THE DISTRIBUTION AND EXCRETION OF PHENOBARBITAL [1]

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The 5,5-disubstituted barbituric acids used medicinally are weak acids with ionization exponents in a range such that their dissociation is influenced by physiological changes of pH. There is much evidence that cellular membranes in general are permeable to the undissociated forms of weak acids and impermeable to the ionized forms (1). The experiments of Clowes, Keltch, and Krahl (2) are interpreted as indicating that a number of barbituric acid derivatives penetrate *Arbacia* eggs only as the undissociated molecules. The influence of urinary pH on the renal excretion of weak acids has been explained in terms of tubular permeability to the undissociated species and impermeability to the ionic species (3, 4). Changes in the concentration of the undissociated form of a weakly acidic drug accompanying physiological changes in the pH of blood or urine can have important effects on the distribution, excretion, and pharmacological actions of the drug.

The rate of change of the concentration of undissociated acid with pH is maximal when the pK' of the acid equals the pH. However, of greater interest is the *proportion* by which the concentration of undissociated acid changes with a change of pH, *i.e.*, the rate at which the concentration of undissociated form is changing divided by that concentration. This function is expressed by the following equation,

$$\frac{\frac{d(HA)}{d(pH)}}{(HA)} = -\log_e 10 \frac{10^{pH}}{10^{pH} + 10^{pK'}},$$

in which (HA) is the concentration of undissociated acid. In Figure 1 is shown the proportional rate of change of the concentration of the undissociated form of a weak acid with pH as a func-

tion of pK' for the pH value of 7.4. The function is without a maximum. It is greater the lower the value of pK'.

The pK' of phenobarbital at 37° C. and ionic strength of 0.16 (approximately the ionic strength of the inorganic ions of plasma) is 7.2. Owing to the strong electron-attracting force of the phenyl group in phenobarbital, this drug is a considerably stronger acid than the commonly used barbituric acid derivatives having only alkyl or alkenyl groups in the 5-position. The drugs of the latter types for the most part have pK' values under the same conditions in the range of 7.6 to 8.0 (5).[3] N-methylated barbituric acid derivatives, such as hexobarbital, mephobarbital, and metharbital, have pK' values about 0.4 unit higher than the corresponding non-methylated compounds (6). Thiobarbituric acid derivatives are stronger acids than the analogous oxygen compounds. Thiopental has a pK' value of 7.3 at 37° C. and ionic strength of 0.16.[4] Thus phenobarbital has a lower pK' value than any other commonly used barbituric acid derivative. It is evident from Figure 1 that the proportional change in the concentration of undissociated form resulting from a small change in pH at pH 7.4 is much greater for phenobarbital than for other hypnotic barbituric acids with pK' values of 7.6 to 8.0. For instance, it is 1.8 times as great as for barbital or amobarbital, which have pK' values of 7.7.

Because of the important influence that physiological shifts in pH might be expected to have on the distribution and excretion of phenobarbital, an

[3] By spectrophotometry and by potentiometric titration we have found higher values of pK' for several barbituric acid derivatives at 25° C. than did Krahl and also lower temperature coefficients. Our directly measured values at 37° C. and ionic strength of 0.16 are about 0.2 unit higher than the values calculated from Krahl's data.

[4] This value was determined spectrophotometrically and by potentiometric titration. The value of 8.2 reported by Brodie *et al.* (7) was obtained by titration in ethanol-water solution.
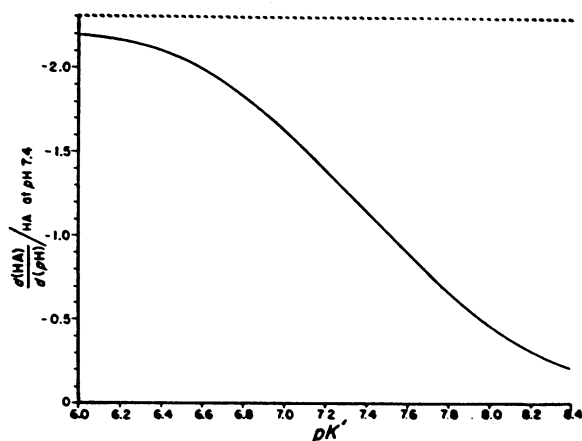
FIG. 1. THE PROPORTIONAL RATE OF CHANGE OF THE CONCENTRATION OF THE UNDISSOCIATED FORM OF A WEAK ACID WITH pH AT pH 7.4 AS IT IS RELATED TO THE pK' OF THE ACID

The dotted line is the asymptote approached by the function as pK' decreases.

investigation has been made of these effects. It was anticipated that the information to be gained might be of value in the treatment of phenobarbital poisoning. An investigation has also been made of other factors influencing the renal excretion of phenobarbital.

