Reports

Effect of "Tranquilizing" Drugs on Amphetamine Toxicity in Aggregated Mice

In 1940, Gunn and Gurd reported that mice which were grouped with other mice were more susceptible to the excitatory effects of amphetamine and allied drugs than were single mice (*1*). In 1946, Chance reported experiments (*2*) investigating the relationship of "confinement" and "aggregation" to amphetamine toxicity. Mice that were crowded together were observed to die from much smaller doses of sympathomimetic amines than were mice in a less crowded environment. Amphetamine was particularly striking in this regard, the LD_{50} changing tenfold as a result of changing the population density. In a second paper (*3*), Chance further investigated the factors contributing to variability in amphetamine toxicity and concluded that "confinement" (that is, decrease in available space per mouse) and "aggregation" (that is, presence of other mice) were the most potent variables, overshadowing such factors as strain of mouse, sex, body weight, hydration, noise, light, and temperature.

It occurred to us that the agitated mouse in a crowded environment, suffering serious deleterious effects from the proximity of agitated mouse neighbors, resembled (albeit perhaps in a specious way) the agitated patients in a "disturbed ward" in a crowded state hospital. Specious or not, a simple experiment seemed of interest, to see whether chlorpromazine (allegedly beneficial to the agitated patient) would benefit the agitated mouse (*4*). To provide a crowded milieu, mice were placed, three in a group, in cylindrical metal canisters whose bottoms measured 13 in.^{2} in area. A screen was placed on top to keep the mice from escaping. After approximately 20 to 30 minutes in this situation, the mice were injected with racemic amphetamine sulfate (Benzedrine sulfate solution, SKF). Other mice were injected with amphetamine, but placed, one mouse to a pan, in ordinary rectangular enamel laboratory pans, measuring 104 in.^{2} in area and similarly covered with a screen. White male Swiss mice were used (obtained from Carworth Farms or Rockland Farms), each mouse usually weighing 20 to 30 g. Injections were made intraperitoneally in volumes of 0.1 ml/20 g of body weight. Experiments were arbitrarily terminated at 4 hours after amphetamine injection, for only an occasional death occurred after this time.

Our experiments confirmed Chance's observations (see Table 1, *I*): The grouped animals showed an LD_{50} approximately one-eighth that of the individual animals (*5, 6*).

We next simultaneously investigated the effects of sodium pentobarbital, sodium phenobarbital, or chlorpromazine hydrochloride (given as Thorazine solution, SKF) (*7*) administered 20 to 30 minutes after the mice had been placed in the canisters, and followed in about 30 minutes by amphetamine. All injections were made intraperitoneally and in volumes of 0.1 ml/20 g of body weight. Pentobarbital sodium given prior to the amphetamine yielded no protection to either the grouped or individual mice (see Table 1, *I*). After doses of phenobarbital of 50 mg/kg (which produced no particular gross behavioral defect), a trend toward elevation of the amphetamine LD_{50} was seen (see Table 1, *I*) in both the grouped and the single animals. Raising the dose of phenobarbital to anesthetic levels conferred striking and significant protection against amphetamine on the grouped mice, but provided no additional protection to the individual mice. For this protection, however, the mice paid a heavy price, in that they were obviously affected (ataxic, "sleepy," and so forth) for many hours after the experiment and indeed often on the following day.

A dose of chlorpromazine (1 mg/kg) which was devoid of gross behavioral effect on the mice raised the LD_{50} of the grouped mice somewhat, without altering the LD_{50} for the individual mice. A larger dose of chlorpromazine (5 mg/kg) raised the LD_{50} of grouped animals significantly and altered the LD_{50} of individual animals only slightly (see Table 1, *I*). It should be pointed out that, even with the higher dose of chlorpromazine, animals were not rendered unconscious, and the behavior of the surviving mice seemed essentially normal at the end of the experiment. In addition, these doses of chlorpromazine are well below the LD_{50} of intraperitoneal chlorpromazine for mice (*8*), whereas in the case of phenobarbital a doubling of the 150-mg/kg dose results in deaths from the phenobarbital per se.

