REVIEW ARTICLE

THE DISTRIBUTION IN THE BODY AND METABOLIC FATE OF BARBITURATES

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The metabolic fate and distribution of barbituric acid derivatives in the body has been the subject of a vast number of studies since they first began to be used in therapeutics in the early years of this century. The reviews of Lundy and Osterberg[1], Tatum[2,3] and Maynert and van Dyke[4] are useful sources of information on articles on this subject printed before 1949. The aim of the present review is to describe recent advances in this field, together with the methods used. In it the author has tried to report, if not all, at any rate the most important contributions on this subject that have been published since that date. It should be mentioned that the expression barbiturate has been used for both barbituric and thiobarbituric acid derivatives used clinically.

Methods

The study of the distribution of barbiturates in the body and their excretion has been facilitated by the introduction of new methods of estimation more sensitive and specific than those used in the past.

The colorimetric methods of estimation of barbiturates based on the formation of cobalt complexes with barbituric and thiobarbituric acid derivatives[5] and copper complexes with thiobarbituric acids[6] have been used in recent studies and the conditions of these reactions have been studied during recent years by some authors[7,8]. Their application to the estimation of the barbiturate content of biological materials is not easy because of the low specificity and sensitivity of these methods. In addition, the ether or chloroform extracts of animal tissues and urine may contain impurities that give a positive reaction with those metals. Although these extracts can be partially purified by treating them with charcoal or alumina, it may often happen that impurities which modify the colour reactions of barbiturates may remain in the extracts.

Modern methods of estimation of these substances are based on their ultra-violet spectra described by Elvidge[9] and by Stuckey[10,11]. The ultra-violet spectrum of barbituric acids has a maximum at 220 mμ in acid media and at 255 mμ in alkaline media[12], whilst the thiobarbituric acids are characterised by a maximum at 290 mμ, when examined in acid media, and 305 mμ in alkaline media, so that the intensity of absorption of the ultra-violet light by a tissue extract at those wavelengths may be used as a measure of the barbiturate content of the sample. These methods have been made extremely easy by the development of modern spectrometers. The first method of estimation based on this property was that of Hellman *et al.*[13] for the estimation of thiopentone, which was modified by Jailer

and Goldbaum[14] later on. Similar methods for the estimation of barbituric acids were described by Walker *et al*[15] and by Goldbaum.[12]

These methods are more sensitive than those based on the formation of metal complexes, but they are not completely specific since the ultraviolet spectrum of all barbituric and thiobarbituric acids, and of some of their metabolites, is identical so long as their ring structure remains intact. Therefore if an estimate of the amounts of one of these compounds in some biological material is required, methods of extraction must be used that will extract only the barbiturate without extracting its metabolites at the same time. By using different solvents, Brodie *et al.*[16] have been able to estimate separately the amounts of thiopentone and its carboxylic acid derivative, present in the tissues and urine of man and animals injected with thiopentone.

TABLE I

REACTIONS FOR VISUALISATION OF BARBITURATES IN PAPER CHROMATOGRAMS

Reagents		1 Ultra-violet light	2 Ultra-violet light + sodium hydroxide and fluorescence screen	3 Mercuric nitrate	4 Mercuric sulphate (Lamaire reagent) + thiodiphenyl carbazone	5 Silver acetate + thiodiphenyl carbazone	6 Potassium permanganate	7 Copper sulphate + pyridine	8 Copper sulphate + pyridine + diethyl dithiocarbamate	9 Potassium-nitroprusside (Grote's reagent) + sodium carbonate
Barbiturates	Saturated 5·5 radicals	none	black	grey	purple	brown	none	pale pink	brown	none
	unsaturated 5·5 radicals	none	black	grey	purple	brown	brown	pale pink	brown	none
Thiobarbiturates		black	black	grey	purple	brown	brown	green	brown	orange-red

References:—1, 4, 5 and 6, Algeri and Walker[23]; 2, Grieg[24]; 3, Romano[25]; 1, 6, 7, 8 and 9 Raventós[26].

The application of chromatographic methods to the purification of tissue extracts prior to the estimation of their barbiturate content, was first used by Raventós[6]. By his method he was able to remove most impurities of the extracts and also to separate the barbituric acids from the thiobarbituric acids present in the extracts. He has applied this method of fractionation to the estimation of barbituric acids present in the urine of animals injected with thiobarbiturates. Bush, Butler and Dickison[17] have used silica columns in their researches on the metabolism of hexobarbitone.

The identification of the barbiturates can be done by physical methods such as their melting point[18], temperature of sublimation[19], colour reactions and crystallisation pattern[20], X-ray powder pattern[21], or by the preparation of their *p*-nitrobenzyl derivatives[22]. For all these methods, samples

of pure material, larger than 5 to 10 mg., are required and these are sometimes difficult to obtain from biological materials.

More practical methods for the identification of compounds of this type are paper chromatograms. On these one can identify the barbiturates by their R_F values after visualisation with suitable colour reactions (see Table I).

Ultra-violet light[23,24,25], mercury nitrate[26], silver acetate[23], mercury sulphate (Lamaire reagent)[23], potassium permanganate[23,24,25], copper sulphate and pyridine[25], potassium nitroprusside (Grote's reagent) have all been used for the visualisation of barbituric and thiobarbituric acids on paper chromatograms. The sensitivity of these reactions is not very high and a minimum of 50 μg. of most barbiturates is needed for a positive result (see Table II).

