Metabolic Breakdown of Thiopental in Man Determined by Gas Chromatographic Analysis of Serum Barbiturate Levels

Eileen S. Furano, M.D., and Nicholas M. Greene, M.D.

WHILE it is generally agreed that a major metabolic pathway by which thiopental is inactivated consists of oxidation of the methylbutyl side chain to an acid,[1, 2, 3] an alternative metabolic pathway for thiopental as well as other thiobarbiturates has been demonstrated, namely desulfuration to the corresponding oxybarbiturate. Taylor *et al.*[4] reported in 1952 that 26.7 per cent of the radioactivity in the urine of rats given isotopically labelled S^{35}-thiopental appeared as inorganic sulfate, though whether this inorganic S^{35} was derived directly from thiopental or from one of its metabolites was not determined. Winters *et al.*[5] provided more convincing evidence that desulfuration, at least in rat liver minces, was of importance in the metabolism of thiopental. These authors demonstrated spectroscopically the presence of both oxybarbiturates as well as thiobarbiturates in rat liver minces that had been incubated with S^{35}-thiopental. Radioactivity, however, was present only in those compounds which had the absorption spectrum of thiobarbiturates. On the other hand, when thiopental was labelled with C^{14}, up to 29 per cent of the radioactivity was accounted for by the oxybarbiturate metabolite. Infrared spectoscopy, melting point determinations, elemental analysis, and partition coefficients showed the oxybarbiturate to be pentobarbital. These findings were confirmed[6] by the demonstration that the major urinary metabolite of thiopental in dogs is the alcoholic derivative of pentobarbital. On the other hand, Cooper and Brodie[3] showed that rabbit liver microsomes which were able to carry out side-chain oxidation of thiopental in the presence of oxygen and reduced triphosphopyridine nucleotide were unable to convert thiopental to pentobarbital. They suggested that either the cellular localization of the two enzymatic processes are different, or that cofactors necessary for desulfuration were lacking in their preparation. In this regard, Spector and Shideman[7] isolated pentobarbital from rat liver microsomal preparations in which thiopental had been incubated with oxygen, reduced triphosphopyridine nucleotide, and magnesium ions. In such a preparation 11.3 per cent of the thiopental was converted to pentobarbital as determined by ultraviolet and infrared spectral analysis. More recently, Frey *et al.*[8] have demonstrated the presence of pentobarbital in the blood of dogs following thiopental administration. Finally, in 1963 Cochin and Daly,[9] using thin-layer chromatography, studied the urinary metabolites of thiopental in human subjects and found a spot corresponding to pentobarbital. Desulfuration of thiobarbiturates other than thiopental has also been shown, barbital being present in the urine of subjects given thiobarbital,[10] secobarbital being reported in rat liver minces incubated with thiamylal[7] as well as in the urine of subjects having received thiamylal,[9] and butabarbital having been reported in serum of man following thiobutabarbital administration.[8] Desulfuration of methitural has also been reported as occurring in dogs.[8]

While the above reports indicate that thiopental can and does undergo desulfuration to some extent, they do not indicate the extent to which pentobarbital resulting from desulfuration of thiopental is responsible for the central nervous system depression observed

Accepted for publication July 3, 1963. This work was done in the Division of Anesthesiology, Yale University School of Medicine, and the Department of Anesthesiology, Grace-New Haven Community Hospital, New Haven, Connecticut.

following the intravenous administration of thiopental under clinical conditions. The present study was designed to determine whether the conversion of thiopental to pentobarbital occurs in man shortly after induction of thiopental anesthesia and thus to determine whether pentobarbital contributes to the state of anesthesia at a time when the degree of central nervous system depression is most profound. No attempt has been made to determine the degree to which thiopental may ultimately be desulfurated over a period of many hours. A gas chromatographic technique was employed because of its high sensitivity.

Methods

Male and female patients aged 24 to 55 undergoing elective minor surgery and without known liver disease were studied following premedication with meperidine and atropine. In each case venous blood samples (20 ml.) were drawn immediately prior to and again 15 minutes after the intravenous administration of thiopental in fractional doses adequate to maintain levels of anesthesia required by the proposed surgery. In two patients a third sample was obtained 60 minutes following induction of anesthesia. In all cases nitrous oxide and oxygen (3:1 liters per minute) were also administered with the thiopental.

