The time course of fluorescent product formation from ninhydrin is different for these compounds as compared with 5-HT. Therefore, careful study can discriminate between them.

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Keyphrases

5-Hydroxytryptamine in tissue—analysis
Tissue analysis—5-hydroxytryptamine
Fluorometry, specificity—5-hydroxytryptamine analysis
Ninhydrin reagent
Amine interference—5-hydroxytryptamine analysis

Some Conditions Under Which Pentobarbital Stimulates Spontaneous Motor Activity of Mice

By NATHAN WATZMAN, HERBERT BARRY, III, WILLIAM J. KINNARD, JR., and JOSEPH P. BUCKLEY

The effects of 4 doses of sodium pentobarbital (10, 20, 40, 80 mg./kg., p.o.) on spontaneous motor activity of mice were tested in photocell cages during a 2-hr. period immediately after drug or saline administration. A total of 480 albino mice was divided among several experimental conditions. Increasing doses produced progressively increasing activity in the last 0.5 hr. when normal activity was at a low level. In the first 0.5 hr., the usual intense activity was unchanged by the three lower doses and greatly depressed by the highest dose. Throughout the session, the three lower doses had a stimulant effect on animals tested in a lighted environment in groups of five but not singly, and a small but consistent stimulant effect on both single and grouped animals in a dark environment. Animals fasted for 24 hr. prior to the test were greatly stimulated by the lowest dose and were greatly depressed by the highest dose. The results indicate that the stimulant effect of barbiturates requires both inhibitory influences on normal activity and conditions increasing susceptibility to arousal.

CLINICALLY, subhypnotic doses of barbiturates have been known to produce excitement, euphoria, and confusion rather than central nervous system depression (1). Many investigators have reported a stimulant effect in laboratory animals with low doses and depression with high doses. Kinnard and Carr (2) reported that spontaneous motor activity of mice was increased by low doses and decreased by high doses of sodium amobarbital and sodium secobarbital. A stimulant effect of low doses of barbiturates on spontaneous motor activity of mice has also been demonstrated in several subsequent experiments (3–7).

The stimulant action of barbiturates has been analyzed in situations where the drug enhances a previously rewarded response that was subsequently inhibited by punishment or nonreward (8). This stimulant effect has generally been attributed to a disinhibitory action which at certain doses is stronger than the intrinsic depressant action (9). Dews (10) reported that in a variety of food-rewarded tasks, conditions

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which depressed the normal response rate of pigeons increased the stimulant effect of amobarbital.

Spontaneous motor activity, in common with all behavior, is controlled by opposing excitatory and inhibitory influences. Therefore, it is reasonable to assume that the demonstrated disinhibitory action of barbiturates might account for their stimulant effect on spontaneous motor activity. Such an assumption might be tested by comparing the effects of barbiturates under different conditions which are known to influence the strength of the inhibitory tendency. However, the effects of barbiturates on spontaneous motor activity have generally been tested only under a single set of conditions. An exception to this was a brief report by Wright *et al.* (11) that doses of pentobarbital which depressed spontaneous motor activity of single mice had no effect or increased activity of mice tested in groups of three. Evidence for greater susceptibility of aggregated mice to a stimulant effect is also found in a report by Brown (3) that pentobarbital and phenobarbital greatly increased spontaneous motor activity of mice tested in groups of five, whereas observations of isolated mice indicated a depressant effect of the same doses.

In the present study, effects of pentobarbital on spontaneous motor activity of mice were compared under several experimentally varied conditions: test aggregation, illumination of the test environment, and satiation or fasting for 24 hr. prior to the test. Lower activity levels, which might be attributed to inhibitory influences, were previously found (12) in animals tested singly, or in the light, or after satiation. A prolonged, 2-hr. session permitted measurement of the drug effect throughout a period when normal activity decreases greatly, as previously shown in the same test situation (12, 13).

METHOD

Subjects—The subjects were 480 male Swiss-Webster mice (Taconic Farms, N. Y.) weighing approximately 18–20 g. and housed in groups containing 20 animals per cage.