METHODS

Determination of phenobarbital. For dog and human plasma and for dog urine the method of Butler, Mahaffee, and Waddell (8) was used. By measurements of partition coefficients between ether and buffers, the specificity of this method for plasma had previously been demonstrated. By the same procedure it has been shown to have adequate specificity when applied to the determination of phenobarbital in dog urine. This method determines bound as well as unbound phenobarbital in plasma.

Human urine, unlike dog urine, contains unconjugated p-hydroxy phenobarbital (9), which interferes with the determination of phenobarbital. The interfering metabolic product was separated by partitioning between benzene and water. To 1 ml. of urine in a glass stoppered tube was added 1 ml. of 1 N HCl and 12 ml. of benzene. The tube was shaken and centrifuged. The aqueous phase was then transferred by pipette to a second tube, where it was shaken with a fresh 12-ml. portion of benzene. After centrifugation, the aqueous phase was removed and discarded. The first benzene extract was shaken with 1 ml. of 0.1 N HCl. After centrifugation, the aqueous phase was transferred to the second benzene extract. After shaking and centrifugation, the aqueous phase was discarded. The two portions of benzene were combined and evaporated to dryness by drawing a stream of air over the surface of the liquid with an impinger. The

residue was taken up in the 1 M phosphate buffer of pH 8 used in the original method (8), and the subsequent steps of that method carried out as usual. When the specificity of this method was investigated by the procedure described by Butler, Mahaffee, and Waddell (8), the ether/buffer partition coefficients were found to conform closely to the values expected for phenobarbital.

The methods used for the determination of phenobarbital in dog and human urine when applied to urine free of drug gave calculated values that did not exceed 5 per cent of the phenobarbital concentrations that were to be experimentally measured in urine. The various drugs that were administered to dogs did not interfere with the determination of phenobarbital in plasma or urine.

Weighed samples of 1 to 5 Gm. of tissues were homogenized with weighed 5-ml. portions of 1 M phosphate buffer of pH 8. The homogenizer was a device consisting of sharp blades mounted on a shaft driven at high speed by an electric motor. A 2-ml. sample of the homogenate was weighed, 1 ml. of the buffer of pH 8 was added, and the subsequent steps of the analytical procedure were carried out in the same way as previously described for plasma (8). When this method was applied to tissues free of drug, the calculated phenobarbital concentrations did not exceed 1 μg. per Gm. Recovery of phenobarbital added to tissues was complete.

Because higher concentrations of phenobarbital were found in liver than in other tissues, the specificity of the method as applied to liver was investigated. A homogenate of a liver sample from a dog that had received phenobarbital was extracted with ether in the same manner as used in the first extraction step of the regular analytical procedure. The ether extract was evaporated and the residue distributed between ether and 1 M phosphate buffer of pH 8.2 in a 30-cell countercurrent distribution train of the type described by Craig, Hausmann, Ahrens, and Harfenist (10). Twenty-two effluent fractions of ether were withdrawn from the train. Fractions 11 to 22, which would contain p-hydroxy phenobarbital, if any were present, were analyzed for that compound by the method described by Butler (9). No evidence was found of the presence of any p-hydroxy phenobarbital. Fractions 1 to 10 were combined and evaporated and the residue was put through the countercurrent distribution train, the light solvent being a mixture of 30 per cent (v/v) ether and 70 per cent 2,2,4-trimethylpentane and the heavy solvent a 1 M phosphate buffer of pH 7.2. Thirty effluent fractions of light solvent were collected. These were individually extracted with 4-ml. portions of the buffer of pH 11 used in the regular analytical procedure. Each buffer extract was washed with 2 ml. of purified ether and the phenobarbital concentration calculated from the difference between the absorbency of the buffer at 240 mμ and that at 260 mμ. In Fractions 11 to 30 the amounts of phenobarbital measured in this way conformed quite closely to the pattern of distribution to be expected for pure phenobarbital. From the amount in the peak fraction the total phenobarbital in the tissue sample was calculated by the equation given by Bush and Densen (11). The amount so calculated was 93 per cent

of that found by the analytical procedure described in the paragraph above. Thus it has been shown that this analytical method as applied to liver has a satisfactory degree of specificity.

Measurements of binding of phenobarbital to protein. The mechanical dialyzer described by Reiner and Fenichel (12) was used. Plasma or a buffered solution of crystallized bovine serum albumin (Pentex Inc., Kankakee, Ill.) or human serum albumin (Cutter Laboratories) was placed in the dialysis bag, which was made of cellophane dialyzer tubing tied at the bottom. The outer chamber was filled with a buffer. Phenobarbital was added either to the solution in the bag alone or to the solutions on both sides of the membrane. The temperature was maintained at 37° C. and stirring of the solution in the bag was continued for 20 to 24 hours. It was found that this time was sufficient for the attainment of equilibrium. Analyses of the solutions on both sides of the membrane furnished the data for calculation of the extent of binding.