TABLE II

PAPER CHROMATOGRAPHY OF BARBITURATES. R_F VALUES OF SEVERAL BARBITURATES FOUND WITH DIFFERENT SOLVENT MIXTURES

	Solvent mixtures			
	n-butanol saturated with 5N ammonia[23]	*n*-butanol saturated with 1·3N ammonia[25]	Acetone-water saturated with diethyl-amine vapour[26]	*n*-butanol saturated with water-saturated with diethylamine vapour[26]
Amylobarbitone	0·73	0·79	—	—
Barbitone	0·47	0·65	0·90	0·81
Butobarbitone	0·69	—	0·90	0·90
Cyclobarbitone	—	0·68	0·92	0·84
Allobarbitone	0·55	0·71	0·94	0·86
Hexethal	0·76	—	—	—
Hexobarbitone	0·71	0·78	—	—
Methylphenobarbitone	—	0·65	0·94	0·90
Pentobarbitone	0·73	0·85	—	—
Phenobarbitone	0·50	0·65	0·92	0·84
Propallylonal	—	—	0·95	0·86
Rutonal	—	—	0·90	0·74
Quinalbarbitone	0·76	—	—	—
Thipentone	0·77	0·87	1·00	0·92
Thialbarbitone	—	0·86	—	—
Thiobarbitone	—	0·68	—	—
Thiophenobarbitone	—	0·72	—	—

Barbiturates labelled with heavy or radioactive elements have been used for the study of the fate of these substances in the body. Maynert, van Dyke and their co-workers[27,28,29,30,31] administered ^{15}N labelled barbiturates to animals and followed their distribution and excretion by mass spectrography methods. Roth *et al.*[32] and Kahn[33,34] have used pentobarbitone labelled with ^{14}C in position 2 and Taylor, Richards and Tabern[35] have studied the excretion of ^{35}S in animals injected with ^{35}S-labelled thiopentone. The use of these preparations has shed some light on problems connected with the excretion and metabolism of barbiturates, especially when the urine of animals treated with these compounds is submitted to paper chromatographic methods[35] or to cold isotope dilution methods[28,29,30,31,35]. Perhaps the more interesting technique, using radio isotopes in combination with chromatographic methods, has been described by Titus and Weiss[36]. They chromatographed on paper the urine of

animals treated with ^{14}C–labelled pentobarbitone and from these chromatograms they isolated several ^{14}C–containing fractions that were later used as tracers in large-scale experiments with normal pentobarbitone. This method has the advantage over the cold isotope dilution methods of other authors, that it is not necessary to assume beforehand the chemical structure of any given metabolite.

Distribution of Barbiturates in the Tissues

After their absorption from the alimentary canal or after their intravenous administration, the barbiturates are found in the blood and they are distributed throughout all the tissues of the body. This process of distribution has recently been studied with modern methods of estimation, and the results of these studies have made understandable some aspects of the mode of action of these drugs.

It had been observed in the past that the maximum concentration of drug in the blood was found immediately after its intravenous administration. This concentration declined quickly during the first 20 to 30 minutes, but at the end of this time the disappearance of drug from the blood proceeded at a much slower rate[37]. It was assumed that the initial fall in drug concentration was due to its distribution in the tissues and that the second fall was, in reality, the result of the metabolic destruction of the drug. As the methods of estimation, however, were not sufficiently sensitive it was impossible to follow the fall in blood concentration for many hours after the administration of a dose of barbiturate.

Recent studies by Brodie and co-workers[16,38] have shown that after a single intravenous injection of thiopentone or pentobarbitone, the concentration of drug in the blood follows the pattern described above, but the second fall is slower than that observed previously by other authors and traces of drug can still be detected in the blood several hours after its administration.

A different result is seen when large doses of pentobarbitone or thiopentone were administered by intravenous infusion lasting for 50 to 60 minutes. With such a method of administration the drug was found to be evenly distributed throughout the body during the injection period and the concentration of drug in the blood decreased progressively at a slow rate as soon as the administration had stopped. The rates of disappearance of short-acting barbiturates are, in general, very low (about 10 to 15 per cent. per hour) and these are about the same for thiopentone, hexobarbitone, thialbarbitone and thiamylal sodium.

The rate of disappearance of pentobarbitone from the blood is about 4 per cent. per hour and this is even slower in the case of long-acting barbiturates such as barbitone or phenobarbitone.

The concentration of barbiturates in the tissues depends to a great extent on its concentration in the blood. The maximum concentration of drug in the tissues is reached a short time after its intravenous administration and there are no big differences between the concentrations of drug in the tissues and that in the blood. Table III gives the tissues and blood concentrations observed with two well-known barbiturates.

The adipose tissue is an exception to this rule. The maximum concentration of barbiturate is not found in the fatty tissue until some time after its administration and this maximum may be several times higher than that of the blood. The extent of the fixation of barbiturates may be sufficiently high to account for a high proportion of the administered drug, about 2/3 to 3/4 in the case of thiopentone[38]. The immediate result of this fixation is to remove a large proportion of the drug circulating in the blood

TABLE III

CONCENTRATIONS OF BARBITURATES IN TISSUES

Tissue	Pentobarbitone[44] ml./l.	Thiopentone[46] mg./l.	*N*-methyl-thiopentone[40] mg./l.
Blood plasma	34·4	14·7	2·0
Plasma water	18·7	7·0	–
Cerebrospinal fluid	18·2	—	–
Red cells	36·0	—	–
Liver	64·4	27·8	–
Brain	42·3	23·9	–
Muscle	27·5	22·2	–
Kidney	45·8	17·6	–
Heart	38·4	18·4	–
Lung	20·8	13·6	–
Spleen	41·8	13·0	–
Fat: Lumbrosacral	37·3	119·0	–
Perineal	—	222·0	–
Omental	—	192·0	–

and to form a kind of deposit of the drug in the fat from which it is released later. The intensity of this fixation is seen in the values of the ratios between the drug concentrations in the blood and in the fat. This is about or slightly higher than 1·0 in animals treated with barbiturates with long duration of action, but this ratio is increased to 6 to 10 for short-acting barbituric acids such as thiopentone and it can be as high as 100 in the case of *N*-methyl-thiopentone[40].