Each blood sample was allowed to clot, being kept at 0–4° C. until being centrifuged at 2,000 r.p.m. for 10 minutes, following which as much serum as possible was removed and immediately extracted according to a modification of the method of Parker and Kirk [11] as follows: 8.0 ml. of serum was brought to *p*H 1 with 6N HCl and then shaken vigorously for 5 minutes with 50 ml. of chloroform in a separatory funnel. Following separation of the aqueous and organic phases, 40 ml. of the chloroform layer was filtered through Whatman no. 1 filter paper, the barbiturates then being extracted from the chloroform by shaking vigorously with 5.0 ml. of 0.45N NaOH. The chloroform was then discarded and the aqueous phase centrifuged at 2,000 r.p.m. for 5 minutes to remove any debris carried over from the serum. Exactly 4 ml. of the NaOH extract was transferred to a third separatory funnel and brought to *p*H 1 with 6N HCl and shaken with 3 ml. of chloroform. The organic phase containing the barbiturates was then carefully transferred to a small vial and evaporated under an air stream. This step was repeated three times and the final chloroform extracts were pooled in the same vial and evaporated to dryness. A slightly yellow powder appeared in the bottom of the vial. This powder was dissolved in 20 μl. of acetone before its injection into the gas chromatography machine.

A Barber-Colman gas chromatograph fitted with an ionization detector was used. After a number of preliminary experiments, it was found that a 4 foot column packed with neopental adipate terminated (NAT-3%) and kept at a temperature of 200° C. resulted in the optimal separation of mixtures of pentobarbital and thiopental. The following conditions were also established as yielding the maximal efficiency for analysis of the barbiturates: flash heater temperature, 260° C.; cell temperature, 200° C.; gas (argon) pressure, 30 pounds; gain, 1,000; voltage, 1,225. No sample was analyzed in the 24 hour period immediately following insertion of a new column so as to allow complete equilibration of the column. Finally, any deviations from the above conditions were corrected before every analysis.

Aliquots of the samples which had been dissolved in acetone were injected into the column with a 10 μl. Hamilton microsyringe. Determinations on each sample as well as thiopental and pentobarbital standards were run in duplicate. Identification of thiopental and pentobarbital was made by comparing the retention times of the barbiturates in the sample with the retention times of known thiopental and pentobarbital.

Results were calculated after the output of the ionization detector were fed into a strip recorder, the areas under the well-separated curves associated with the retention times of pentobarbital and thiopental being planimetrically measured. The results obtained for unknown samples were compared with the area of curves obtained using known amounts of thiopental and pentobarbital, it thus being possible to obtain an accurate estimate of the amount of thiopental and pentobarbital in each unknown blood sample.

TABLE 1. Serum Levels (μg per 8 ml. plasma) of Thiopental (T) and Pentobarbital (P) Following Thiopental Administration

Patient	Total Thiopental Administered (mg.) in First 15 Minutes	Barbiturate	Time (min.) 0	15		60	
			(μg)	(μg)	(% of total)	(μg)	(% of total)
2	450	T	0.0	105.0			
		P	0.0	1.8	1.7		
3	550	T	7.4	361.0			
		P	0.0	6.9	1.6		
4	1275	T	0.0	108.7			
		P	0.0	1.7	1.5		
5	475	T	5.6	84.6			
		P	0.0	1.5	1.7		
6	450	T	0.0	81.7			
		P	0.0	1.1	1.3		
7	500	T	0.0	66.7			
		P	0.0	2.3	3.3		
8	700	T	2.0	32.1			
		P	0.0	1.2	3.6		
9	750	T	2.0	80.6		120.2	
		P	0.2	1.3	1.6	2.9	2.4
11	1250	T	0.0	64.6		74.6	
		P	0.9	0.8	1.2	5.2	6.5

Preliminary experiments were carried out to determine the efficiency of the extraction procedure in removing thiopental and pentobarbital from serum and it was found that approximately 80 per cent of thiopental and 50 per cent of pentobarbital added to serum was recovered. This difference in the recovery of the two barbiturates was for all practical purposes offset by the fact that the gas chromatograph was more sensitive in detecting pentobarbital as compared to thiopental, the area of the curve associated with 1 μg. of pentobarbital being 1.6 to 2.3 times larger than the area of the curve produced by the same amount of thiopental.