Determination of creatinine. Phillips' modification of the method of Folin and Wu (13) was used.

Administration of drugs to dogs and men. Phenobarbital was administered to dogs intravenously as a solution of the sodium salt. The men in whom clearance studies were made were receiving phenobarbital in the form of tablets by mouth. Creatinine was administered to dogs subcutaneously in aqueous solution in a dose of 100 mg. per Kg. about an hour before a clearance determination. All other drugs used in dogs were given intravenously.

Production of acidosis and alkalosis. A mixture of 29 per cent CO_2 + 71 per cent O_2 was administered to dogs with an anesthetic gas machine. A respiration pump was used to produce hyperventilation in dogs. Intravenous injections of $NaHCO_3$ in a solution of 8 Gm. per 100 ml. were given to the dogs and the men. Mice received $NaHCO_3$ intraperitoneally in a dose of 1 Gm. per Kg. as a solution of 10 Gm. per 100 ml.

Collection of urine. Urine was collected by catheter from the dogs and from one of the men in the clearance studies. The other man voided voluntarily.

Anesthetic doses in mice. Carworth Farms CF No. 1 male mice were used. In the experiments with each drug the mice were assigned to the treatments and the doses by the use of a table of random numbers. In the mice in which alkalosis was to be induced, $NaHCO_3$ was injected intraperitoneally 5 minutes before administration of the anesthetic. Phenobarbital and barbital were given intravenously as solutions of the sodium salts. A mouse was considered anesthetized if it could not gain and maintain the standing posture after stimulation by pinching of the tail. Ether was introduced in liquid form into a vessel of 1.9 L. capacity containing a mouse. The mouse was observed for 30 minutes. If at the end of that time the mouse had lost the ability to maintain the standing posture and rolled over continuously when the vessel was rotated, it was considered anesthetized. The calculated concentrations of ether were those present initially before any absorption by the mouse had taken place.

Median anesthetic doses were calculated on the assumption that the curve relating log dose and proportion anesthetized is the integrated normal frequency curve.

Measurements of pH. A Cambridge Instrument Co. Model R glass electrode pH meter with water jacketed electrode assembly maintained at 37° C. was used. The primary standard of pH was a solution containing 0.025 mole of KH_2PO_4 + 0.025 mole of Na_2HPO_4 per L. It was assigned a pH value of 6.84 at 37° C. (14). Venous blood from dogs and men was used for pH measurements. Blood was taken from mice by cardiac puncture. The blood was handled with strict anaerobic precautions, and the measurements were made within 10 minutes after drawing the blood. The pH of urine samples was measured within 10 minutes after their collection.

RESULTS

Binding of phenobarbital to protein

The binding of phenobarbital to serum albumin was measured by the dialysis method. Table I shows the percentage of phenobarbital bound in a 4 Gm. per 100 ml. solution of bovine albumin as it is related to the pH of the solution. The binding is affected only to a small degree by pH over the range studied. Binding decreases as the albumin concentration decreases. In a 1 Gm. per 100 ml. solution of albumin the percentage of phenobarbital bound is about half as great as in a 4 Gm. per 100 ml. solution. In a 4 Gm. per 100 ml. solution of albumin the proportion of phenobarbital bound is almost independent of the phenobarbital concentration over the range of 20 to 100 mg. per L. (total concentrations). Binding to human serum albumin did not differ significantly from that to bovine albumin. Binding in dog and human plasma was found to be approximately the same as in a solution of pure albumin of the same concentration as the plasma albumin. Because the

TABLE I

*Binding of phenobarbital in solutions of bovine serum albumin, 4 Gm. per 100 ml. in phosphate buffers of 310 milliosmoles per L.**

pH	Per cent of phenobarbital bound
6.1	36
7.2	40
7.6	46
8.1	42
8.6	41

* The concentration of unbound phenobarbital at equilibrium was 45 mg. per L.; the temperature was 37° C.
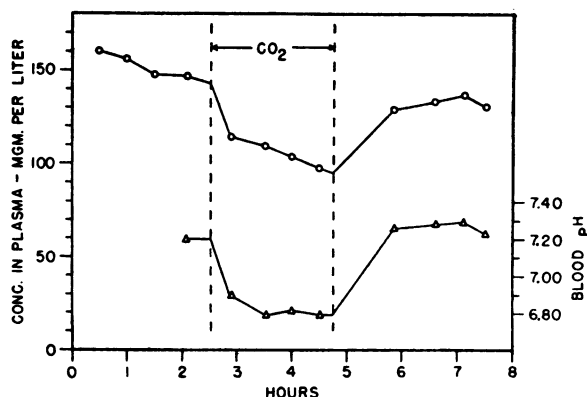
FIG. 2. EFFECTS OF INHALATION OF 29 PER CENT CO_2 IN A DOG ANESTHETIZED WITH AN INTRAVENOUS DOSE OF 125 MG. PER KG. OF PHENOBARBITAL