According to Brodie *et al.*[38] the duration of action of a barbiturate depends more on the amounts of drug fixed in the fat than on the rate of their metabolic inactivation. Herman and Wood[42] have found that in rats a 5 per cent. decrease in the body fat resulted in a 100 per cent. increase in the duration of action of a given dose of thiopentone. In these fat-depleted animals the dose of thiopentone required for a given duration of anæsthesia was 40 per cent. smaller than in normal animals. In contrast, the duration of action of barbiturates, such as pentobarbitone, that are not accumulated in the fat to a great extent, was not affected by fatness of the animals.

The rate of penetration of barbiturates in the central nervous system has been studied by Butler[43], who injected mice intravenously with barbitone and hexethal and measured the barbiturate content of the brain at different times after the injection of these drugs. He found that there is a close relation between the depth of anæsthesia and the concentration of drug in the central nervous system and that the rate of penetration of the drug into the brain is paralleled by the time of onset of the effects. With a drug like barbitone which has a long period of onset, it penetrated slowly

and its maximum concentration in the brain was not observed until 30 to 40 minutes after the injection. Hexethal, a quick-acting barbiturate, was found in maximum concentrations immediately after its administration, and its concentration decreased at a steady rate afterwards. The concentration of barbitone in the brain found by Butler is about 10 per cent. lower, and that found in the same tissue by Maynert and van Dyke[28] with ^{15}N labelled barbitone is slightly higher, than the concentrations calculated assuming that the drug is evenly distributed. Hexethal, on the other hand, was found by Butler, in the brain, in concentrations about 40 per cent. higher than those calculated for even distribution of the drug throughout the body.

TABLE IV

RATIOS BETWEEN THE CONCENTRATION OF SEVERAL BARBITURATES IN THE TISSUES AND IN THE PLASMA

	Ratio $\frac{\text{Tissue concentration}}{\text{plasma concentration}}$							
	Fat	Liver	Kidney	Lung	Spleen	Brain	Muscle	Reference
Thiopentone	6·0	1·6	1·2	0·8	0·7	1·2	1·3	39
Thialbarbitone.. ..	8·0	1·3	0·8	0·5	—	—	0·8	
Thiamylal sodium ..	8·0	1·6	0·9	0·9	0·7	0·7	1·3	
Hexobarbitone.. ..	3·2	1·3	1·1	—	—	—	0·9	
Pentobarbitone ..	1·1	1·9	1·4	0·6	1·2	1·2	0·8	
N-methylthiopentone ..	100·0	—	—	—	—	—	—	40

TABLE V

CONCENTRATION OF BARBITURATES IN THE BLOOD AT DIFFERENT LEVELS OF ANÆSTHESIA

		Concentration mg. per cent				
	Blood	Light anæsthesia	Surgical anæsthesia	Respiratory arrest	Cardiac arrest	Reference
Thialbarbitone.. ..	arterial	3·55	8·82	18·8	—	37
Thiopentone	arterial	2·7	4·78	7·42	—	37
	arterial	—	3·71	4·97	18·0	45
	venous	—	2·32	3·47	13·8	
5-*iso*Propyl-5(2-methyl-2-phenyl) 2-thiobarbituric acid	arterial	—	1·95	3·77	5·6	45
		—	1·27	2·0	4·4	

The distribution of barbiturates in different parts of the central nervous system has been investigated by Maynert and van Dyke[31] and Brodie *et al.*[38] Both groups of authors have found no significant differences between the concentrations of barbitone and thiopentone in the cortex, diencephalon, medulla, pons and cord.

The relation between the concentration of barbiturates in the blood and their pharmacological action has been investigated in the last few years. It is difficult, however, to assess the real relationship between the active drug concentration and the intensity of action because most available data have been obtained with whole blood or plasma, without taking into account the extent of the fixation of barbiturates in the plasma proteins.

This has been measured by Brodie *et al.*[38,44], who found that 75 per cent. of the thiopentone and 55 per cent. of the pentobarbitone present in the plasma is bound to the proteins.

In spite of this reservation, it seems from the results of Carrington and Raventós[37], and Peterson, Gould and Shiedeman[45] that there is a close parallel between the depth of anæsthesia produced by short-acting barbiturates and their concentration in the blood. Peterson *et al.*[45] found in their experiments that the concentration of drug in the arterial blood was slightly higher than that in the venous. Both groups of authors think the ratios of the concentrations of drug in the blood at the levels of anæsthesia and at respiratory arrest are a good indication of the therapeutic safety margin of any barbiturate drug.

The passage of thiopentone into the cerebrospinal fluid has been studied by Brodie *et al.*[38], who found that after a lag of about 15 minutes the concentration in the fluid was proportional, although 20 per cent. lower than that in the plasma water. It seems that the initial lag in the appearance of the drug in the cerebrospinal fluid is due to the time required for the drug to reach the cisterna magna after crossing the blood-brain barrier.

The process of the transfer of barbiturates from the blood to the tissues probably depends on many factors, such as the physico-chemical characteristic of the drug. Of these the water/lipoid solubility coefficient seems to be an important one and could account for the accumulation of barbiturates in the fat, but if the lipoid content of a tissue is compared with its barbiturate content one finds that only a small proportion of the drug can be fixed in these lipoids. Brodie *et al.* have found that the tissue concentration of thiopentone is in general higher than that of the plasma. As only about 25 per cent. of the drug present in the blood is diffusible it is evident that there is more drug in the tissues than can be accounted for on the basis of its free concentration in the body water, and that a large proportion must be fixed in the tissue cells. At the same time, they measured the fat content of the tissues and calculated the amount of drug localised in the tissue fraction, assuming that it would be in the same concentration as in the lumbo-dorsal fat. These calculations showed that only a small proportion of thiopentone was fixed in the cellular lipoids and it is possible that most of the drug is fixed in the proteins of the tissues.

