarbitone	*iso*Pentyl Ethyl	Intermediate	0·0292	0·0052	41·7	55·8
Cyclobarbitone	Ethyl *cyclo*Hexenyl	Short	0·0302	0·0078	44·7	91·7
Quinalbarbitone	Allyl 1-Methylbutyl	Short	0·0288	0·0078	47·5	97·6
Pentobarbitone	Ethyl 1-Methylbutyl	Short	0·0268	0·0060	48·1	98·2

* Cf. *The Extra Pharmacopoeia* (Martindale, W.), 23rd ed., vol. I, p. 247 (London: The Pharmaceutical Press).

distinguish barbitone from butobarbitone, but does enable a partial identification to be made rapidly and on small quantities of material.

DISCUSSION

Detection

The reaction of barbiturates with cobaltous salts (Koppanyi *et al.* 1934) is widely used as a qualitative test. At least 0·2 mg. of barbiturate is necessary for a positive result and the method, which is by no means specific (Riley, Krause, Steadman, Hunter & Hodge, 1940), cannot be readily applied to blood.

Ultraviolet spectrophotometric methods are rapid and sensitive, and can be used with a wide variety of biological material. An absorption maximum at 240 mμ. at pH 10 is not, however, specific for barbiturates. Goldbaum (1952) showed that barbiturate could be detected by its characteristic absorption spectra at pH 13 and 10, and the present work has confirmed that this is specific. The method, and examples of the characteristic barbiturate absorption spectra, given in this paper may be used to prove the presence of barbiturate in biological material.

Estimation

Goldbaum (1952) did not give the complete experimental evidence for the choice of conditions used in his method. A systematic investigation of the variables in this method largely confirmed the choice of conditions made by Goldbaum. The use of 0·6 M boric acid–KCl, instead of 0·5 M, is recommended, since this increases the buffering of the solution and results in greater reproducibility of extinction readings E_B. Modifications in technique are recommended, and the proposed method is rapid, sensitive and specific.

Salicylic acid and sulphonamides do not have equal extinctions at 240 mμ. in solutions at pH 10 and in acid, and they may therefore interfere in methods for the determination of barbiturate, such as that of Walker *et al.* (1948), based upon extinction differences, $E_{pH\,10} - E_{acid}$, at 240 mμ. These authors recognized this fact, but it remains difficult to detect this interference from the absorption spectra obtained. Salicylic acid and sulphonamides should also interfere in the method of Goldbaum (1952) since $E_{pH\,13}$ is not equal to $E_{pH\,10}$ at 260 mμ. This author stated that sulphadiazine will not interfere, since $E_{pH\,13} = E_{pH\,10}$ for this compound at 260 mμ., but the work reported in the present paper shows that sulphonamides and their metabolites can interfere with this determination and their detection from the absorption spectra is described. Interference by Megimide in this determination may be recognized from the absorption spectra obtained and by its rapid decomposition in 0·45 N-NaOH.

Identification

With the development of quantitative methods it became obvious that the significance of a given blood barbiturate concentration depended on the type of drug present, and this determination was of limited value in poisoning cases unless the barbiturate could, at least, be partially identified

(Wright, 1954, 1955; Broughton, Higgins & O'Brien, 1956).

Of the methods generally used for the identification of barbiturates, organic analysis (Raventos, 1954) needs comparatively large amounts of material, colour reactions (Turfitt, 1948) are only reliable on carefully purified extracts, and paper chromatography (Algeri & Walker, 1952; Allgen, 1953; Wickström & Salvesen, 1952) uses 25–100 μg. of material, but takes 4–7 hr. for a good separation of barbiturates to be achieved. Wright (1954) used ultraviolet spectrophotometry for the determination, and paper chromatography for the identification, of barbiturates in biological material. Goldbaum (1952) found that barbiturates could be partially identified by measuring ratios of $E_N - E_B$ at various wavelengths, but the present author has been unable to confirm this.

Wolff (1951) used micro-analytical methods for the identification of the substituents of barbiturates, including alkaline hydrolysis to the dialkylurea derivatives, some of which had a characteristic crystalline appearance. The present method of identification is a quantitative development of this author's observations on the alkaline hydrolysis of barbiturates. It is intended as a rapid partial identification; further analysis by any of the methods described above may be made on the extracts after spectrophotometric measurements.

Metabolites of barbiturates, in which the pyrimidine ring is intact, have absorption spectra similar to those of the original compounds (Maynert & Dawson, 1952; Lous, 1954), but it is probable that they will be hydrolysed in alkali at a rate different from that of the original barbiturate. Values of R, determined on material obtained from patients with acute poisoning, do not differ significantly from values obtained with pure solutions, and this suggests that the influence of these metabolites is not sufficient to interfere in this method.

The hydrolysis constant R and, with one exception, the calibration constant F appear to be inversely proportional to the duration of action of the different barbiturates (Table 1). These facts are often useful in the analysis of specimens from cases of acute poisoning, where it may be sufficient to classify the barbiturate in this way, rather than to attempt complete identification. Results obtained with this method in cases of acute poisoning are discussed by Broughton *et al.* (1956).

SUMMARY

1. A rapid method is described for the determination of barbiturates in biological material by ultraviolet spectrophotometry.

2. Characteristic absorption spectra of barbiturates in varying concentration are given, and the criteria for their detection are listed. Absorption spectra of salicylate and sulphonamides, which can interfere with the determination, are given and their recognition or removal is described. Interference by Megimide (β-ethyl-β-methylglutarimide) in the detection of barbiturate by this method, and by its reaction with cobaltous salts, is described.

3. A method is described for the partial identification of barbiturates by measurement of the amount remaining after alkaline hydrolysis under standard conditions.

I wish to thank Mr J. R. P. O'Brien for advice and criticism during this work. I also wish to thank British Drug Houses Ltd. for a gift of cyclobarbitone and Nicholas Products Laboratories Ltd. for a gift of Megimide.

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