We have also studied promazine hydrochloride (used as Sparine solution, Wyeth (*9*) and reserpine (*10*), with the volume of injected solution and route of injection the same as previously described. Promazine seemed to be less active (by weight) than chlorpromazine in affecting spontaneous motor activity in the mice prior to amphetamine administration, and also less effective in preventing death from amphetamine (see Table 1, *II*). Because of the reported delay in onset of effect with reserpine, experiments were designed so that there was a 1- to 2-hour lag between pretreatment with reserpine and administration of amphetamine. Two doses of reserpine

Table 1. LD_{50} (± standard error) for amphetamine (mg/kg) in grouped and single mice. The drug was administered intraperitoneally.

Pretreatment	Grouped	Single
	I	
Control	14.8 ± 6.7	111.1 ± 13.1
Pentobarbital		
10 mg/kg	16.0 ± 7.9	118.1 ± 3.5
30 mg/kg	29.2 ± 7.6	117.0 ± 13.8
60 mg/kg	26.2 ± 7.6	121.5 ± 8.1
Phenobarbital		
50 mg/kg	66.9 ± 26.8	147.9 ± 6.7
150 mg/kg	112.5 ± 17.1	135.4 ± 7.5
Chlorpromazine		
1 mg/kg	42.9 ± 13.6	118.1 ± 5.7
5 mg/kg	120.8 ± 18.6	143.8 ± 7.8
	II	
Control	31.1 ± 6.6	
Chlorpromazine		
5 mg/kg	> 150	
20 mg/kg	141.7 ± 26.5	
Promazine		
10 mg/kg	55.8 ± 19.7	
20 mg/kg	68.8 ± 18.7	
	III	
Control	12.5 ± 4.3	
Chlorpromazine		
5 mg/kg	125.0 ± 14.4	
Reserpine		
1 mg/kg	108.3 ± 19.8	
5 mg/kg	96.5 ± 21.5	

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were chosen, one which produced little if any effect per se on the mice (1 mg/kg) and one which made mice definitely "sleepy" and quiet (5 mg/kg). With both doses there was significant protection against amphetamine for grouped mice (see Table 1, *III*).

In all this work, it must be emphasized that arbitrary timing in drug administration and termination of experiments has been employed. One could conceivably miss a "protective" effect of short duration, and the superior performance of phenobarbital over pentobarbital might be explained on such a basis. On the other hand, pentobarbital deaths were seen on occasion within an hour after injection. Doses of pentobarbital larger than 60 mg/kg were not employed because of the toxicity of this barbiturate at such levels. No attempt was made to improve the performance of pentobarbital or promazine by using repeated doses of these drugs.

It thus appears that the "single" animals die with about the same frequency after administration of amphetamine whether they are "untreated" beforehand or "treated" with phenobarbital, pentobarbital, or chlorpromazine. On the other hand, phenobarbital, chlorpromazine, or reserpine (in appropriate doses) afford definite protection to grouped animals, and indeed transform the three-per-canister situation to what is, in essence (from the standpoint of mortality), a one-per-can situation.

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References and Notes

1. J. A. Gunn and M. R. Gurd, *J. Physiol. (London)* 97, 453 (1940).
2. M. R. A. Chance, *J. Pharmacol. Exptl. Therap.* 87, 214 (1946).
3. ———, *ibid.* 89, 289 (1947).
4. This work was supported in part by a grant from the U.S. Public Health Service, National Institutes of Health (B-865-C), and in part by a grant from Eli Lilly and Company.
5. The LD_{50} and standard error values were obtained by the Spearman-Kärber method (*6*). The usual formula for variance (q_i) which is given by Finney as $p_i\ q_i/n_i$ assumes the independence of the results on all animals at the i^{th} dose. In the experiments reported here, in which animals were grouped in canisters, it would appear likely that the death of one animal in a cannister alters the situation with regard to the effects of amphetamine on the remaining two mice. Certainly, in our experiments, the animals in a given canister did not invariably either all live or all die. Our data indicated that an assumption of no correlation within canisters was false, as was an assumption of complete correlation within canisters. Because of the size of the experiments, it was deemed unwise to get an unbiased estimate of the variance from the differences between canisters. Accordingly, rather than use either of these assumptions in estimating the variance, a conservative procedure was adopted. For doses of amphetamine in the range at which not all mice died and not all survived, the variance of the proportion surviving was estimated by $(\frac{1}{2})\ (\frac{1}{2})/k = \frac{1}{4}k$, where k is the number of canisters at the i^{th} level. A similar estimate of variance was applied to the highest dose of amphetamine at which all animals survived, and to the lowest dose of amphetamine at which all animals died. (For doses outside this range, the variance estimate was set equal to zero.) Because of the conservative nature of this variance term, the confidence limits on the LD_{50} values are probably somewhat broader than they deserve to be. A special debt of gratitude is owed to Paul Meier for his suggestions on how to deal with the statistical problems raised in these studies.
6. D. J. Finney, *Probit Analysis* (Cambridge Univ. Press, Cambridge, ed. 2, 1952).
7. Chlorpromazine was generously supplied by Smith, Kline and French Laboratories.
8. N. C. Moran and W. M. Butler, Jr. [*J. Pharmacol. Exptl. Therap.* 118, 328 (1956)] report the LD_{50} as 190 mg/kg; Paul Mattis of Smith, Kline and French Laboratories reports it to be 92 mg/kg (personal communication).
9. Promazine was generously supplied by Wyeth Laboratories.
10. Reserpine was generously supplied by Ciba Pharmaceutical Products, Inc.

