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THIOPENTONE AND BUTHALITONE: THE RELATIONSHIP BETWEEN DEPTH OF ANAESTHESIA, PLASMA CONCENTRATION AND PLASMA PROTEIN BINDING

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For 24 hr. after intravenous administration of buthalitone or thiopentone, plasma concentrations in young human subjects have been followed. Buthalitone was distributed to the tissues more rapidly but was metabolized at a slower rate than thiopentone. The relationships between these findings and differences in plasma protein binding and oil/water partition coefficients were studied. It is suggested that some of the differences observed in potency between the substances is a reflection of differences in their modes of distribution. No relationship was found between speed of recovery from anaesthesia and plasma barbiturate concentrations.

Of the new ultra-short-acting thiobarbiturates, the most commonly employed is buthalitone sodium, the sodium salt of 5-allyl-5-isobutyl-thiobarbituric acid. It was introduced as an anaesthetic by Weese and Koss (1954) and has achieved its greatest popularity on the European continent. In Britain some authors regard it as useful for anaesthesia of brief duration (Young, 1956; Henderson and Mackett, 1957), while others conclude that it offers no advantage over thiopentone sodium, the sodium salt of 5-ethyl-5-(1-methylbutyl)-thiobarbituric acid (Simmons and Blanshard, 1957; O'Mullane, 1957). It is generally agreed that buthalitone appears to be a less potent anaesthetic than thiopentone when given in the same dose. The present investigation had three main objects: first, to compare the rates of elimination of the two substances from the blood stream after intravenous injection and thereby to deduce, if possible, any difference there might be in the rates of metabolism of the two; second, to attempt to relate changes in plasma concentrations with the clinical signs of recovery from anaesthesia; and third, to compare two physical attributes, the plasma protein binding and the oil/water partition coefficients, in an attempt to account for the differences in clinical activity of the two drugs.

METHODS

Clinical.—The patients were otherwise healthy male subjects, aged 18 to 37 years, weighing 58.5 to 86.2 kg., who underwent minor operative procedures such as incisions, manipulations, and excisions of cysts. Premedication consisted solely of atropine sulphate (0.6 mg. given subcutaneously) 45 min. before operation. Doses both of buthalitone (16 patients) and of thiopentone (10 patients), based on 11 mg./kg. body weight, were administered as 10 and 5% (w/v) solutions respectively at a rate of 1 ml./sec. The drug was injected into a vein in the antecubital fossa, and blood samples were withdrawn from a vein in the opposite arm at −1, 2, 4, 8, 16, 32 min., and 1, 2, 4, 8, and 24 hr. from the mean time of the injection. In most cases some supplementary anaesthesia was required, and nitrous oxide, oxygen, and trichlorethylene or cyclopropane and oxygen were administered. In a few cases muscle relaxants, with or without controlled respiration, were needed. Three patients were given a second dose of buthalitone 16 min. after the first. Three tests were applied to six subjects who received buthalitone but no supplementary anaesthesia whenever blood samples were taken. The observations were recorded by a witness otherwise independent of the investigation, and consisted of: (1) Response to pin-prick. A movement resulting from two or more out of three such stimuli applied to the back of the hand was recorded as being a positive response. (2) Response to simple commands such as "Put out your tongue" and "Lift up your right hand." Obedience to any single such command was recorded as positive. (3) The ability of the patient to walk unaided.

Laboratory.—Blood was transferred to tubes containing potassium oxalate (1 mg./ml.) and centrifuged within 1 hr. of collection. Thiobarbiturate concentrations were estimated by the method of Crawford and Kane (1956) which involved extraction

of the substance into chloroform. Two ml. of plasma was used for each estimation. To establish the suitability of the method for the estimation of buthalitone, analyses were performed on plasma samples, taken from patients receiving no barbiturates, to which known amounts of buthalitone in the range 0 to 50 μg. had been added. A linear relationship was found between "corrected" absorbance (Crawford and Kane, 1956) and amount of buthalitone. The agreement between replicate estimates was of the order ±10%.