Phenobarbital concentrations are designated by circles, blood pH's by triangles. Times on the abscissa are measured from the time of phenobarbital administration.

extent of binding is so nearly independent of pH and of phenobarbital concentration in the ranges of interest, plasma binding was not directly measured in the experiments to be described below. In the calculations of renal clearances the assumption was made that 40 per cent of all measured plasma phenobarbital concentrations represented bound drug. This assumption is sufficiently accurate to support the conclusions derived from the calculations.
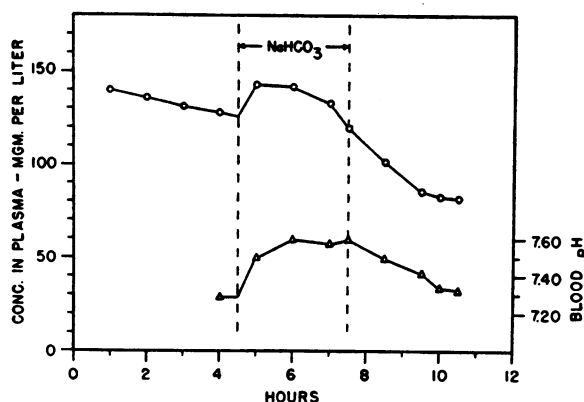

FIG. 3. EFFECTS OF INTRAVENOUS INJECTION OF $NaHCO_3$ IN A DOG ANESTHETIZED WITH AN INTRAVENOUS DOSE OF 125 MG. PER KG. OF PHENOBARBITAL

The total dose of $NaHCO_3$ administered over a period of 3 hours was 2.1 Gm. per Kg. Phenobarbital concentrations are designated by circles, blood pH's by triangles. Times on the abscissa are measured from the time of phenobarbital administration.

TABLE II

Tissue/plasma phenobarbital concentration ratios in the dog as affected by treatments changing the pH of the blood [*]

Treatment	Blood pH	Tissue/plasma ratio			
		Brain	Fat	Liver	Muscle
CO_2	6.76	1.2	1.0		1.0
CO_2	6.78	1.5		2.4	1.4
CO_2	6.80	1.4	1.0		1.1
CO_2	6.80	1.4	0.9	1.8	1.1
None	7.27	0.9	0.6		0.8
None	7.29	1.0	0.7		
None	7.42	0.9	0.6		0.8
Resp. pump	7.60	0.8	0.5	1.1	0.5
Resp. pump	7.70	0.7	0.6	1.1	0.6
$NaHCO_3$	7.70	0.6	0.4	1.0	0.5

[*] The ratios are calculated from total (bound plus unbound) concentrations in plasma. The samples were taken approximately an hour after the beginning of the treatment.

Effect of pH on the distribution of phenobarbital

In several experiments on dogs in which the blood pH was lowered by CO_2 inhalation or raised by hyperventilation or intravenous infusion of $NaHCO_3$, it was consistently observed that the plasma phenobarbital concentration fell as the blood pH fell and rose as the blood pH rose. The results of representative experiments in which acidosis was produced by CO_2 inhalation and in which alkalosis was produced by $NaHCO_3$ infusion are shown in Figures 2 and 3, respectively.

In order to investigate the changes in equilibrium between tissues and plasma that result in changes in the plasma concentration of phenobarbital, tissues were analyzed from ten dogs, three with no treatment to change pH, four made acidotic by CO_2 inhalation, and three made alkalotic by hyperventilation or $NaHCO_3$ infusion. The results are shown in Table II. In all of the tissues studied—brain, fat, liver, and muscle—the tissue/plasma concentration ratio varies in a direction opposite to that of the blood pH.

Effect of pH on anesthesia

It was noted that alkalosis produced by hyperventilation or by $NaHCO_3$ infusion caused a lightening of phenobarbital anesthesia in dogs. A quantitative assessment of this effect was made by measurement of median anesthetic doses in mice without treatment and in mice rendered alkalotic by $NaHCO_3$ administration. The dose of $NaHCO_3$ used, 1 Gm. per Kg., raised the average value

of the blood pH from the normal of 7.23 to 7.41 one-half hour after dosage. As is shown in Table III the median anesthetic dose of phenobarbital is 20 per cent higher in the mice treated with NaHCO₃ than in the normal. Measurement of phenobarbital in urine withdrawn by needle from the urinary bladder showed that the alkalotic mice excreted no more than about 3 per cent of the dose during the interval between the injection and the onset of anesthesia (average, about 15 minutes).

