Table II.—(*Continued*)

Dragstedt Curves

25	307.5	306.0	100.5
40	315.0	319.0	98.8
50	320.0	327.0	97.9
60	325.0	337.0	96.4
75	337.5	344.0	98.1

It will be noted that in the experimental curves a mortality of 25 per cent is shown at three dosage levels for the U. S. P. Reference Standard tincture and at two dosage levels for the unknown tincture, thus giving six possible ratio strengths for the unknown tincture at this percentage mortality, ranging from 75.0 to 113.4 per cent of the U. S. P. potency. The application of the double integration procedure to the two experimental curves, however, completely smooths out these irregularities and gives curves for the corresponding mortality percentages of the two preparations to be composed, which run almost parallel throughout the 25–75 per cent mortality range.

Taking the figures from the Dragstedt curves the potency of the unknown tincture varies through the corresponding percentage mortality values only between 96.4 and 100.5 per cent of U. S. P. potency, with a mean value of 98.3 per cent and a probable error of the mean ±0.403 per cent.

SUMMARY

1. A simple statistical method is presented for the rapid and accurate calculation of comparative potencies in digitalis assays.

2. The method employs the simultaneous integration of survivals and deaths at all dosage levels.

3. The method does not require any transposition of units and the results obtained are directly comparable with those obtained by other workers.

4. The integrated results have an accuracy of approximately ±1 per cent throughout the 25–75 per cent mortality range.

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A Pharmacological Study of Some New Synthetic Hypnotics*

By John W. Nelson, Stanley C. Lyster and George F. Cartland

The studies here reported were undertaken with the purpose of evaluating in a preliminary way, the hypnotic activity of a large group of synthetic compounds, most of which have not previously been described in the literature. All of the compounds here studied, with the exceptions noted in Table I, were prepared by Blicke and Centolella (1, 2). The rat has generally been regarded as the most suitable animal for such studies, and the intraperitoneal route has been adopted by us as the most sensitive method of estimating hypnotic activity in a group comprising compounds of widely different solubilities. Fitch and Tatum (3) have suggested the intraperitoneal route as closely approximating slow intravenous injection. For references to the literature and a discussion of methods we refer to this paper.

Further studies on the more effective members of the series, involving measurement of induction time, and duration of sleep by oral administration in rabbits and intraperitoneal injection in rats are included in this report.

EXPERIMENTAL

Rat Experiments.—Healthy young adult male Wistar strain albino rats raised in our animal colony and weighing 175 to 300 Gm. were used in all experiments included in this report. No animal received more than one injection in order to avoid any tolerance for the material injected. The rats were not starved previous to injection since preliminary ex-

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Table I.—Hypnotic Activity by Intraperitoneal Injection in Rats

