[CONTRIBUTION FROM THE ABBOTT LABORATORIES, NORTH CHICAGO, ILL.]

Physico-Chemical Properties and Hypnotic Action of Substituted Barbituric Acids

BY D. L. TABERN AND E. F. SHELBERG

Henderson,[1] in a recent review, has enumerated no less than ten distinct theories that have been proposed to account for the mechanism of hypnotic action. The one which has best stood the test of time, or perhaps we should say, has developed the fewest shortcomings, is that of Meyer and Overton. This states that all chemically indifferent bodies which are soluble in fats or lipoids act as narcotics to living protoplasm; the relative degrees of activity of such narcotics depend on their affinity for fat-like substances on the one hand and for other body constituents on the other; as an approximate measure of this ratio, one may determine the distribution coefficient of the substances between oil and water.

Within the last ten years, barbiturates have come to occupy a prominent place among hypnotics, not only in routine medical practice, but in the more specialized fields of pre-anesthetic sedation and the prevention of local anesthetic reactions. These newer uses have been made possible by the development of new members possessing specifically valuable properties. For instance, certain members containing the (1-methyl butyl) group are not only the most potent yet described,[2] but are quickly broken down in the body. This leads to a period of intense hypnosis followed by rapid recovery without the prolonged depression characteristic of longer acting substances. The advantage of such rapid recovery following operative procedures is self obvious. Tatum[3] has recently shown that *n*-butylethylbarbituric acid is particularly effective in the prevention of local anesthetic intoxication.

There have now been synthesized and accurately evaluated pharmacologically more than one hundred barbiturates of relatively similar structure; over sixty have been tested in our own laboratories by a standard procedure; in addition, we have available a considerable mass of clinical data regarding prominent members. We may not only vary the number of carbon atoms in each of the alkyl groups present, but in the higher members we have many possibilities of isomerism.

It seemed of interest, therefore, to study the applicability of certain of the above-mentioned theories of hypnotic action to the barbiturates.

Of practical importance was the possibility that, if a definite relation could be established between certain physical-chemical properties and the hypnotic activities, a chemical method could be made available for the

(1) Henderson, *Physiol. Rev.*, **10**, 171 (1930). An excellent bibliography is included.

(2) Volwiler and Tabern, THIS JOURNAL, **52**, 1676 (1930); Shonle, Keltch and Swanson, *ibid.*, **52**, 2440 (1930).

(3) Tatum, personal communication.

preliminary evaluation of newly synthesized compounds. We have selected fifteen members, some because of their commercial importance, others because they contained interesting chemical groupings. The total length of the carbon chains attached to the "5" carbon varied from 2 to 11 atoms, covering not only the rise in hypnotic efficiency, but also its subsequent decrease in higher members. Two isomeric butyl and three isomeric amyl groups were included. Two compounds contained asymmetric carbon atoms.

Distribution coefficients of the barbituric acids were determined between water and a specially purified fatty acid free olive oil at 20°. Equal amounts of the oil and the aqueous solutions were continuously agitated for a number of hours until equilibrium had been established. Analyses of the aqueous phase were made by the Kjeldahl method.

TABLE I

No.	Barbiturate	Mol. wt.	Sol. g. per liter	Dist. coeff., av.	Efficiency[a] rating
1	Dimethyl	156	2.419	0.066	0
2	Diethyl (Barbital)	184	6.00	.214	+
3	Ethyl *i*-propyl (Ipral)	198	1.36	.73	+
4	*i*-Propyl allyl	212	4.02	1.12	+++
5	Diallyl (Dial)	208	1.465	0.85	++
6	*n*-Butyl ethyl (Neonal)	212	1.90	2.58	+++
7	*Sec.*-butyl ethyl	212	1.987	1.36	+++
8	*Sec.*-butyl allyl	224	2.16	2.48	++++
9	*n*-Amyl ethyl	226	0.554	2.92	+++
10	*i*-Amyl ethyl (Amytal)	226	.530	2.895	+++
11	Ethyl (1-methyl butyl) (Nembutal)[b]	226	1.20	4.4	++++
12	*Sec.*-butyl β-bromoallyl (Pernocton)	304	0.684	4.3	++++
13	Phenyl ethyl (Phenobarbital)	234	.970	1.34	++
14	Allyl (methylhexylcarbinyl)	280	3.06	0.306	0
15	Ethyl (methylhexylcarbinyl)	267	0.414	.408	0

[a] Since variations of technique such as the route of administrations, etc., lead to slightly divergent numerical data, it seems most satisfactory to employ only comparative values. [b] Nembutal (pentobarbital sodium) is the sodium salt of this acid.