Two other anesthetics were compared with phenobarbital—barbital as an example of a drug with an ionization exponent (7.7) higher than that of phenobarbital, and ether as an example of an undissociable drug. The median anesthetic dose of barbital is raised by only 10 per cent by NaHCO₃ treatment. With ether there is no significant difference between the doses in mice with and without alkalosis. The probability is 0.004 that equivalent doses could differ by as much as 10 per cent.
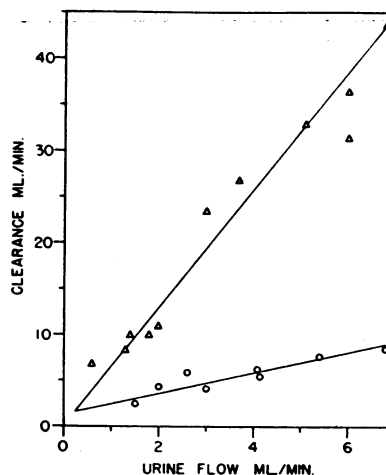

FIG. 4. RENAL CLEARANCE OF PHENOBARBITAL IN THE DOG AS IT IS RELATED TO RATE OF URINE FLOW

The values designated by circles are from experiments in which diuresis was induced by oral water, intravenous mercaptomerin, or intravenous Na₂SO₄ and in which the urinary pH was below 7.0. The values designated by triangles are from experiments in which NaHCO₃ was given intravenously and in which the urinary pH was 7.8 to 8.0. Clearances are calculated from the concentrations of unbound drug in plasma.

Renal excretion of phenobarbital

The renal clearance of phenobarbital was measured in dogs anesthetized with 125 mg. per Kg. of the drug. All clearances will be expressed in terms of the unbound drug in plasma (assumed to be 60 per cent of the total). Clearances based on total plasma concentrations would be 60 per cent of the values to be reported here. Under phenobarbital anesthesia without other treatment the urine flow was scanty, usually about 0.2 ml. per minute, and the phenobarbital clearance was less than 1 ml. per minute. Figure 4 shows the results of experiments in which diuresis was produced by administration of 30 ml. of water per Kg. by stomach tube, 130 mg. of mercaptomerin sodium intravenously, 100 ml. of 0.95 M Na₂SO₄ intravenously, or NaHCO₃ intravenously in isotonic solution or in a solution of 8 Gm. per 100 ml. and in volumes of 50 to 500 ml. Values from experiments in which diuresis was produced by water, mercaptomerin, or Na₂SO₄ are designated by circles. The pH of the urine in these experiments was less than 7.0. Values from experiments in which NaHCO₃ was administered are designated by triangles. In these experiments the pH of the

TABLE III

Anesthetic effects of phenobarbital, barbital, and ether in normal mice and mice treated with 1 Gm. per Kg. of NaHCO₃ intraperitoneally *

Drug and dose	No. of mice anesthetized/total	
	Without NaHCO₃	With NaHCO₃
Phenobarbital		
146 mg./Kg.	2/10	
161	6/10	
177	9/10	1/10
195		6/10
215		10/10
Barbital		
322 mg./Kg.	5/10	1/10
354	8/10	5/10
Ether		
80 mg./L.	6/20	4/20
88	11/20	11/20
97	18/20	17/20

Calculated median doses with their standard errors

Drug	AD 50	
	Without NaHCO₃	With NaHCO₃
Phenobarbital	158± 4 mg./Kg.	191±3 mg./Kg.
Barbital	322±11 mg./Kg.	354±8 mg./Kg.
Ether	85± 2 mg./L.	87±2 mg./L.

* The doses of phenobarbital and barbital are expressed in terms of the acid forms. The values for ether are concentrations in air.
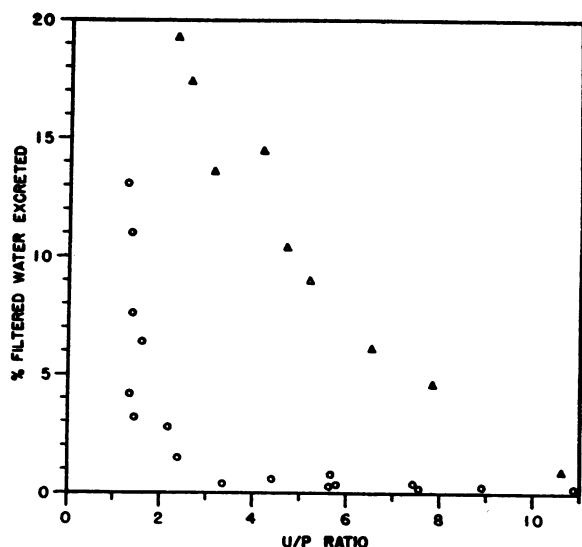
FIG. 5. THE URINE/PLASMA CONCENTRATION RATIO OF PHENOBARBITAL AS IT IS RELATED TO THE PER CENT OF FILTERED WATER EXCRETED BY THE DOG