	Substituted Acetylureas	Therapeutic Index M. L. D./M. H. D. =
1.	*N*-Methyl-diethyl	700/300 = 2.3
2.	Ethyl-propyl	>750/>750 = ...
3.	*N*-Methyl-ethyl-butyl	500/300 = 1.7
4.	Ethyl-butyl-α-bromo	>740/>740 = ...
5.	Ethyl-amyl	>1000/>1000 = ...
6.	Ethyl-hexyl	>500/>500 = ...
7.	Ethyl-β-cyclohexylethyl	>1000/>1000 = ...
8.	Di-β-cyclohexylethyl	>950/>950 = ...
9.	β-Phenylethyl	>500/>500 = ...
10.	Ethyl-benzyl	>600/>600 = ...
11.	Ethyl-β-phenylethyl	>1000/>1000 = ...
12.	Di-β-phenylethyl	>500/>500 = ...
13.	Propyl-β-phenylethyl	>500/>500 = ...
14.	Isopropyl-β-phenylethyl	>600/>600 = ...
15.	Allyl-β-phenylethyl	>750/500 = >1.5
16.	Butyl-β-phenylethyl	>500/>500 = ...
17.	Isobutyl-β-phenylethyl	>600/>600 = ...
18.	β-Cyclohexylethyl-β'-phenylethyl	>500/>500 = ...
19.	Ethyl-γ-phenylpropyl	>750/>750 = ...
20.	Ethyl-δ-phenylbutyl	1000/1000 = 1.0
21.	Ethyl-ε-phenylamyl	>500/>500 = ...
22.	Ethyl-ζ-phenylhexyl	>1000/>1000 = ...
23.	Ethyl-cinnamyl	>500/>500 = ...
24.	Ethyl-methyl	>500/350 = >1.4
25.	Diethyl	500/250 = 2.0
26.	Ethylbutyl	>500/400 = >1.25
27.	α-Bromodiethyl	380/130 = 2.9
	Substituted Acetamides	
28.	Diethylthio	100/80 = 1.25
29.	*N*-Methyldiethyl	>600/500 = >1.2
30.	*N*-Butyldiethyl	>600/500 = >1.2*
31.	Ethylbutyl	350/100 = 3.2
32.	*N*-Methylethylbutyl	300/150 = 2.0*
33.	*N*-Ethylethylbutyl	250/200 = 1.25*
34.	*N*-β-Hydroxyethylethyl-butyl	500/300 = 1.6
35.	*N*-Butylethylbutyl	300/... = ...*
36.	Ethylamyl	400/100 = 4.0
37.	Ethylhexyl	>450/150 = >3.0
38.	Ethyl-β-cyclohexylethyl	>500/>500 = ...
39.	*N*-Methylethyl-β-cyclo-hexylethyl	>500/>500 = ...
40.	*N*-Ethylethyl-β-cyclo-hexylethyl	>500/>500 = ...
41.	*N*-β-Hydroxyethylethyl-β'-cyclohexylethyl	>500/200 = >2.5
42.	*N*-Butylethyl-β-cyclo-hexylethyl	>1000/>1000 = ...
43.	Di-β-cyclohexylethyl	>650/>650 = ...
44.	*N*-Methyldi-β-cyclohexyl-ethyl	>750/>750 = ...
45.	*N*-Ethyldi-β-cyclohexyl-ethyl	>750/>750 = ...
46.	Butyl-β-cyclohexylethyl	.../... = ...
47.	*N*-Methylbutyl-β-cyclo-hexylethyl	>800/>800 = ...
48.	*N*-Ethylbutyl-β-cyclo-hexylethyl	>500/>500 = ...
49.	*N*-β-Hydroxyethylbutyl-β'-cyclohexylethyl	>500/>500 = ...
50.	*N*-(Diethylacetyl)-morpho-line	600/400 = 1.5
51.	*N*-(Ethylbutylacetyl)-morpholine	450/175 = 2.6
52.	*N,N'*-bis-(Diethylacetyl)-ethylenediamine	>2000/>2000 = ...
53.	Ethylbenzyl	425/175 = 2.4
54.	Ethyl-β-phenylethyl	325/120 = 2.7
55.	*N*-Methylethyl-β-phenyl-ethyl	400/200 = 2.0*
56.	*N*-Ethylethyl-β-phenyl-ethyl	200/150 = 1.3*
57.	Di-β-phenylethyl	>500/>500 = ...
58.	*N*-Methyldi-β-phenylethyl	>500/>500 = ...
59.	*N*-Butyldi-β-phenylethyl	>500/>500 = ...
60.	β-Cyclohexylethyl-β'-phenylethyl	>300/>300 = ...
61.	Propyl-β-phenylethyl	500/200 = 2.5
62.	*N*-Methylpropyl-β-phenyl-ethyl	>200 <500/200 = ...*
63.	*N*-Ethylpropyl-β-phenyl-ethyl	400/200 = 2.0*
64.	*N*-β-Hydroxyethylpropyl-β'-phenylethyl	400/200 = 2.0
65.	*N*-Butylpropyl-β-phenyl-ethyl	>500/>500 = ...*
66.	Isopropyl-β-phenylethyl	400/150 = 2.6
67.	*N*-β-Hydroxyethyliso-propyl-β'-phenylethyl	450/250 = 1.8*
68.	Allyl-β-phenylethyl	350/125 = 2.8
69.	Butyl-β-phenylethyl	>1000/>1000 = ...
70.	*N*-Methylbutyl-β-phenyl-ethyl	>1000/500 = >2.0*
71.	*N*-Ethylbutyl-β-phenyl-ethyl	600/600 = 1.0*
72.	*N*-β-Hydroxyethylbutyl-β'-phenylethyl	400/200 = 2.0*
73.	*N*-Butylbutyl-β-phenyl-ethyl	>1000/>1000 = ...
74.	Isobutyl-β-phenylethyl	450/125 = 3.6
75.	Ethyl-γ-phenylpropyl	>500/400 = >1.25
76.	Ethyl-δ-phenylbutyl	600/250 = 2.4
77.	Ethyl-ε-phenylamyl	>750/150 = >5.0
78.	Ethyl-ζ-phenylhexyl	>1000/>1000 = ...
79.	Ethylcinnamyl	400/200 = 2.0
80.	Ethylmethyl	>1000/>1000 = ...
81.	Diethyl	>500 <1000/>300 = ...
82.	Ethylpropyl	500/200 = 2.5
83.	Cyclohexyl	600/600 = 1.0
84.	β-Phenylethyl	>500/300 = >1.7
85.	*N*-α-Hydroxymethyldi-ethyl	>1000/>800 = ...
86.	Tetramethylsuccinimide†	15/... = ...*
87.	Ethyl isoamyl	425/110 = 3.8

Compounds 1 to 23, inclusive, described in Table II (Reference 1).
Compounds 28 to 51, inclusive, described in Table I (Reference 2).
Compounds 53 to 79, inclusive, described in Table II (Reference 2).
Compounds 24 to 26, inclusive, and 80 to 86 inclusive prepared by Blicke and Centolella but not published.
Compound 27 (carbromal U. S. P.) was used as reference standard.
Compound 87 prepared by Dr. H. G. Kolloff.
* Convulsant action.
† Compound 86 is not a substituted acetamide.

periments showed that only a 5 per cent decrease in body weight resulted after 16 hours' fasting.