Apparatus—The experimental work was performed in four circular, 6-beam photocell activity cages (Actophotometers, Metro Industries, Inc., N. Y.), 33 cm. (13 in.) in diameter, operating simultaneously in a sound-attenuated room. The same units have been used in prior studies (12, 13); characteristics of the apparatus were discussed in another prior study (14) and in a review article (15).

Procedures—The 2-hr. test session was initiated immediately after oral administration of sodium pentobarbital (10, 20, 40, or 80 mg./kg.) or placebo (saline, 0.1 ml./10 g. body weight). The groups tested under these five dosage conditions were each subdivided according to three additional experimental conditions: test aggregation (singly or in groups of five mice), illumination (light or dark test environment), and feeding condition (satiated or fasted for 24 hr. prior to the test session). A complete factorial design for the five dosage conditions and the two conditions of each of the other three variables resulted in 40 combinations of conditions ($5 \times 2 \times 2 \times 2$). Each of these was replicated in all four actophotometer units, thus giving a total of 160 conditions, 80 comprising single mice and 80 comprising aggregations of five mice. In order to provide variation in experimental conditions among the four animals or groups tested simultaneously in the different units, each of these animals or groups was under a different dosage condition. The activity counts for each 0.5 hr. and for the total 2 hr. were transformed into square root scores for the reasons discussed previously (13). Statistical significance was tested by an analysis of variance program (BMD 02V, for the IBM 7090 computer), according to the methods described previously (12).

RESULTS

The first 0.5 hr. of the session might not be expected to provide sufficient time for the full effects of the drug because of the oral route of administration and the very brief interval prior to the beginning of the session. Nevertheless, Fig. 1 shows a very strong depressant effect of the highest pentobarbital dose (80 mg./kg.) on activity in the first 0.5 hr. Inspection of the counts recorded for the first 15 min. showed that the effect occurred also in that shorter time period. The test of statistical significance for the first 0.5 hr. revealed a highly reliable difference among the five dosage conditions ($F = 82.1$, $df = 4/117$, $p < 0.001$). Figure 1 shows that the difference was almost entirely attributable to the effect of the highest dose, and a statistical analysis for the same period, omitting the highest dosage condition, shows no significant differences among the four remaining dosage groups.

The activity of the group with the highest drug dosage increased rapidly after the second 0.5 hr., and was the highest among the five groups in the

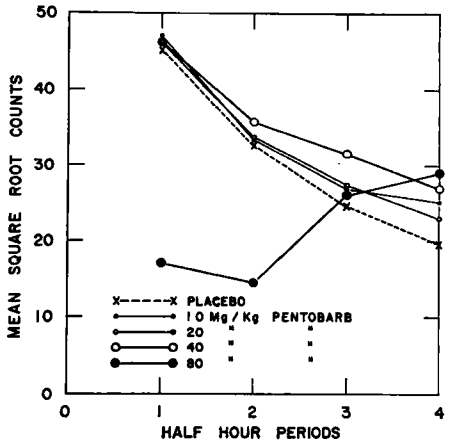

Fig. 1—Spontaneous motor activity of the five dosage groups in each 0.5-hr. period.

fourth 0.5 hr. Meanwhile, the activity of the other four groups decreased progressively. A statistical test of this trend for these four dosage groups during the successive 0.5 hr. periods, with the orthogonal polynomials used previously (12–14), revealed a linear decrease in activity which was highly reliable ($F = 540.0$, $df = 1/288$, $p < 0.001$). The activity of the groups given the three lower doses of pentobarbital decreased more slowly than the activity of the placebo group, so that each of these drug groups was higher than the placebo group at the end of the 2-hr. session. The gradual emergence of the difference among these four dosage conditions, during the successive 0.5-hr. periods, is indicated by a significant interaction between the linear trend for the four periods and the linear trend for the four dosage conditions ($F = 5.86$, $df = 1/288$, $p < 0.05$). A comparison among all five dosage groups in the fourth 0.5 hr. shows an almost uniformly progressive increase in activity with increasing doses. This linear trend was statistically highly reliable ($F = 7.91$, $df = 1/117$, $p < 0.01$).