Brodie, Mark, Papper, Lief, Bernstein, and Rovenstine (1950) have found that methods of barbiturate estimation involving extraction into a highly polar solvent such as chloroform may give results which are falsely high if plasma concentrations are followed for prolonged periods. This is due to the extraction of a metabolic product, a pharmacologically inactive carboxylic acid derivative, which has a similar ultra-violet absorption spectrum. They therefore used a method involving extraction into light petroleum in order to obtain "true" thiopentone concentrations. To establish whether the chloroform-extraction method estimates the barbiturate alone or in addition its metabolic product, a number of 4 hr. plasma samples were estimated in duplicate, employing the method of Brodie *et al.* (1950) and the present method. In addition, one 4-hr. sample was estimated by infra-red as well as ultra-violet spectrophotometry with the same object in view, since the infra-red spectrum of a metabolite is very unlikely to be the same as that of the drug. Twenty ml. of plasma was extracted with 250 ml. chloroform. The chloroform phase was filtered through a No. 1 Whatman filter and extracted with 50 ml. of 0.1 N-NaOH. The aqueous phase was acidified with HCl and re-extracted with 4 × 10 ml. of chloroform. The chloroform extracts were dried with Na_2SO_4 and evaporated on to 300 mg. KBr. A pressed disc was made (Stimpson and O'Donnell, 1952) and the infra-red spectrum was obtained on a Perkin-Elmer model 21 spectrophotometer over the range 6 to 10 μ. The quantity of buthalitone had been found from trial experiments to be proportional to the "corrected" absorbances at 6.55 and 8.65 μ. An attempt to develop an infra-red method for thiopentone failed owing to the lack of strong bands for this compound in regions of the infra-red spectrum in which interference from extracted constituents of plasma was not encountered. The results of these preliminary experiments (Table I) confirmed that for thiopentone the concentrations recorded by the method of Crawford and Kane (1956) were probably too high. For buthalitone, however, the different methods showed good agreement, indicating a probable lack of interference from metabolic products.

TABLE I

COMPARISON OF METHODS OF ESTIMATING BARBITURATES

Duplicate results on 5 separate 4-hr. samples, expressed in μg./ml.

No.	Barbiturate	Ultra-violet		Infra-red
		Chloroform Extraction	Light Petroleum Extraction	Chloroform Extraction
1	Thiopentone	4·4	3·3	—
2	,,	7·1	4·1	—
3	Buthalitone	9·7	11·6	—
4	,,	0·8	0·6	—
5	,,	10·3	—	9·7

Plasma protein binding was estimated by dialysing fresh oxalated human plasma whose protein concentration was found to be 70 mg./ml. (albumen 46 mg./ml., globulin 24 mg./ml.) in ¼ inch "Visking" tubing bags against solutions of the barbiturate. These solutions were made up in M/15 phosphate buffer of *p*H 7.4 with olive oil at 37° for 3 hr., and 37° for 24 hr. Trial experiments revealed that binding increased during the first 24 hr., thereafter gradually decreasing, probably because of slow protein denaturation. Penicillin 100 units/ml. and streptomycin 100 μg./ml., which were found not to interfere with either the extent of binding or the protein or barbiturate estimations, were added to the barbiturate solutions in order to prevent the growth of bacteria during the incubation period. Because the plasma protein became diluted by 10 to 15% during the experiments, binding is expressed as the amount of barbiturate/mg. protein.

Oil/water partition coefficients were found by shaking the barbiturate solutions in phosphate buffer of *p*H 7.4 with olive oil at 37° for 3 hr., and estimating the amounts of barbiturate in the aqueous layer before and after shaking.

RESULTS

Blood Barbiturate Concentrations.—Fig. 1 shows the mean plasma concentrations after injections of buthalitone and thiopentone. Although the general trend of the plasma concentrations was similar in the two groups, there were two main differences. In the first place, in spite of the similarity of dose, the concentrations of thiopentone during the first 32 min. after a single dose were significantly higher than those of buthalitone ($P<0.01$). On the other hand, the concentration of thiopentone from 2 hr. onwards declined more rapidly and the difference in concentrations of the two substances again became significant at 8 ($P<0.05$) and 24 hr. ($P<0.001$).