It having been observed [10] that thiopental and, to a lesser extent, pentobarbital may undergo ring cleavage at alkaline *p*H's, it was determined whether the extreme alkaline and acid conditions during portions of the present extraction procedure could produce spurious serum barbiturate levels by spectrophotometrically following for 3 hours the ultraviolet absorption of solutions of both thiopental and pentobarbital at *p*H's of 1.0, 7.0, and 11.0. The optical density did not change for either barbiturate and, since the ultraviolet absorption of these compounds depends on an intact ring structure,[3] it was concluded that changes in *p*H during the extraction did not result in ring cleavage.

Finally, analyses were made to determine whether the extraction process itself resulted in desulfuration of thiopental. This was done by adding thiopental to distilled water and then immediately carrying out the entire extraction procedure. The results showed that 1.1 per cent of the barbiturate recovered appeared as pentobarbital. Comparable results were obtained when thiopental was added to

serum. On the other hand, only pentobarbital was recovered after pentobarbital had been added to serum.

Results

The results of determinations of serum pentobarbital and thiopental levels in 9 patients following the intravenous administration of thiopental are summarized in table 1. Though the absolute amount of pentobarbital in serum varied from patient to patient (as did the amount of thiopental), pentobarbital accounted for 1.2 to 1.7 per cent of the total barbiturate present in 7 of the 9 subjects 15 minutes after the start of thiopental administration. These figures are not significantly different from those obtained when the effect of the extraction process on desulfuration of thiopental was evaluated as in "Methods" above. In 2 patients (7 and 8) pentobarbital represented 3.3 and 3.6 per cent, respectively, of the total barbiturates present. In these 2 patients the amount of pentobarbital present in serum may possibly represent a small amount of desulfuration of thiopental which occurred *in vivo* rather than desulfuration due to the extraction process, but even in these 2 cases the serum levels of pentobarbital were so low they could not be considered representative of a degree of desulfuration which would significantly contribute to the central nervous system depression produced by thiopental. In the 2 patients in whom serum barbiturate levels were determined 60 minutes after the start of thiopental administration, serum pentobarbital levels constituted slightly greater percentages of total serum barbiturates than they did in the same patients at 15 minutes. This suggests, though does not prove, that desulfuration of thiopental is a function of time, but even in these 2 patients the amount of pentobarbital present which cannot be ascribed to the extraction process is so slight as to indicate a pharmacologically insignificant degree of desulfuration in view of the degree of central nervous system depression exhibited by these patients. In several patients unidentified compounds with retention times of thiopental or pentobarbital appeared in serum samples obtained before the administration of thiopental but did not impair barbiturate analysis.

Discussion

The present results indicate that desulfuration of thiopental to pentobarbital occurs little if at all shortly after the intravenous administration of thiopental. It is concluded that pharmacologically pentobarbital does not contribute significantly to thiopental anesthesia at a time when the level of anesthesia is most profound. Whether desulfuration of thiopental occurs to a significant extent many hours after thiopental administration and so results in a situation wherein pentobarbital contributes to postoperative central nervous system depression cannot be determined on the basis of the present data, but such a possibility must be considered in view of published reports of thiopental desulfuration. If such occurred, it would be analagous to the fact that the hypnotic effect of chloral hydrate immediately after its administration is due to unmetabolized chloral hydrate whereas later the hypnotic effect is due both to chloral hydrate and to its metabolic product, trichlorethanol.[12]

The fact that the extraction procedure for thiopental can account for the majority if not all of the pentobarbital observed under the conditions of the present investigation emphasizes the necessity for evaluation of the effects of chemical methodology on desulfuration, especially in view of the previous demonstration by Bush[10] that the extraction of thiobarbital with diethyl ether results in the formation of its oxy-analogue, barbital. A review of the reports demonstrating desulfuration of thiopental[4-7, 9] reveals that in the majority of such studies this possibility has not specifically been looked for and specifically excluded. In many reports the percentage recovery of thiobarbiturates has been reported but pentobarbital as a breakdown product of the extraction procedures has not been measured. An exception to this is the report by Frey.[13] Using paper chromatography and ultraviolet spectrophotometry he found traces of oxy-analogues following extraction of thiopental with ether but not following extraction with chloroform. The present results indicate, however, that extraction of thiopental even with chloroform may result in desulfuration.