The effects of two of the other three experimental variables also depended on the portion of the 2-hr. session. The analysis for the four 0.5-hr. periods, excluding the highest pentobarbital dose, showed no difference between the fasted and the satiated animals in activity for the first 0.5 hr., but the subsequent decrease in activity was greater for the satiated animals, resulting in a progressively greater superiority in activity of the fasted animals in successive 0.5-hr. periods. This interaction between fasting condition and the linear function of 0.5-hr. periods was statistically highly reliable ($F = 19.0$, $df = 1/288$, $p < 0.001$). Similarly, the animals tested in the dark showed a superior level of activity which was very slight in the first 0.5 hr. and progressively increased in successive 0.5-hr. periods, with a statistically reliable interaction between illumination and the linear function of 0.5-hr. periods ($F = 8.48$, $df = 1/288$, $p < 0.01$). Activity was much higher for the aggregated than single animals throughout the 2-hr. session ($F = 168.6$, $df = 1/288$, $p < 0.001$), but this difference persisted at approximately the same level throughout the session, as is indicated by the lack of a significant interaction between aggregation and 0.5-hr. periods.

The influence of the experimental conditions on the response to the drug may be measured by the interaction between dosage conditions and the experimental variables. The analysis of the four 0.5-hr. periods showed no significant effect of the periods on these interactions, measured by the three-way interactions for periods, dosages, and any of the other variables. Therefore, the drug effects are compared under the different experimental conditions for the total 2-hr. session.

Figure 2 shows the effect of fasting on the response to pentobarbital. For the fasted animals, the drug had a strong stimulant effect at the lowest dose but a marked depressant effect at the highest dose, whereas the satiated animals were less strongly affected by the various dosage conditions. The superior activity level of the fasted animals at an intermediate dosage condition (10 mg./kg.) but not at the extreme conditions (placebo and the higher doses) is measured by the quadratic orthogonal polynomial. A significantly different drug effect for the satiated and fasted animals is found in the interaction between fasting and the quadratic trend of dosage with all five dosages ($F = 19.1$, $df = 1/117$, $p < 0.001$) and also with the highest dosage omitted ($F = 8.16$, $df = 1/93$, $p < 0.01$). Thus, fasting enhanced sensitivity both to the stimulating effect of a low dose and to the depressant effect of a high dose.

The relationship of the aggregation and illumination conditions with each other influenced their effects on the response to pentobarbital, as indicated by a statistically significant three-way interaction among the four dosage conditions (excluding the highest dose), aggregation and illumination ($F = 3.09$, $df = 3/93$, $p < 0.05$). Figure 3 divides these complex influences on the response to pentobarbital into their simple components by the use of two graphs, both of which compare the single with the aggregated animals under a single illumination condition. In the lighted environment, the three lower pentobarbital doses substantially stimulated the aggregated but not single animals. The interaction between aggregation and the linear function of dosage (omitting the highest dose) was statistically

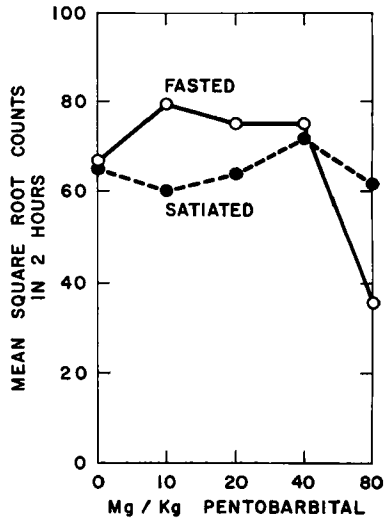

Fig. 2—Comparison of fasted with satiated animals under the different dosage conditions for the total 2 hr.