The values designated by circles are from experiments with and without diuresis in which the urinary pH was below 7.0. The values designated by triangles are from experiments in which NaHCO₃ was given intravenously and in which the urinary pH was 7.8 to 8.0. The U/P ratios are calculated from the concentrations of unbound drug in plasma.

urine was 7.8 to 8.0. The clearance of phenobarbital increases with increasing urine flow in acid or alkaline urine, but at any given rate of flow the clearance is much higher in alkaline urine than in acid urine. At the same levels of urine flow and urinary pH, clearances after administration of acetazoleamide were somewhat lower than after administration of NaHCO₃. Probenecid did not affect the clearance of phenobarbital.

Creatinine clearances, determined simultaneously with those of phenobarbital in dogs, showed that the excretion ratio of phenobarbital was always less than unity. In acid urine it varied from 0.02 at low flows to 0.2 at high flows. In alkaline urine it varied from 0.3 at low flows to 0.7 at high flows.

Figure 5 shows the urine/plasma concentration ratio of phenobarbital plotted against the per cent of filtered water excreted, as calculated from the creatinine clearances. This chart contains some of the experiments of Figure 4 as well as some in which the dogs were not subjected to diuretic treatment. Values from experiments with or

without diuretic treatment in which the urinary pH was below 7.0 are designated by circles. Values from experiments in which NaHCO₃ was administered and in which the urinary pH was 7.8 to 8.0 are designated by triangles. The effect of alkalinization of urine in interfering with the reabsorption of phenobarbital is again evident. With any given percentage of filtered water excreted, the U/P ratio is much higher for alkaline than for acid urine. With high flow rates the U/P ratio approaches a value somewhat over 1 for acid urine and somewhat over 2 for alkaline urine.

The renal clearance of phenobarbital was studied in two men receiving usual therapeutic doses of the drug. The results are shown in Table IV. The effects of water diuresis and of NaHCO₃ treatment are similar to those found in dogs. The clearance increased with increasing rate of urine flow. At comparable flows clearances were higher with alkaline than with acid urine.

DISCUSSION

Protein binding

The extent of plasma binding of phenobarbital that we have found is about the same as was measured by Lous (15, 16) in human serum by ultrafiltration. Goldbaum and Smith (17) found a higher degree of binding to albumin than we did in comparable experiments. Their calculated

TABLE IV

Phenobarbital clearances in two men receiving therapeutic doses of phenobarbital *

Man	Treatment	Urine pH	Flow	Clearance	U/P Ratio
			ml./min.	ml./min.	
	Before treatment	6.7	0.8	2.5	3.1
1	After water p.o.	6.6	9.0	7.5	0.9
	After water p.o.	5.5	6.7	4.3	0.7
1	Before treatment	6.1	0.8	2.0	2.6
	After NaHCO₃ p.o.	8.0	2.3	9.8	4.4
	Before treatment	6.1	1.2	3.0	2.6
2	Before treatment	5.8	0.8	2.0	2.4
	After NaHCO₃ i.v.	8.0	8.0	29.0	3.6
	After NaHCO₃ i.v.	8.0	2.8	13.8	4.9

* The weights of Man 1 and Man 2 were 82 and 70 Kg., respectively; the dose of water was 1 L., of oral NaHCO₃, 50 Gm., and of intravenous NaHCO₃, 14 Gm.; clearances and U/P ratios are calculated from the concentrations of unbound drug in plasma.

binding constants predict considerably more extensive binding than was found in any of our experiments. A possible explanation is that their experimental method entailed an approximate doubling of the protein concentration during the course of the ultrafiltration.

So long as the concentration of phenobarbital is low relative to the number of available binding sites on protein, the proportion bound will be nearly independent of the concentration. These conditions apparently do prevail with normal concentrations of plasma albumin and with concentrations of phenobarbital that can occur in a living animal. The small effect of pH on the extent of binding to albumin is indicative that the intrinsic binding constants for the binding of the undissociated and ionic forms of phenobarbital are nearly equal. The two forms may or may not be bound to the same sites on the protein molecule. In the physiological range of pH, binding of phenobarbital varies so little that it cannot be a factor of any importance in the changes of distribution and excretion accompanying acidosis and alkalosis.