Although it has been stated above that the intensity of action and the concentration of barbiturates in the blood are related to each other, there are indications that this relation is not very exact. Brodie *et al.*[46] have measured the blood concentrations of thiopentone in men who had received doses of this drug ranging from 20 to 65 mg./kg. In these experiments it was found that for the same levels of anæsthesia the blood concentration was higher after high doses than after small doses of thiopentone. These results show that men, at least, develop a tolerance to thiopentone in a very short time; this is probably due to an increased resistance of the tissues to the drug.

Changes in the sensitivity of tissues to barbiturates have been described

by Lamsom *et al.*[47,48], who found a return to anæsthetic levels when strong solutions of glucose, other sugars and substances involved in the metabolism of carbohydrates, were injected into animals recovering from the effects of barbiturates. At the same time, the LD50 of barbiturates is decreased, and the time of onset of the action of slow-acting barbiturates is reduced by the previous administration of these sugar solutions. Richard, Bertcher and Tabern[49] confirmed Lamsom's results but they found not only that the "glucose" effect could be produced by glucose and other sugars but that the administration of a concentrated solution of mineral salts produced the same effect. This makes it unlikely that carbohydrate metabolites are directly connected with this glucose effect, as suggested by Lamsom, and it is probable that it is due to some osmotic changes.

The glucose effect is produced only in some animal species and here it is produced during a short period at the beginning of the recovery. Lamsom *et al.*[48] studied the glucose effect in mice injected with hexobarbitone and found that it was produced only when the barbiturate content of the brain was between 2 and 3 mg. per cent. If the concentration of hexobarbitone in the brain was higher than 3 mg. per cent. the mice were anæsthetised, but no return to anæsthetic levels was observed when the sugar solution was administered, if the concentration of drug had fallen below 2 mg. per cent.

Changes in the *p*H of the blood may modify the concentration of barbiturates in the blood. In dogs injected with thiopentone, Brodie *et al.*[39] measured the blood drug concentration during the inhalation of carbon dioxide and observed that the amount of drug decreased by 40 per cent. when the *p*H of the blood had fallen from normal to 6·8. Both the blood drug concentration and *p*H returned to normal levels a short time after the animals respired normal air. As the renal excretion of thiopentone during these experiments was negligible, it may be assumed that the fall in plasma concentration is due to a transfer of drug from the plasma to the tissues, presumably to the adipose tissue.

Metabolism of Barbiturates

Up to a few years ago practically nothing was known about the metabolic fate of most barbiturates. With the introduction of new methods for the estimation and isolation of barbiturates and their metabolites, the schools of Butler and Bush, of van Dyke, of Brodie and of Richards have studied different aspects of this problem. As a result we now possess some valuable information about the possible chemical reactions involved in their inactivation.

As a rule, these drugs are metabolised and only small amounts of them are excreted unchanged in the urine. The great complexity of these problems has been demonstrated by Roth *et al.*[32], and Kahn[33], who found several metabolites in the urine of animals injected with ^{14}C-labelled pentobarbitone. Taylor *et al.*[35] found at least 12 sulphur-containing metabolites in the urine of animals injected with ^{35}S-labelled thiopentone.

The only exception to this rule is barbitone. Practically 100 per cent.

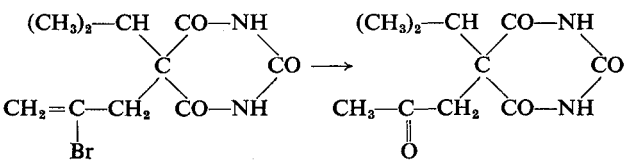
of the administered drug is excreted unchanged in the urine and although a great proportion of it is excreted during the first 24 hours, the excretion is not completed for about a week, during which traces of barbitone are found in the urine. The more stable long-acting barbiturates are excreted unchanged in a larger proportion than the less stable short-acting ones. Phenobarbitone, for instance, is excreted in amounts of 50 per cent. of the dose, while only 0·3 per cent. of thiopentone is found unchanged in the urine.

Recent studies have shown that there are several chemical reactions that may be involved in the metabolic fate of barbiturates. In order to facilitate the description of these metabolic reactions, the author has divided them into the following types:—

a = oxidation of radicals in position 5 with the formation of keto, hydroxy and carboxy barbituric acids,
b = loss of *N*-alkyl radicals,
c = desulphuration of thiobarbiturates,
and *d* = hydrolytic opening of the barbiturate ring.

Oxidation of radicals in position 5: The oxidation of the radicals in position 5 of barbiturates is the most important of the processes of their inactivation. It was demonstrated first in barbiturates that contain bromine such as propallylonal.

After the administration of propallylonal, Halberkann and Reiche[50] were able to isolate from the urine some unchanged material and 5-acetonyl-5-*iso*propyl barbituric acid which could account for 12 to 16 per cent. of the dose. Fretwust, Halberkann and Reiche[51] also found that after the administration of butallylonal only traces of unchanged drug appeared in the urine, but this contained 5 to 17 per cent. of the dose as 5-acetonyl-5-*sec.*-butyl barbituric acid.

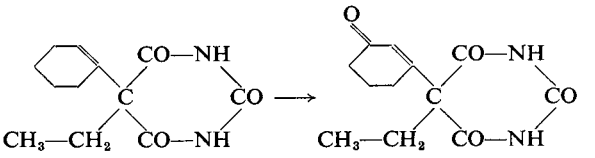


Propallylonal
5-bromoallyl-5-*iso*propyl barbituric acid

5-acetonyl-5-*iso*propyl barbituric acid

The same group of workers[52] investigated the metabolic fate of cyclobarbitone. They found that 2 to 7 per cent. of the drug was excreted unchanged and that 12 to 20 per cent. was excreted as a non-toxic compound which they isolated in pure form. From the elementary analysis of this compound they concluded that the substance was 5-*cyclo*hexenonyl-5-ethyl barbituric acid, but this structure was not confirmed by other methods.