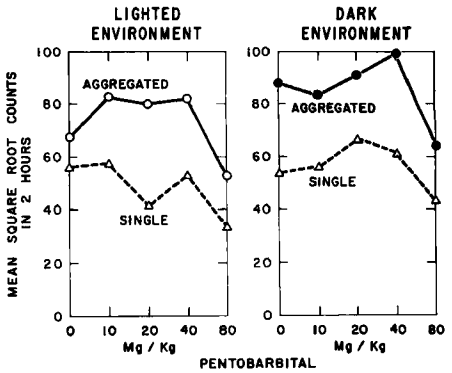

Fig. 3—Effects of test illumination and aggregation under the different dosage conditions for the total 2 hr.

significant ($F = 6.72$, $df = 1/48$, $p < 0.05$). In the dark environment, there was no significant differential effect of aggregation on the response to pentobarbital, but the drug had a stimulant effect on both single and aggregated animals, with a statistically significant linear function of dosage, excluding the highest dose ($F = 6.49$, $df = 1/48$, $p < 0.05$). Thus, in the lighted environment, pentobarbital stimulated only the aggregated animals, whereas in the dark environment the drug had a stimulant effect which was smaller but not dependent on aggregation condition.

DISCUSSION AND CONCLUSIONS

The theory of the disinhibitory effect of barbiturates (8, 9) implies that conditions which strengthen the inhibitory tendencies on behavior, thus reducing the normal activity level, will enhance the stimulant effects of the drug. Some of the present findings clearly support this theory. A stimulant drug effect was not found in the period of intense exploratory activity, during the initial 0.5 hr. in the novel environment. The stimulant drug effect at the end of the session might be attributed to a disinhibitory action on the behavioral mechanisms which cause the activity of the placebo animals to decrease to a low level. This stimulation is not due to compensation for earlier depressed levels of activity, because a depressant effect was seen only with the highest of the four drug doses. The later portions of the session were necessarily associated with longer intervals after drug administration, which enabled recovery from the depressant effect of the highest dose, but none of the prior studies (3–7) has given evidence that the stimulant effect on spontaneous motor activity occurs only at a long interval after drug administration.

Further indication of a disinhibitory effect of pentobarbital is seen in the strongly stimulant drug effect on aggregated animals tested in the light. The lighted environment decreases normal activity in mice, and the aggregation condition may have enhanced this depressant tendency, as indicated by much lower activity scores in the light than in the dark. The three lower doses of pentobarbital increased these scores in the light to the level for the placebo animals tested in the dark. Aggregated animals have been observed to cluster together and become quiescent late in the session; the higher activity counts of the aggregated animals are due to the larger number of opportunities to interrupt the photobeams, and a measure of counts per animal would yield lower scores in the aggregated than in the isolated condition.

Some of the present findings suggest that the stimulant drug effect is enhanced by conditions which increase rather than decrease the normal activity level. The lowest dose of pentobarbital stimulated the fasted but not the satiated animals. The three lower doses had a consistent although small stimulant effect in the dark environment, regardless of aggregation condition. Prior findings have also given evidence that a certain degree of arousal may enhance the stimulant effect of barbiturates. Aggregation was considered to have such an arousing effect in a prior report (3) that barbiturates increased spontaneous motor activity of aggregated but not isolated mice. Quigley *et al.* (16) observed that pentobarbital, amobarbital and barbital evoked excitement in dogs with a nervous temperament, whereas simple depression occurred in quiet, well-behaved animals. The dogs had been trained to lie down quietly for an extended time period in the test situation, which thereby required considerable inhibition of natural activity. In general, a combination of inhibitory influences and susceptibility to arousal appears to be necessary for a stimulant effect of barbiturates. If the animals are already maximally aroused, as when initially introduced into a new environment, the barbiturate cannot induce a further stimulation. If the conditions are predominantly inhibitory, the normal quiescence appears to be enhanced rather than reversed by barbiturates. The stimulant effect of barbiturates appears to be maximized by strongly conflicting behavior tendencies, such as approach and avoidance in an instrumental learning situation (8, 9) or excitatory and inhibitory tendencies after the initial introduction to a novel situation, as in the later portion of the test reported in the present paper.