All compounds were suspended in 5 per cent acacia solution immediately before injection in order to avoid any decomposition of the compounds. Recourse to solvents other than 5 per cent acacia solution is precluded for the reason that they would be toxic in the volume range required by the tests. The material was injected intraperitoneally as a 5 per cent suspension into rats and the time of injection, onset and duration of sleep were recorded. The rats were observed closely for sleep during the first 3 hours after injection and any dose which failed to produce sleep during this period was considered sub-hypnotic. All animals were observed for delayed deaths for a period of seven days after injection.

Table II.—Hypnotic Activity by Oral Administration in Rabbits

	Compound	M. L. D.	M. H. D.	M. L. D. / M. H. D.	Induction Time at Approx. 60% M. L. D.	Duration of Sleep at Approx. 60% M. L. D.
36.	Ethyl-*n*-amyl acetamide	1750	400	4.4	20 min.	>5 hours
54.	Ethyl-β-phenylethyl acetamide	>2000	750	>2.6	35 min.	>4 hours
31.	Ethyl-*n*-butyl acetamide	1250	150	8.3	17 min.	3 hours
87.	Ethyl-isoamyl acetamide	>2000	300	>7.0	.....	.....
68.	Allyl-β-phenylethyl acetamide	>2000	>2000	...	.....	.....
53.	Ethyl-benzyl acetamide	>3000	1000–1500	>2.0	.....	.....
37.	α-Bromodiethyl acetylurea (carbromal U. S. P. XI)	1500	175	8.6	Approx. 1 hr.	>6 hours

The minimum hypnotic dose, or M. H. D., is defined as the minimum dosage at which at least 50 per cent of the injected animals are put to sleep. "Sleep" is defined as the period during which the animals do not assume a normal position when placed on their backs, and during which the animals respond to pinching the tip of the tail by only unsuccessful attempts at righting themselves. The minimum lethal dose, or M. L. D., is defined as the smallest dose killing at least 50 per cent of the injected animals. The therapeutic index is the ratio of minimum lethal dose to minimum hypnotic dose or M. L. D./M. H. D.

Compounds which failed to produce sleep at a dose of 500 mg. per Kg. were considered as relatively inactive and their therapeutic ratios were not determined. The more effective hypnotics were evaluated using 20 to 40 rats on each compound. Thus, it is possible to select from the entire group of 87 compounds, six which were most effective for producing hypnosis in rats. These compounds were subjected to more detailed study in rats and also orally in rabbits. Carbromal (No. 27) was injected in parallel as a reference standard.

Rabbit Experiments—Mature healthy rabbits weighing 1500 to 3000 Gm. were used for testing the effectiveness of the six more active compounds by oral administration. The material was suspended in 5 per cent acacia solution and administered by stomach tube to rabbits starved for 12 hours. The tube was rinsed with about 5 cc. of 5 per cent acacia solution to insure that the full dose was administered. The M. H. D., M. L. D. and therapeutic ratio is defined as in the rat experiments except that sleep in the rabbit is defined as the period during which the animals do not assume a normal position when placed on their backs, and during which the animals do not right themselves when pinched in the thigh muscles with thumb and forefinger.

Results.—The pharmacologic data based on the results obtained from 1053 rats and 97 rabbits are recorded in Tables I and II. Of the compounds studied, the acid amides are outstanding as a group in that all of the hypnotics having a satisfactory M. H. D. and therapeutic ratio are included in this group. As a group, the acetyl ureas are relatively inefficient hypnotics for the rat, which may in part be due to their poor solubility. It is interesting to observe that, with the exception of number 86, all of the compounds showing convulsant action are *N*-substituted acid amides. This convulsant action is apparently reduced by the substitution of an ^{-}OH in the alkyl group attached to *N*- or by β-cyclohexylethyl in the α-position.

Of the six compounds studied orally in rabbits, only one, ethyl-*n*-butyl acetamide, appears to be more active than carbromal. The other five are much more effective by intraperitoneal injection in rats than by oral administration in rabbits. This discrepancy may be due to poor absorption from the stomach or possibly to destruction in the gut.

SUMMARY

Eighty-seven compounds have been studied by intraperitoneal injection in rats for estimation of the therapeutic ratio Minimum Lethal Dose/Minimum Hypnotic Dose. Six of the most promising compounds of this group have been studied further by oral administration in rabbits. Only one compound, ethyl-*n*-butyl acetamide, was found to be more active than carbromal by both tests.

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"If you, or I, can ultimately give to the world a single volume, or line, or thought which will enable our fellow men and women to enjoy life a little more fully, or endure it a little more easily, then we shall not altogether have laboured in vain."—Ian Hay