These results are similar to those more recently obtained by Bush, Butler and Dickison[17], who studied the excretion of hexobarbitone and its homologue 5$\triangle^{1:2}$-*cyclo*hexenyl-5-ethyl barbituric acid (norhexobarbitone). They found that both compounds are oxidised to their keto derivatives.



Cyclobarbitone

5 $\triangle^{1:2}$-*cyclo*hexenyl-5-ethyl barbituric acid

5 $\triangle^{1:2}$-*cyclo*hexenonyl-5-ethyl-barbituric acid

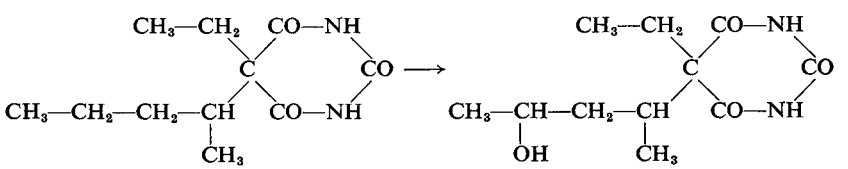
In the case of norhexobarbitone they found that it was excreted unchanged in quantities accounting for about 13 per cent. of the dose and about 20 per cent. in the form of its keto-derivative. The total excretion of these two fractions represents 1/3 of the dose administered and 2/3 is unaccounted for. The fate of hexobarbitone is complicated by the loss of the methyl radical in position 1 and this is reflected in the appearance in the urine of hexobarbitone, norhexobarbitone, ketonorhexobarbitone and two keto derivatives of hexobarbitone. In these experiments it was found that 5 to 6 per cent. of the dose was excreted as ketonorhexobarbitone. Beside this fraction, the authors were able to identify two isomeric forms of ketohexobarbitone, I and II, which account respectively for 1 per cent. and 0·3 per cent. of the administered drug. Hexobarbitone and norhexobarbitone were found in the urine in very small amounts. Table VI reproduces the results of Bush *et al*,[17] on the excretion of ketobarbiturate in dogs injected with hexobarbitone and norhexobarbitone.

TABLE VI

Compound	Norhexobarbitone, per cent.	Hexobarbitone, per cent.	Norketohexobarbitone, per cent.	"Ketohexobarbitone I," per cent.	"Ketohexobarbitone 2,") per cent.	Total, per cent.
Norhexobarbitone..	13	—	21	—	—	34
Hexobarbitone ..	traces	traces	5·5	1·0	0·5	>7·1

Raventós[25] has studied in rabbits the fate of thialbarbitone (5 $\triangle^{2:3}$ *cyclo*hexenyl-5-allyl-2-thiobarbituric acid). He has been able to isolate from the urine of these animals a thiobarbiturate that, according to elementary analysis, contains one oxygen atom more than the administered compound. He has not been able to determine, however, where this extra oxygen atom is incorporated in the molecule of thialbarbitone. By analogy with hexobarbitone and *cyclo*barbitone it is probable that this compound is the cyclohexenonyl derivative of thialbarbitone.

Another type of oxidation of the 5-alkyl radicals of barbiturates is that



Pentobarbitone

5-ethyl-5(3-hydroxy-1-methylbutyl) barbituric acid

described by Maynert and van Dyke[54]. They isolated from the urine of dogs injected with pentobarbitone a new type of metabolite, 5-ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid.

This work was followed by Maynert and Dawson[55], who administered by mouth 50 mg./kg. of pentobarbitone to dogs until 8 g. of drug had been administered. The pooled urine of these dogs was extracted with ether in a continuous extractor for 24 hours, and the ether extract was reduced to about 500 ml. This concentrate was washed several times with about one-fifth of its volume of water and the aqueous extract was concentrated to a small volume under vacuum. On standing, 435 mg. of a dextrorotatory substance ($[\alpha]_D^{28^\circ C.} + 26{\cdot}6^\circ$) was obtained which after several recrystallisations had a melting point of 209° to 210° C. After evaporation of the liquors they also obtained 780 mg. of a lævorotatory compound ($[\alpha]_D^{28^\circ C.} -5{\cdot}6^\circ$) which after recrystallisation had a melting point of 152° to 153° C. The two substances had an ultra-violet spectrum characteristic of barbituric acids and an absorption band at 2·8 mμ in the infra-red spectrum characteristic of hydroxyl groups. As these metabolites gave a positive iodoform reaction on treatment with $NaIO_3$, and they formed 5-ethylbarbituric acid after treatment with sulphuric acid, the authors confirmed that the hydroxyl group of these metabolites was attached to the third carbon of the 1-methylbutyl radical of pentobarbitone. Analysis of the urine of dogs treated with pentobarbitone, showed that about 33 to 36 per cent. is excreted as the dextro-hydroxy metabolite and about 15 per cent. as the lævo-hydroxy compound. The total excretion of the two compounds accounts for about 45 per cent. of the administered pentobarbitone.

Brodie *et al.*[44] have studied the fate of pentobarbitone in men. They found that the urine of these subjects contained a metabolite identical with the hydroxy barbiturate isolated by Maynert and van Dyke from the urine of dogs treated with this drug. In men the excretion of this metabolite accounts for 15 per cent. of the administered pentobarbitone.

Maynert[56,57] extended his researches to the study of the metabolic fate of butobarbitone, 5-ethyl-5-butylbarbituric acid, and amylobarbitone, 5-ethyl-5-*iso*amylbarbituric acid.