The present study gives evidence of multiple and complex factors which influence the effect of pentobarbital on spontaneous motor activity in mice. Various excitatory and inhibitory tendencies determine the activity counts registered at a particular time, under particular conditions. The present study has identified only a few of the conditions which influence the drug effect. In a prior study by Harris *et al.* (7) 90 mg./kg. pentobarbital, administered orally, greatly increased spontaneous motor activity of mice in a 30-min. test beginning 30 min. after drug or placebo administration. A somewhat lower dose (80 mg./kg.) had a depressant effect under the most nearly equivalent conditions of the present experiment (satiated animals tested singly during the second 0.5 hr.). Some characteristics of the animals or of the test situation in the present experiment evidently caused either greater resistance to the drug's stimulant effects or greater susceptibility to its depressant effects. Further studies, comparing the drug effects under experimentally varied conditions, are needed to identify more comprehensively the conditions which determine the stimulant and depressant effects of barbiturates.

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Keyphrases

Phenobarbital effects—motor activity, mice
Stimulant effects—phenobarbital
Motor activity—phenobarbital administration
Environment effect—phenobarbital activity
Fasting, satiation effects—phenobarbital activity

Mechanisms of Action of Cryptenamine

By MARIE L. JANDHYALA and JOSEPH P. BUCKLEY

The hypotensive effect of cryptenamine has been reported to be markedly attenuated by adrenalectomy or by treatment with *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286) in atropinized animals. Cryptenamine also appeared to potentiate the response of isoproterenol on β-adrenergic receptors. This present study was conducted to further investigate these two phenomena. Cryptenamine potentiated the response of epinephrine on the β-adrenergic receptors in the intact dog heart, partially blocked the effect of isoproterenol on the isolated guinea pig auricle, and had no effect on the isoproterenol-induced relaxation of the isolated guinea pig tracheal chain. Cryptenamine produced positive inotropic and chronotropic effects on the isolated guinea pig heart and potentiated the inotropic effect of epinephrine and inhibited its chronotropic effects in this preparation. These data suggest that the effects of cryptenamine on β-adrenergic receptors are variable and that either potentiation or inhibition may be observed depending on the effector organ and on the species of animal studied. Cryptenamine also significantly decreased the epinephrine content of the adrenal venous blood while increasing the norepinephrine content suggesting that the drug may inhibit the methylation of norepinephrine in the adrenal medulla.

CRYPTENAMINE, an alkaloidal mixture, prepared from *Veratrum viride* by a nonaqueous benzene triethylamine extraction procedure, has been reported to have a ratio of emetic to effective hypotensive dose superior to that of other veratrum preparations (1, 2). Finnerty (2) reported that in humans the divergence between the hypotensive and emetic doses of cryptenamine was apparent on intravenous administration. McCall and his colleagues (3) studied the effects of cryptenamine on cerebral circulation and cerebral oxygen consumption in patients with toxemia of pregnancy. They observed that on intravenous administration of cryptenamine cerebral blood flow and cerebral oxygen metabolic rate were increased significantly while the respiration quotient of the brain remained normal. In these experiments, comparison with other veratrum preparations indicated fewer side effects. Although a satisfactory ambulatory treatment of hypertension by the oral administration of cryptenamine has been reported (4), Abreu (5) failed to demonstrate any superiority of cryptenamine over protoveratrine *A* in dogs as to the ratio of emetic to hypotensive doses.

Recently, Jandhyala and Buckley (6) reported that cryptenamine sensitized β-adrenergic receptors and that it might possibly stimulate the release of epinephrine from the adrenal medulla and that these factors might contribute to the overall hypotension. They reported that the hypotensive effects of cryptenamine were inhibited or abolished by pretreatment with *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286), bretylium, pronethalol, reserpine, α-methyl-dopa, and by adrenalectomy in atropinized animals. Cryptenamine also potentiated the isoproterenol-induced relaxation of the vasculature in the denervated perfused hind limb of the dog. Cryptenamine potentiated the

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