The validity of the present investigation

depends in part upon a conversion rate of thiopental to pentobarbital which is more rapid than the rate at which pentobarbital is converted to its breakdown products. Brodie *et al.*[14, 15] showed that the rate of metabolism of thiopental in man was 15 per cent per hour while that of pentobarbital was 4 per cent per hour. If the rate of thiopental metabolism reflects the rate of its desulfuration, pentobarbital formed as a metabolic product of thiopental should therefore be detectable in the serum of patients studied under the conditions of the present experiment, especially since a method as sensitive as gas chromatography was employed. In addition, 98.6 per cent of pentobarbital has been reported as being recovered 15 minutes following its administration in animals,[16] suggesting that any pentobarbital formed would have been observed within the time limits of this experiment.

Summary

Gas chromatographic analysis of serum pentobarbital and thiopental levels 15 minutes following the intravenous administration of thiopental under clinical conditions revealed levels of pentobarbital which were not significantly different from those levels ascribable to the extraction process employed. It is concluded that immediately after thiopental administration in man, at a time when central nervous system depression is maximal, pentobarbital resulting from desulfuration of thiopental does not contribute to the central nervous system depression. It is further concluded that extraction processes, including those employing chloroform, may result in desulfuration of thiopental and so lead to spurious results.

References

1. Brodie, B. B., Mark, L. C., Papper, E. M., Lief, P. A., Berstein, E., and Rovenstein, E. A.: The fate of thiopental in man and a method for its estimation in biological material, J. Pharmacol. Exp. Ther. **98**: 85, 1950.
2. Wood, H. B., Jr., and Horning, E. C.: 5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-barbituric acid and its thio-analog. Metabolites from pentobarbital and thiopental, J. Amer. Chem. Soc. **75**: 5511, 1953.
3. Cooper, J. R., and Brodie, B. B.: Enzymatic oxidation of pentobarbital and thiopental, J. Pharmacol. Exp. Ther. **129**: 75, 1957.
4. Taylor, J. D., Richards, R. K., and Tabern, D. L.: Metabolism of S^{35} thiopental (Pentothal). Chemical and paper chromatographic studies on S^{35} excretion by the rat and monkey, J. Pharmacol. Exp. Ther. **104**: 93, 1952.
5. Winters, W. D., Spector, E., Wallach, D. P., and Shideman, F. E.: Metabolism of thiopental-S^{35} and thiopental-2-C^{14} by a rat liver mince and identification of pentobarbital as a major metabolite, J. Pharmacol. Exp. Ther. **114**: 343, 1955.
6. Winters, W. D.: Urinary metabolites of thiopental in rat, dog and man, Fed. Proc. **16**: 347, 1957.
7. Spector, E., and Shideman, F. E.: Metabolism of thiopyrimidine derivatives: thiamylal, thiopental and thiouracil, Biochem. Pharmacol. **2**: 182, 1959.
8. Frey, H. H., Doenicke, A., and Jäger, G.: Quantitative Bedeutung der Desulfurierung im Stoffwechsel von Thiobarbituraten, Med. Exp. **4**: 243, 1961.
9. Cochin, J., and Daly, J. W.: The use of thin-layer chromatography for the analysis of drugs. Isolation and identification of barbiturates and nonbarbiturate hypnotics from urine, blood and tissues, J. Pharmacol. Exp. Ther. **139**: 154, 1963.
10. Bush, M. T., Mazel, P., and Chambers, J.: The metabolic fate of thiobarbiturates: thiobarbital in man, J. Pharmacol. Exp. Ther. **134**: 110, 1961.
11. Parker, K. D., and Kirk, P. L.: Separation and identification of barbiturates by gas chromatography, Anal. Chem. **33**: 1378, 1961.
12. Mackay, F. J., and Cooper, J. R.: A study of the hypnotic activity of chloral hydrate, J. Pharmacol. Exp. Ther. **135**: 271, 1962.
13. Frey, H. H.: Möglichkeit der Desulfurierung von Thiobarbituraten im Verlauf der Extraktion mit Äther aus biologischem Material, Naturwissenschaften **47**: 471, 1960.
14. Brodie, B. B.: Physiological disposition and chemical fate of thiobarbiturates in the body, Fed. Proc. **11**: 632, 1952.
15. Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M.: The fate of pentobarbital in man and dog and a method for its estimation in biological material, J. Pharmacol. Exp. Ther. **109**: 26, 1953.
16. Kozelka, F. L., Nelson, D. E., and Tatum, H. J.: A method for the determination of barbituric acid derivatives in tissue and body fluids, J. Pharmacol. Exp. Ther. **67**: 71, 1939.