From the urine of dogs treated with amylobarbitone a hydroxybarbiturate was isolated and identified by the same reactions as he used in his work on pentobarbitone. The structure of this metabolite, 5-ethyl-5(3-hydroxy*iso*amyl)barbituric acid, was confirmed by its synthesis by two different methods. Quantitative measurements of the excretion of this metabolite were carried out with the urine of dogs treated with ^{15}N-labelled amylobaritone by isotope methods. The results of these experiments revealed that about 35 to 40 per cent. of the drug was excreted as its hydroxy derivative.

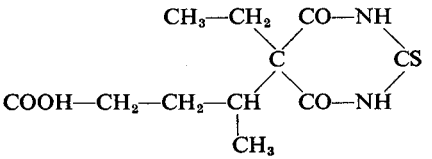
Butobarbitone is also excreted as its hydroxy derivative, 5-ethyl-5(3-hydroxybutyl) barbituric acid, in amounts of about 10 to 20 per cent. of the administered dose.

The last type of metabolite of barbiturates produced by oxidation of their 5-alkyl radicals is the formation of carboxylic acid derivatives.

From the urine of men treated with large doses of thiopentone, Brodie

et al.[16] isolated a metabolite identified as a carboxylic acid derivative of thiopentone in amounts that accounted for 10 to 25 per cent. of the dose. The authors established by ultra-violet spectrography that this compound was a thiobarbituric acid derivative and, by potentiometric titration with sodium hydroxide, found that it had two acidic groups, one with a pKa = 5·2 and another with pKa = 8·2, which is the same as that of the only acidic group of thiopentone.

In their original paper, the authors were not sure of the position of the carboxylic group of the metabolite, but Horning and Wood[58] have demonstrated by its synthesis that this metabolite is 5-ethyl-5(4-carboxy-1-methylbutyl)2-thiobarbituric acid.



So far, we have very little information about the pharmacological action of the metabolites of barbiturates produced by the oxidation of their radicals in position 5. The carboxylic acid derivative of thiopentone of Brodie, the hydroxy barbiturate studied by van Dyke, Maynert and collaborators, and the keto derivatives of hexobarbitone of Bush *et al.*, have been found to be devoid of hypnotic activity.

Loss of N-*alkyl radicals*: *N*-Alkyl barbiturates are metabolised in the body by a different method. Bush and Butler[59] found that the urine of animals treated with *N*-alkyl barbiturates contained in some cases considerable amounts of the parent homologue. They measured the excretion of barbitone in animals injected with a series of its *N*-alkyl derivatives and found that the extent of dealkylation depended on the length of the *N*-radical of the tested substance. Table VII summarises their results.

TABLE VII

N-alkyl homologue of barbitone	Excretion of barbitone, percentage of dose
methyl	69
ethyl	30 to 40
*iso*propyl	traces
allyl	
butyl	
phenyl	

They have extended their researches to the study of the fate of methylphenobarbitone and hexobarbitone, the two *N*-methyl barbiturates most commonly used in practice. Butler and Bush[60] recovered phenobarbitone from the urine of animals treated with methylphenobarbitone. Butler[61] and Butler *et al.*[62] have measured the blood levels of phenobarbitone and methylphenobarbitone in animals and men treated with the last-mentioned drug. In some of their experiments on dogs the excretion of the two barbiturates in the urine was measured during the 7 days after the administration of methylphenobarbitone. During this period they recovered as

unchanged material less than 1 per cent. of the dose and about 20 per cent. as phenobarbitone. The excretion of phenobarbitone in these animals shows that at least one half of the administered methylphenobarbitone is demethylated.

Bush, Butler and Dickison[17],[53] in their studies on the metabolic fate of hexobarbitone found that this was demethylated and excreted as norhexobarbitone and its keto derivative—(see Table VI). In these experiments, the excretion of norhexobarbitone was very small, but that of its keto derivative accounted for 5 to 6 per cent. of the administered hexobarbitone. As in parallel experiments with norhexobarbitone they found that 20 per cent. of the dose was excreted as its keto derivative, the extent of demethylation of hexobarbitone can be calculated. After making allowances for the different molecular weights of the compounds, it appears that only about 25 per cent. of the administered hexobarbitone has been demethylated in the body.

Desulphuration of thiobarbiturates: Recent researches on the disposal of the sulphur of the molecules of thiobarbituric acids in the body will be summarised here. The introduction of ^{35}S in the molecule of thiopentone has allowed Taylor, Richards and Tabern[35,63,64] to study the disposal of the radioactive S in different animals species. In the 4 days after the administration of anæsthetic doses (40 mg./kg.) of this preparation to rats, it was possible to isolate from the urine several ^{35}S-containing fractions. The total excretion of the radioactive element accounted for 89 to 90 per cent. of the administered ^{35}S-labelled thiopentone. This excretion was made up of the following fractions: A—7 to 14 per cent. was extractable with chloroform and presumably contained the excreted thiopentone and derivatives; B—26 per cent. was found as sulphur precipitable by barium chloride; C—7·7 per cent. was precipitable by barium chloride after hydrolysis and D—40 per cent. called "remainder" by the authors, was the non-precipitable and non-hydrolysable fraction of ^{35}S. This last fraction was calculated by the difference between the total ^{35}S and the sum of the chloroform-soluble, precipitable and hydrolysable ^{35}S present in the urine. The fæces of these animals contained 5·3 per cent. of the radioactive S and 2 per cent. was found in the carcase. The demonstration of ^{35}S in the carcase of the experimental animals 4 days after the administration of radioactive thiopentone indicates that some of its sulphur may be used in the normal metabolism of the animals in the formation of sulphur-containing amino acids and proteins. Taylor *et al.* were also able to demonstrate the excretion of thiourea by cold isotope dilution methods. The excretion of ^{35}S thiourea during the first 24 hours of the experiment accounts only for 2 to 8 per cent. of the administered thiopentone.