Table I demonstrates a definite parallelism between the partition coefficients and hypnotic efficiency, both increasing from a low value in dimethyl to a maximum in ethyl (1-methyl butyl), *sec.*-butyl allyl, and *sec.*-butyl (β-bromoallyl)-barbituric acids, and then decreasing rapidly to small figures for ethyl and allyl octyls. The only outstanding exception is diallylbarbituric acid, although in general the unsaturated members are somewhat more effective than the coefficients would lead us to expect.

Within the last year, Barlow and associates[4] have carefully compared a number of barbiturates with particular reference to efficiency in preanesthetic sedation. They place Nembutal (Pentobarbital) at the top,

(4) Barlow, Duncan and Gledhill, *J. Pharmacol.*, **41**, 366 (1931).

followed by Pernocton and Dial; barbital and phenobarbital were found relatively ineffective. If we employ, instead of the pharmacologic data recently published comparisons of certain members when used intravenously in human beings, the correlation is even closer; for instance, both Fitch, Waters and Tatum[5] and Lundy[6] have reported Nembutal twice as effective as Amytal and "somewhat" more effective than Pernocton, and we find the coefficients of 4.4, 2.8 and 4.3, respectively.

Since the theory of Meyer and Overton is based essentially upon the high lipoid content of nerve tissue, it seemed of interest to determine the distribution of various barbiturates between water and a chemically neutral medium such as ash-free charcoal.[7] Adsorption (by 1 g. of charcoal) from (300 cc. of a 0.12%) aqueous solution was remarkably rapid, equilibrium with resultant removal of 75% or better of the barbiturate being complete in less than fifteen minutes. To our surprise the order of the members studied follows qualitatively at least the order of hypnotic efficiency.

TABLE II

Barbiturate	% Adsorp.
Dimethyl	79
Diethyl (Barbital)	88
Diallyl (Dial)	92.5
n-Butyl ethyl (Neonal)	96
i-Amyl ethyl (Amytal)	95 app.
Ethyl (1-methyl butyl) (Nembutal)	96
Octyl ethyl	83.5

Traube has suggested[8] that the surface tension of the hypnotic solution may be the deciding factor in determining its efficiency. We have, therefore, determined the surface tension of the aqueous solutions of the above barbiturates at two concentrations. Measurements were made by the De Nouy tensiometer at 20°, and the results expressed as the percentage of the normal value for water determined at the same time. From Table III, it is seen that while surface activity does increase with increasing hypnotic efficiency, it does not decrease in the octyl homologs as the theory would lead us to expect. Furthermore, Amytal is more surface active than Nembutal (Pentobarbital acid) but much less effective.

Chemical stability and rate of excretion are becoming important factors now that we appreciate the superiority of short-acting barbiturates as preanesthetic sedatives. Barbital is largely (70–80%) excreted unchanged in the urine, while with certain higher homologs the majority is apparently decomposed in the body.[9] Shonle and Keltch[10] have stated that neither Amytal nor Nembutal is eliminated as such in the urine.

(5) Fitch, Waters and Tatum, *Am. J. Surgery*, **9**, 110 (1930).
(6) Lundy, *Anesthesia and Analgesia*, **9**, 210 (1930).
(7) Kindly supplied by Dr. E. J. Miller.
(8) Traube, *Arch. ges. Physiol.*, **153**, 276 (1913); **160**, 51 (1915); **161**, 530 (1915).
(9) Herwick, *J. Pharmacol.*, **39**, 267 (1930).
(10) Shonle and Keltch, Indianapolis Meeting, A. C. S., April, 1930.

TABLE III

No.	Barbiturate	Mol. wt.	% S. T (satd.)	% S. T. 1:2000	Efficiency rating
1	Dimethyl	156	98.5	100.0	0
2	Diethyl (Barbital)	184	90.0	99.1	+
3	Ethyl *i*-propyl (Ipral)	198	94.5	98.5	+
4	*i*-Propyl allyl	212	84.0	97.5	+++
5	Diallyl (Dial)	208	87.5	97.5	++
6	*n*-Butyl ethyl (Neonal)	212	75.5	89.5	+++
7	*Sec.*-butyl ethyl	212	84.0	96.0	+++
8	*Sec.*-butyl allyl	224			++++
9	*n*-Amyl ethyl	226	75.0	76.5	+++
10	*i*-Amyl ethyl (Amytal)	226	77.5	76.5	+++
11	Ethyl (1-methylbutyl) (Nembutal)	226	75.0	85.5	++++
12	*Sec.*-butyl β-bromoallyl (Pernocton)	304	87.0	91.5	++++
13	Phenyl ethyl (Phenobarbital)	234	95.0	97.5	++
14	Allyl (methylhexylcarbinyl)	280	67.2	79.0	0
15	Ethyl (methylhexylcarbinyl)	267	75.5	..	0