Clinically, most of the six patients who received buthalitone but no supplementary anaesthesia were completely unresponsive to all stimuli at 2 min., and at 32 min. were able to walk unaided. Individually, however, their plasma concentrations varied only slightly during this period and no relation was found between recovery from anaesthesia and the plasma thiobarbiturate concentration. For example, one patient was

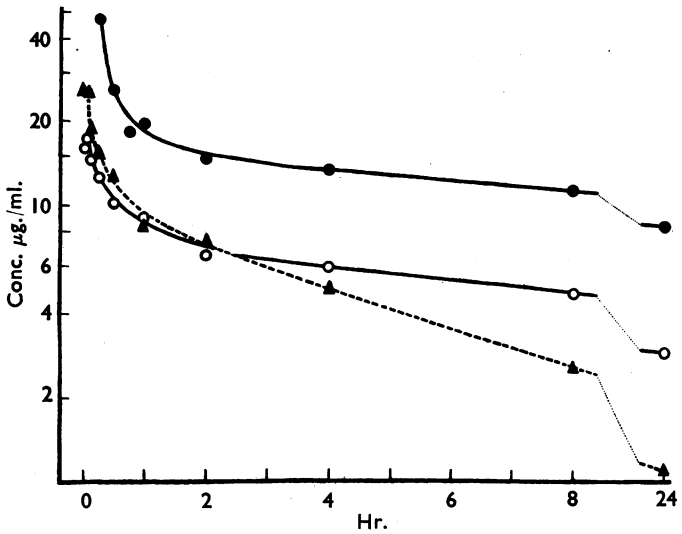

FIG. 1.—Mean plasma concentrations after intravenous administration of buthalitone 11 mg./kg. injected at 0 and 16 min. (n=3), ●——●; buthalitone 11 mg./kg. at 0 min. (n=16), O——O; thiopentone 11 mg./kg. at 0 min. (n=10), ▲- - -▲; standard errors are smaller than symbol used for mean except at 8 and 24 hr.

completely unconscious at 2 min. with a plasma concentration of 13.4 μg./ml., and at 4 min. he responded to pin-prick and obeyed simple commands, although he then had a plasma concentration of 13.9 μg./ml. Another subject was unconscious at 2 min. (10.0 μg./ml.), able to respond to pin-prick at 4 min. (12.3 μg./ml.), obeyed commands at 8 min. (13.8 μg./ml.) and at 32 min. was able to walk unaided, although his plasma concentration was then 10.1 μg./ml.

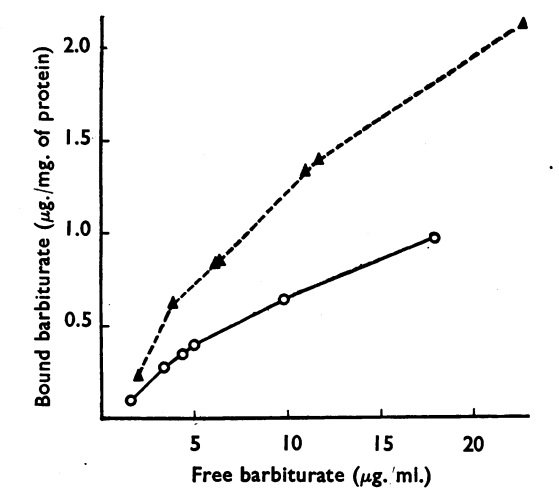

FIG. 2.—Barbiturate bound by plasma proteins. Thiopentone, ▲- - -▲; buthalitone, O——O.

The mean plasma concentrations found in the three subjects who received two doses of buthalitone are illustrated from 18 min. onwards in Fig. 1. The concentrations attained 2 min. after the second dose are more than twice as high as those after one dose, but from 4 hr. onwards the decline runs parallel to that following the single dose. Recovery of consciousness in these three patients was delayed until about 20 min. after the second dose, but again no relation was found between this recovery and the plasma thiobarbiturate level.

Plasma Protein Binding.—Dialysis experiments showed that thiopentone was bound to a much greater extent than was buthalitone, and that increasing barbiturate concentrations led to a relative fall-off in the degree of binding, as partial saturation of the protein occurred (Fig. 2).