Effect of pH on the distribution of phenobarbital

The change of plasma phenobarbital concentration resulting from change of pH is due to a change in the concentration of the ionic form. The calculated concentration of the undissociated form in plasma undergoes little change or may even change in a direction opposite to that of the total concentration. The shift of phenobarbital between tissues and plasma can be most simply explained by assuming that the intracellular pH remains relatively constant and that the cellular membrane is permeable to the undissociated form but not to the ionic form of the drug.

Brodie et al. (7) found that the plasma concentration of thiopental decreased during a period of acidosis. With this drug the effect is explicable in large part in terms of partitioning of the undissociated form between plasma and fat. It is surprising that alkalosis was not found to change the plasma concentration of thiopental significantly. Jailer, Zubrod, Rosenfeld, and Shannon (18) found that the plasma concentrations of quinacrine and chloroquine increased as the blood pH decreased. This effect is indicative that it is the undissociated forms of these bases that are in-

volved in the equilibration between plasma and tissues.

Effect of pH on anesthesia

That the effect of alkalosis on barbiturate anesthesia is not a nonspecific stimulatory effect on the central nervous system is shown by the absence of any effect on ether anesthesia. The proportional change in the median anesthetic dose of phenobarbital is about twice as great as that in the dose of barbital. This can be correlated with the greater proportional change in the concentration of the undissociated form of phenobarbital produced by a change of pH. The effect of alkalosis on barbiturate anesthesia appears then to be due to the passage of some of the drug out of the brain consequent to increased ionization in the plasma.

Because of the abnormally large amount of the drug in the brain in acidosis, patients in a condition of acidosis from any cause would be expected to be unusually sensitive to the depressant effects of phenobarbital. Acidosis could be a factor in the abnormal sensitivity to phenobarbital in patients with renal disease, which was reported by Brodwall and Stöa (19), and which was attributed by them to impaired excretion of the drug.

Renal excretion of phenobarbital

Lous (15, 16) found phenobarbital clearances in man of about the same magnitude as those we have measured and observed the increase of clearance with increase of urine flow. Brodwall and Stöa (19) also reported clearances in the same range, but they did not study the relationship to urine flow. In the clearances reported by Wright (20) there is a suggestion of increasing clearances with increasing flow, but all the measurements were made at low rates of urine flow. The spectrophotometric methods used by all these workers are subject to some interference from unconjugated p-hydroxy phenobarbital in urine. In the method of Lous (15), which was also used by Brodwall and Stöa, we have found that a concentration of 100 mg. per L. of p-hydroxy phenobarbital in urine would be interpreted as a concentration of 8 mg. per L. of phenobarbital. The relative proportions of phenobarbital and unconjugated p-hydroxy phenobarbital in human urine undoubtedly vary considerably, but it is unlikely

that the clearances measured by Lous' method are in error by more than about 10 per cent owing to interference from the hydroxylated product. The method of Wright and Johns (21), which was used by Wright, may be subject to more interference than that of Lous. Cutting and Koppanyi (22) reported that the diuresis resulting from massive intravenous infusions of isotonic saline or glucose was accompanied by increased excretion of phenobarbital. The quantitative accuracy attainable with the cobalt color reaction which they used is questionable, but the effect of diuresis was so great that there can be little doubt of the qualitative validity of the observation. Only Wright (20) has attempted to study the effect of alkalinizing and acidifying treatments on phenobarbital excretion. He did not consider the effects significant, but his measurements were limited to "casual samples" of urine from one patient.

The relationship between water excretion and the urine/plasma concentration ratio as shown in Figure 5 can be interpreted, as was done by Giotti and Maynert (23) for barbital, in terms of reabsorption of phenobarbital by a process of passive back-diffusion, the distal tubule absorbing a small amount of water and being relatively impermeable to phenobarbital. The influence of the pH of the urine on excretion of phenobarbital is explicable on the assumption that the tubule is permeable only to the lipid soluble undissociated form of the drug and that equilibration between phenobarbital in the tubule and in the plasma is established either in the same region of the tubule in which the pH of the tubular fluid is changed or distal to that region. For instance if equilibrium were established between tubular fluid of pH 7.9 and plasma of pH 7.4, the concentration in the tubule would become 2.3 times as high as the unbound concentration in plasma, and the urine/plasma concentration ratio would approach this value with high rates of flow. If tubular fluid of pH 6.0 were equilibrated with the same plasma, the concentration in the tubule would be only 0.45 times the unbound plasma concentration. Thus a U/P ratio of less than unity, such as was actually observed in one of the men of Table IV, is not indicative of an active transport system but can be adequately accounted for on the assumption of passive diffusion.