A definite amount of the pure sodium salt was dissolved in carbon dioxide free water in a sealed tube and held at 100° for sixteen hours. After cooling, the contents were acidified with sulfuric acid and the liberated carbon dioxide absorbed in "Ascarite." From this, the amount of decomposition was calculated. We find that stability toward hydrolysis of the sodium salts in aqueous solution to the corresponding acetyl ureas does not run inversely parallel to hypnotic efficiency but is dependent upon the groups attached to the "5" carbon atom. The presence there of any "secondary" linkage increases stability threefold. The additional presence of the unsaturated negative β-bromoallyl group still further enhances resistance to hydrolysis of the ester linkage. In other words, we find the unexpected fact that other things being equal the powerful barbiturates containing a secondary group are on the one hand very stable in the test tube, and very unstable in the body.

TABLE IV

	Barbiturate	% Hydrolysis
1	Diethyl (Barbital)	80
2	*n*-Butyl ethyl (Neonal)	85
3	*i*-Amyl ethyl (Amytal)	79
4	Cyclohexenyl ethyl (Phanodorn)	41
5	Phenyl ethyl (Phenobarbital)	96
6	*Sec.*-butyl ethyl	28
7	Ethyl (1-methylbutyl) (Nembutal)	24
8	Ethyl *sec.*-octyl	25
9	*Sec.*-butyl β-bromoallyl (Pernocton)	165

Bancroft and Richter[11] have recently revived Bernard's theory of narcosis and presented evidence that hypnosis is in most instances associated with reversible coagulation of cell colloids. While there has been no

(11) Bancroft and Richter, *J. Phys. Chem.*, **35**, 215 (1931).

opportunity for us to study in this way the action of the barbiturates upon living cells, it does not seem to us that their findings conflict with the evidence here presented. Coagulation may be the mode of action within the cell, but there remains in clinical practice the all-important necessity for transporting the hypnotic from the point of administration to the interior of the cell through a series of transferences. Here, surface tension, absorption and more particularly lipoid solubility appear to be the limiting factors in the chain of events.

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Adsorption Experiments with Vitamins B(B_1) and G(B_2)

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Seidell's adsorption of the antineuritic vitamin upon Lloyd's reagent[1] has led to considerable experimentation with this and other forms of hydrous aluminum silicate looking to partial separation of vitamins B(B_1) and G(B_2).[2]

The experiments here recorded were planned in quantitatively graded series, and were carried out upon protein-free milk as being a material of special scientific interest in this connection because of the extent to which it has been used as a source of the vitamin-B complex in numerous researches, beginning with the work of Osborne and Mendel.[3] As adsorbent, we have used a preparation of Lloyd's reagent kindly furnished us by Professor John Uri Lloyd. This has been used in the proportions of 5, 10, 20 and 40 g. per liter of protein-free milk, prepared as described below and adjusted to P_H 3.0 and also to P_H 4.0.

The original untreated protein-free milk, the activated Lloyd's reagent and the filtrates remaining after adsorption were tested quantitatively for their vitamin B(B_1) and G potencies according to methods previously developed in this Laboratory.[4] The preparations were fed in graded amounts corresponding to definite quantities of protein-free milk or of the original skimmed milk powder. No attempt was made to study the possible supplementary relation of the two fractions, activated solids and remaining filtrates to each other. In view of the recent evidence suggesting

(1) Seidell, *U. S. Pub. Health Repts.*, **31**, 364 (1916); THIS JOURNAL, **44**, 2042 (1922); *J. Biol. Chem.*, **67**, 513 (1926).

(2) Jansen and Donath, *Proc. k. akad. wetensch. Amsterdam*, **29**, 1390 (1926); Salmon, Guerrant and Hays, *J. Biol. Chem.*, **76**, 487 (1928); *ibid.*, **80**, 91 (1928); Williams and Waterman, *ibid.*, **78**, 311(1928); Hunt, *ibid.*, **79**, 723 (1928); Guha and Drummond, *Biochem. J.*, **23**, 880 (1929); Evans and Lepkovsky, *J. Nutrition*, **3**, 353 (1930).

(3) Osborne and Mendel, Carnegie Inst. Washington, Pub. No. 156 (1911).

(4) Chase and Sherman, THIS JOURNAL, **53**, 3506 (1931); Bourquin and Sherman, *ibid.*, **53**, 3501 (1931).