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Some Factors Affecting Fluorescence Maxima

The recent widespread use of recording spectrophotofluorometers, such as the Farrand and Aminco-Bowman instruments, as well as the use of manual instruments like the modified Beckman model DU (*1*), has resulted in a number of fluorescence maxima being reported in the literature. A recent paper (*2*) has brought to my attention the fact that not all investigators distinguish between "apparent" and "true" fluorescence maxima. An apparent maximum is one that is observed on a curve recorded directly by the instrument, while a true maximum is one that is observed on a curve which has been corrected for the various factors affecting it. The purpose of this report is to suggest some of the factors that affect fluorescence maxima, together with techniques for their correction, and to propose the usage of the terms *true* and *apparent* fluorescence maxima. The factors discussed should not be confused with those that affect quantum yield or relative intensity of fluorescence, which are adequately treated in a number of standard textbooks (*3*).

One factor which affects the fluorescence maximum is the degree of overlap between the long wavelength portion of the absorption spectrum and the short wavelength portion of the fluorescence spectrum. This factor is significant for a large number of compounds. If the fluorescence maximum lies to the short-wavelength end of the fluorescence spectrum (as is the case with many dye molecules and aromatic hydrocarbons), a shift of this maximum will occur toward longer wavelengths as the result of unequal absorption at each wavelength. This phenomenon is concentration-dependent and decreases with dilution.

Another factor is the relationship between the recorder-pen response (recorder time-constant) and the speed of the wavelength drive of the analyzer monochrometer. If the former is too slow or the latter is too rapid, the recorder pen will not be able to register its maximum response at each increment, particularly if the fluorescence peak is sharp. With instruments in which the wavelength dial is driven by a multiple-speed motor, the importance of this phenomenon can be easily evaluated by comparing curves recorded at different drive speeds.

A factor that is often neglected is the variation of detector sensitivity with wavelength. Most detectors do not have linear response characteristics and thus are more sensitive to light of one wavelength than of another. If one is working in the region of the spectrum where the response curve of the detector has a large slope (such as the region beyond 470 mμ, when using an R.C.A. 1P28 photomultiplier tube), a considerable difference between the true and apparent maxima can occur. (In my laboratory I have found that a difference of 15 to 20 mμ is not uncommon.) This factor is inherent in every instrument, and compensation for it can be accomplished only by correction of the recorded spectrum, at each wavelength, for the response of the detector involved. This can be ac-

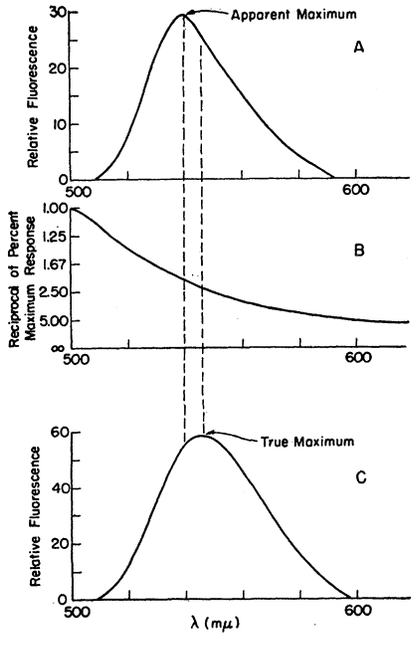

Fig. 1. Effect of detector response on recorded fluorescence curves. (*A*) Fluorescence spectrum as recorded; (*B*) detector response curve; (*C*) corrected fluorescence spectrum.