Increased excretion in alkaline urine has been reported for a number of other organic acids, including salicylic acid (24–27), gentisic acid (28), and some of the sulfonamides (29). Berliner (3) has pointed out that the theory of a tubule permeable only to the undissociated form would explain the effect of pH on excretion of salicylic acid, even though with a pK' of 3 this acid is very largely ionized in the most acid urines. The excretion of weak bases diminishes with increasing urinary pH, an effect that can also be explained in terms of tubular permeability to the undissociated species. The excretion of weak bases has been reviewed by Orloff and Berliner (4), who present a detailed derivation of the theory. This theoretical formulation is also applicable to weak acids.

It is of interest to compare our studies of the excretion of phenobarbital with those of Giotti and Maynert (23) with barbital. There are two important differences in the pattern of excretion of the two drugs. First, the clearances based on total plasma concentrations differ, that for phenobarbital being lower in acid urine and at normal flows. This is attributable to the fact that phenobarbital is extensively bound whereas barbital is not. Second, the clearance of phenobarbital is affected by the pH of the urine to a greater extent than is that of barbital. Barbital, with the higher pK' value of 7.7, is less extensively ionized in alkaline urine than is phenobarbital. The effect of alkalinization of urine would accordingly be expected to be less for barbital.

Implications in the treatment of phenobarbital poisoning

Phenobarbital is normally the most slowly eliminated of the commonly used barbituric acid derivatives. The decrease in plasma concentration in a 24-hour period is of the order of 15 per cent in man (8, 15, 30). Because of the extreme persistence of the drug, the handling of phenobarbital poisoning entails unusual difficulties. Any measures that would increase the rate of elimination would obviously be beneficial.

There have been a number of suggestions that diuresis produced by administration of fluid or fluid together with a mercurial diuretic would be of value in treatment of poisoning with those barbituric acids that are excreted by the kidney (e.g.,

15, 22, 31). However, alkalinizing treatments have apparently not hitherto been advocated in phenobarbital poisoning. From the results of our experiments it would seem that the intravenous administration of NaHCO₃ could shorten phenobarbital coma to a very valuable degree. If the highest clearance obtained in man with intravenous NaHCO₃ in these experiments could be maintained, it can be calculated that the plasma concentration of phenobarbital would decline by about 40 per cent in 24 hours through the process of renal elimination alone. If metabolic inactivation accounted for an additional 5 or 10 per cent, the total elimination would be at a rate approximately three times that found normally. An additional benefit would come from the raising of the blood pH. In deep coma the depression of respiration lowers the blood pH and this causes more phenobarbital to enter the brain with further deepening of the depression. Raising the blood pH would cause the passage of some of the drug out of the brain and would lighten the depression. Thus through two different mechanisms the administration of NaHCO₃ might be expected to be of great value in the treatment of phenobarbital poisoning. Because of the inhibitory effect of barbituric acids on water diuresis, apprehension has been expressed that efforts to induce diuresis by administration of large volumes of fluid might lead to pulmonary edema (20). In dogs deeply anesthetized with phenobarbital it has been our experience that intravenous infusion of hypertonic solutions of NaHCO₃ has consistently resulted in the excretion of a larger volume of water than that administered. If the kidneys are normal, there should be no risk of fluid retention. Intravenous infusion of a concentrated solution of NaHCO₃ is a procedure entailing minimal risk, easily carried out without special equipment, and offering promise of great benefit in phenobarbital poisoning. It should be emphasized that this treatment could not be expected to be of nearly so much value in poisoning with any of the other familiar barbituric acids.

SUMMARY

Phenobarbital, with a pK' of 7.2, is a stronger acid than any of the other familiar barbituric acid derivatives. The proportional change in the concentration of the undissociated form resulting from change of pH in the physiological range is greater for phenobarbital than for other barbituric acids with higher values of pK'.

In the concentrations of practical interest, phenobarbital is bound to the extent of about 40 per cent in a 4 Gm. per 100 ml. solution of serum albumin. The binding is nearly independent of pH.

In dogs the plasma phenobarbital concentration falls as the blood pH falls, and rises as the blood pH rises. These changes are due to changes in the distribution of the drug between plasma and tissues. Tissue/plasma concentration ratios vary in a direction opposite to that of the blood pH. Alkalosis, by diminishing the amount of drug in the brain, lightens phenobarbital anesthesia.

The renal clearance of phenobarbital increases with increasing rate of urine flow. At any given rate of flow, the clearance is much higher when the urine is alkaline than when it is acid. The excretion of phenobarbital can be explained on the assumptions that the drug is reabsorbed by a process of passive back-diffusion and that the renal tubule is permeable to the undissociated form and impermeable to the ionic form.

Intravenous infusion of a concentrated NaHCO₃ solution is suggested as a procedure of practical value in the treatment of phenobarbital poisoning.

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