The complexity of the problem of the metabolic fate of thiobarbiturates is shown by the results of paper chromatograms of the urine of animals treated with ^{35}S-labelled thiopentone. Taylor *et al.* found at least 12 ^{35}S-containing fractions in these chromatograms, but they were able to identify the presence of inorganic sulphate in the band of $R_F = 0{\cdot}06$ to 0·09 which beside inorganic substances contained two more compounds in the ratio of 5:1. The band of $R_F = 0{\cdot}40$ to 0·49 was composed of

4 components in the ratio 1:1:2:5 and the region of $R_F = 0{\cdot}75$ to 0·8 consisted of only one component. The unchanged thiopentone was found in the band $R_F = 0{\cdot}9$ to 0·95 jointly with another fraction. As no radioactive metabolites of thiopentone were available at that time, they attempted to estimate normal sulphur-containing metabolites present in the urine that could be formed at the expense of ^{35}S-containing thiopentone. Their experiments showed that taurine, thiocyanate, the sulphate ester of *p*- and *o*-cresol and phenol might be present in the urine of the animals injected with labelled thiopentone, but the amounts of these substances were very small.

In the opinion of the reviewer, the finding of inorganic and ethereal ^{35}S-sulphates in the urine of animals injected with radioactive thiopentone shows that thiobarbiturates can lose their sulphur and be transformed into barbiturates which then follow the metabolic fate of these last substances. The significance of the remainder of the ^{35}S fraction, accounting for about 40 per cent. of the radioactivity of these urines, is obscure. It may be composed of thiourea, normal sulphur metabolites and substances formed by the opening of the thiobarbituric ring which will be discussed in the next section.

The transformation of thiobarbiturates into barbiturates has not been fully appreciated because of the difficulty in identifying and isolating both types of barbiturates when they are simultaneously present in the urine. Carrington and Raventós[37] studied in the rabbit the excretion of thialbarbitone and found that 2·15 per cent. of the drug administered was excreted as thiobarbituric acids, probably unchanged, and about 2·7 per cent. as barbituric acids, probably as 5$\triangle^{2:3}$-*cyclo*hexenyl-5-allylbarbituric acid.

Raventós[25] has studied in the rabbit the metabolic fate of thiobarbitone and thiophenobarbitone in the hope of being able to isolate and identify their oxygen homologues in the urine of the animals injected with the thio compounds. By fractionation of the urine extracts by means of alumina columns[6], he was able to measure the excretion in the urine of both types of barbiturates. These were isolated and identified after crystallisation. When thiobarbitone was administered intravenously, about 20 per cent. of the drug appeared in the urine with some barbitone that accounted for 10 per cent. of the sulphur homologue. On the other hand, a different picture was found in experiments where thiophenobarbitone was used. In these about 15 per cent. of the dose was excreted unchanged and about 35 per cent. as phenobarbitone. In comparing, in the same animal, the excretion of thiophenobarbitone with that of its oxygen homologue it was apparent that, if not all, a very high proportion of the drug which was not excreted unchanged, was desulphurised into phenobarbitone. In the case of thiobarbitone, however, the process of desulphurisation accounts for only a small proportion of its metabolic destruction. Table VIII gives a summary of these results. The excretions of oxygen and sulphur homologues were calculated as percentage of the dose, after making corrections for differences in molecular weight. It was assumed that the amounts of sulphur homologue not excreted unchanged would be

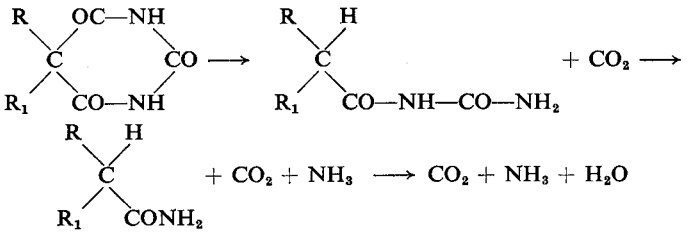
desulphurised and the resulting barbituric acid would be excreted in the same proportion as after the administration of the oxygen homologue.

TABLE VIII

EXCRETION OF OXYGEN AND SULPHUR HOMOLOGUES OF BARBITURIC ACIDS DURING 3 DAYS AFTER ADMINISTRATION

Rabbit	Drug	Unchanged		Theoretical excretion of oxygen homologue if all unaccounted B is desulphurised C	Found excretion of oxygen homologue D	Total unaccounted for (100 − [A + D])
		Excreted A	Unaccounted B			
		per cent.	per cent.	per cent.	per cent.	per cent.
A	Barbitone ..	80	20	—	—	20
	Thiobarbitone ..	20	80	64	10	70
B	Phenobarbitone ..	40	60	—	—	60
	Thiophenobarbitone	15	85	38	35	50

Destruction of the barbituric acid ring: Because of the small amounts of drug found in the urine of animals injected with most barbiturates Scholne *et al.*[65] suggested that the inactivation of these compounds involved the rupture of the barbituric acid ring by hydrolysis with the formation of acetylurea and acetamide derivatives. They explained their failure in demonstrating the presence of these substances in the urine by assuming that these compounds were completely metabolised to carbon dioxide, ammonia and water.



van Dyke *et al.*[27,29,30], with the use of ^{15}N-labelled barbiturates, demonstrated that this hydrolysis is responsible for a very small proportion of the barbiturates metabolised in the body. In their experiments with labelled pentobarbitone, they found that the excretion of ^{15}N in the urine accounted for 95 per cent. of the injected isotope. From this, 0·1 to 0·3 per cent. was excreted as ammonia and 0·4 to 5·0 per cent. as urea. Isotope dilution experiments with the barbituric acid used in these experiments and their hypothetical hydrolytic products showed that a small proportion of these derivatives were present in the urine of these animals. Table IX gives a summary of the experiments of van Dyke *et al.* with pentobarbitone and amylobarbitone. After oral administration to dogs only a small fraction of the excretory product appears to be derived from rupture of the malonylurea ring.