Oil/Water Partition Coefficients.—The mean of three estimations of the coefficients for each substance indicated that thiopentone was relatively more soluble in fat than was buthalitone. The olive oil/water concentration ratios at *p*H 7.4 were 58:1 for thiopentone and 18:1 for buthalitone.

DISCUSSION

The present studies indicate that there are significant biochemical and physico-chemical differences between thiopentone and buthalitone. These differences become of general interest if the two are found to be related. Although Butler (1950) suggested that such relationships are valid only between drugs as closely related as those in a homologous series, it is felt that thiopentone and buthalitone resemble each other sufficiently closely to be able to draw certain conclusions from these investigations.

In the first place, the finding that, following intravenous administration, the initial concentrations of thiopentone were significantly higher than those of buthalitone indicated that the former substance left the circulation less rapidly than the latter. This is to be expected if, as was found, plasma protein binding was more extensive in the case of thiopentone, leaving less free barbiturate to be distributed to the tissues. The finding, that, from 2 hr. after injection onwards, the slope of thiopentone concentrations was considerably steeper than that of buthalitone,

indicated a more rapid rate of metabolism. That this slope does indicate a rate of metabolism is suggested by the parallelism of the slopes following the different doses, reported here for buthalitone, and by Brodie *et al.* (1950) for thiopentone. It is possible that differences in protein binding are manifest not only in the plasma but also in the liver, and that the rates of metabolism are influenced by binding to the enzymes concerned. However, other factors, such as chemical structure and polarity, probably influence the rate as well, and it is not therefore suggested that protein binding is the only one. As far as the nature of the metabolic pathway is concerned, thiopentone undergoes terminal oxidation of its 1-methyl-butyl side chain (Brodie *et al.*, 1950; Cooper and Brodie, 1957), but the changes which occur to buthalitone are not known. Our findings suggest that terminal oxidation is unlikely as we were unable to find evidence of a carboxylic acid derivative with the extraction procedures used.

As a central depressant agent, thiopentone is much more potent than buthalitone. Simmons and Blanshard (1957) and Orkin, Morales, Fujita, and Gabuya (1958) both reported a clinical potency ratio of about 2:1. Rivett (personal communication) found that, when administered intravenously to mice, the anaesthetic dose of buthalitone was three times that of thiopentone. O'Mullane (1957) showed that the same relative amounts were needed to produce equivalent electroencephalographic changes in human subjects. The question arises as to whether it is possible to account for any of this difference in potency. Mark, Burns, Brand, Campomanes, Trousof, Papper, and Brodie (1958) showed a remarkable correlation between oil/water partition coefficients of various barbiturates and the speed with which they enter the brain after intravenous injection, the ones with the highest coefficients entering most rapidly. On this basis, thiopentone probably enters the brain faster than buthalitone as it has a higher coefficient. There appears, therefore, to be a tendency for more thiopentone to enter the brain, but, because less is bound to plasma protein, more buthalitone appears to enter the tissues generally. It seems highly probable that this may account for some of the difference in potency between the two substances. In other words, potency may be partly a manifestation of physical distribution, and that one of the factors which makes thiopentone a more potent central depressant than buthalitone is simply that more reaches the brain in the first few minutes. However, other factors are involved, as shown by the recent findings of Parker and Aldridge (personal communication). They have assessed the inhibitory action of various barbiturates on oxidative phosphorylation of brain and liver mitochondria, and have shown that, in this respect, thiopentone is between two and three times as effective as buthalitone. While not proven, it is probably reasonable to assume a parallelism between such inhibition and central depressant action.

Wyke (1957) has pointed out that one implication of protein binding is that alterations in plasma protein levels may affect sensitivity of patients to administered barbiturates, and that subjects who are protein depleted or acidotic become more sensitive to their action, because a greater proportion of the administered dose remains unbound. However, Goldbaum and Smith (1954), who investigated binding by an ultra-filtration method and employed relatively low concentrations of protein for most of their experiments, suggest that variations in protein concentration or in *p*H found under clinical conditions are unlikely to influence to any significant extent the proportion of barbiturate remaining unbound.

Whether the physical attributes of these two substances determine their potency or not, it seems probable that they influence their mode and rate of distribution and metabolism and also their duration of action.

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