The excretion of ^{15}N in the form of urea found by these authors was

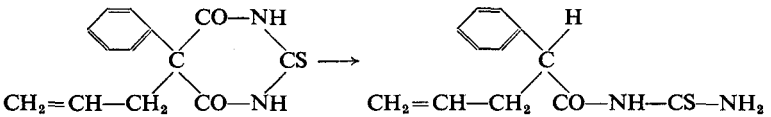
similar to that of the excretion of thiourea found by Taylor *et al.*[35] in the urine of animals injected with ^{35}S thiopentone (see above). Raventós[25] has been able to demonstrate by paper chromatographic methods that the urine of rabbits injected with thialbarbitone contains amounts of thiourea

TABLE IX

EXCRETION OF ^{15}N-CONTAINING FRACTIONS IN THE URINE OF DOGS TREATED WITH ^{15}N-LABELLED BARBITURATES, AS PERCENTAGE OF ADMINISTERED ^{15}N

	Pentobarbitone per cent.	Amylobarbitone per cent.
Total excretion ..	89	80
Ammonia	0·3	0·1
Urea	0·4 to 10·0	7·1
Barbituric acid ..	0·5 to 1·6	0·4 to 0·9
Acetylurea ..	0·0 to 0·2	0·2 to 0·4
Acetamide ..	0·0	0·0 to 0·1
Malonuric acid ..	0·2 to 0·4	0·2 to 0·4

which represents about 0·5 to 2·0 per cent. of the dose. In the same experiments he has obtained a neutral sulphur-containing fraction having the same ultra-violet spectrum as the thiourea derivative of thialbarbitone.



From these results, it is clear that the hydrolytic break of the barbituric ring structure of the barbiturates, is a method of inactivation common to all barbiturates. It plays, however, a minor rôle in these cases in which it has been demonstrated.

CONCLUSIONS

Owing to the fact that most authors have been interested either in the fate of a specific barbiturate or in one aspect of this problem, the recent results of work on the metabolic fate of barbiturates has been presented here in a form that resembles that of a jig-saw puzzle. It is natural that the most recent work should be carried out with drugs of wide application in clinical medicine, but as they are, as a rule, compounds which suffer intense metabolic changes, they and their metabolites are excreted only in small quantities. This has made it difficult to state this problem in general terms. The possible metabolic reactions responsible for the inactivation of these drugs are reproduced in Table X. In it, the reviewer has tried to specify the metabolites of different barbiturates where these have been identified or isolated. The italicised metabolites are included in the table because although they have not been demonstrated as yet, it is possible that they may be produced in the body.

From the work reviewed here, it is apparent that the body can inactivate these drugs by different mechanisms. These processes may act simultaneously on a single compound so that, in theory, the number of metabolites depends on the number of reactions brought into play for the destruction of the drug used in the experiments. A barbiturate

such as thiopentone, for instance, is perhaps metabolised by reactions similar to those of its oxygen homologue, pentobarbitone. Owing to the desulphuration of the sulphur homologue, some pentobarbitone may be formed, and the presence of both oxygen and sulphur metabolites, such as hydroxy and carboxy derivatives, can be expected in the urine of animals injected with thiopentone.

TABLE X

				Demonstrated in experiments with	Reference
N-alkyl barbiturates	Excretion unchanged	ketobarbiturates		Hexobarbitone	17, 53
		hydroxy barbiturates			
	loss of *N*-alkyl radical	barbiturates		Methylphenobarbitone	60, 61, 62
				Hexobarbitone	
				other *N*-alkyl barbiturates	17, 53, 59
Barbiturates	Excretion unchanged			Propallylonal	50
				Butylonal	51
		ketobarbiturate		Cyclobarbitone	52
				Hexobarbitone	17, 53
				Norhexobarbitone	17, 53
	Oxidation of 5-radicals	hydroxy barbiturates		Pentobarbitone	54, 55, 44
				Amylobarbitone	56
				Butobarbitone	57
		carboxy barbiturates			
	hydrolysis of ring	acetylurea / acetamide / urea / ammonia		Pentobarbitone	29
				Amylobarbitone	30
	Barbiturate			Thiobarbitone	25
				Thiophenobarbitone	25
				Thialbarbitone	25, 37
Thiobarbiturates	loss of S			Thiopentone	64, 35
	Oxidation	ketothiobarbiturates		Thialbarbitone	25
		hydroxythiobarbiturates			
		carboxythiobarbiturates		Thiopentone	16
	hydrolysis of ring	acetyl thiourea / *acetamide* / thiourea / ammonia		Thialbarbitone	25
				Thiopentone	35

At the same time, it is evident that the structure of a barbiturate determines the reactions put into action by the body for the inactivation of the drug. In every case in which the formation of hydroxy or carboxy derivatives has been demonstrated, it has been found that the oxidation takes place in a radical other than an ethyl group. The resistance of the ethyl groups of barbiturates to oxidation, is probably the reason for the high proportion of barbitone, 5 : 5-diethylbarbituric acid, excreted in the urine.

It is reasonable to assume that the ethyl radicals of thiobarbitone are as resistant to oxidation as those of barbitone. As the animals injected with thiobarbitone excrete only a small proportion of the administered drug as barbitone, it is evident that most of that barbiturate is inactivated by some reaction that, as a first step, does not involve the loss of sulphur. It is possible that the hydrolytic opening of the barbituric acid ring which does not play a big rôle in the metabolism of other barbiturates, is the main reaction for the inactivation of thiobarbitone.

Although the recent advances in the problem of the metabolic fate of barbiturates have been very important, there are still some points and aspects of the problems which have not been studied yet, but it is hoped that this will be done in the next few years.

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