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Effects of Aggregation and Temperature on Amphetamine Toxicity in Mice*

By

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Introduction

GUNN and GURD observed that grouped mice were more susceptible to the excitatory effects of amphetamine and allied drugs than single mice. CHANCE (1946) estimated the LD 50 of adrenaline, methedrine, ephedrine and amphetamine under varying degrees of confinement and aggregation. When the confining area was kept constant and the number of mice increased from one to 10, the toxicity of adrenaline was doubled and that of amphetamine was increased nearly tenfold. The increase of mortality by ephedrine and methedrine fell between these extremes. Most of these studies were carried out at a temperature of 80° F. A decrease of temperature to 60° F markedly reduced the effects of aggregation on the toxicity of amphetamine, the lethality of the drug dropping almost to the figure obtained with solitary mice under the same conditions. The animals were almost inactive at 60° F.

CHANCE (1947) also investigated other factors influencing the toxicity of sympathomimetic amines, including amphetamine, in mice. Variables increasing the toxicity were: higher weight of the mouse, environmental noises, confinement of the area in which the animal was placed, and aggregation (the presence of other mice). The latter was felt to be the factor having the greatest effect under conditions normally employed in pharmacological laboratories. Increased hydration and particularly lowering of environmental temperature proved to be protective. Although the strain of mouse was found to be important, sex seemed unimportant. The intensity of illumination and the transparency of boundary walls did not alter the results.

From his observations CHANCE concluded "that the essential feature of these amines is that, unlike other central nervous stimulants, they induce in the mouse a state of excitability rather than of excitement.

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Lethal doses almost invariably kill the solitary mouse as a result of violent convulsions, which, therefore, may appear when the excitation in the central nervous system reached a critical level. Doses themselves not lethal may thus become so as a result of external factors raising the excitation to a critical level."

LASAGNA and MCCANN confirmed CHANCE's observations that amphetamine is more toxic in aggregated mice. They postulated that the agitated mouse in a crowded environment, suffering deleterious effects from the proximity of agitated mouse neighbors, might benefit from "tranquilizing" drugs. To the single mouse pentobarbital, phenobarbital, and chlorpromazine conferred no or only slight protective effect against the toxicity of amphetamine. In grouped mice, pentobarbital had no effect on the LD 50. A marked protection, however, was achieved with phenobarbital at doses producing ataxia and „sleepiness" while with chlorpromazine a significant protective effect was observed in doses well below those producing unconsciousness. Reserpine and promazine were also found protective, the latter, however, less so than chlorpromazine.

It appeared of interest to investigate in greater detail the interaction of confinement, aggregation, and temperature on the toxicity of amphetamine in mice. An attempt was made to discover any quantitative relations existing between those variables when other factors were kept as constant as possible.

Methods

Wire mesh cages of various sizes were used to vary the confining area. Male Swiss albino mice of 18 to 24 g weight obtained from Research Supply Co., Philadelphia, were used. Before experiments animals were kept in an animal room lit by daylight during the day and kept dark at night. To avoid possible variations due to the physiological daily rhythms in mice described by HALBERG et al., the experiments were usually started at 10 a.m. and stopped at 4 p.m. Single mice and mice in groups[1] of 3 were placed in wire mesh cages with floor areas of 25, 56, 100, and 185 cm^2; Animals in groups of 5 were placed in cages with floor areas of 100, 185, and 500 cm^2. The wire mesh allowed for adequate observation of the animals and dissipation of any local increase in temperature within the cage due to body heat. The mice were injected intraperitoneally with racemic amphetamine sulfate (Benzedrine sulfate solution, 20 mg/ml, SKF) in a volume of 0.25 ml per 20 g mouse. The amphetamine was diluted in 0.9 per cent NaCl solution. Mice were placed in an air-conditioned room (23° C to 25° C, also referred to as "room temperature"), or in a cold room (8° C to 12° C). The usual temperatures for these rooms were 25° C and

[1] The mice were grouped so as to combine mice housed prior to the experiment in different cages (10 to a cage).

10° C respectively, and both were artificially lighted. In most instances mice were injected at room temperature with a given dose and then randomly allocated either to the 25° C or to the 10° C environment. Some of the animals, however, were injected immediately after being placed in the cold. The mice were observed for 5 hours after injection and the number of dead animals recorded at hourly intervals. The dosages of amphetamine given to single animals were 10, 25, 50, 100, and 150 mg/kg, whereas the grouped mice received those doses and also 200 mg/kg. For evaluation of the effects on amphetamine toxicity of shorter exposure to cold, some mice were injected at 10° C and then moved to 25° C after varying periods (from 5 to 180 minutes) of exposure to cold. For comparison, groups of mice, injected with same dosage as these animals, were simultaneously observed at 25° C.

To estimate the effects of "pre-treatment" with cold, mice were exposed to 10° C for 30 minutes, then moved into room temperature and injected immediately with amphetamine. The results were compared with mice simultaneously injected but not "pre-treated" in the cold.

In one experiment on 24 mice injected with 150 mg/kg and placed 3 per container, the time from injection to death was measured with a stop watch over the first 30 minutes.

Uninjected single and grouped mice were exposed for 5 to 6 hours to 10° C to evaluate the effects of cold on non-medicated animals.

Results

Aggregation (see Table 1). At amphetamine doses of 25 to 100 mg/kg, at 25° C, fewer mice died when kept individually than when mice were grouped in threes or fives. At 10° C, however, no such deleterious effect of aggregation was demonstrated. Increasing the number of mice per group to five did not accentuate the aggregation effect beyond that seen with three animals per group.

Confinement. The results of decreasing the space for individual mice from 185 to 25 cm^2 had little influence on amphetamine mortality, except possibly for the cold experiment at the 5th hour, where the smallest containers showed the highest mortality (see Table 2). In the case of the grouped mice, the results of decreasing the space available per mouse yielded inconsistent data (see Tables 3 and 4), although such trends as were seen were for the most part in the direction of an inverse relationship between mortality and container size.

Temperature. A baseline study on the effects of prolonged 10° C ambient temperature indicated that unmedicated mice kept singly ($N = 24$), 3 per cage ($N = 48$), or 5 per cage ($N = 40$) were little affected by this degree of cold. Of these 112 animals, only 2 died, both in the 5th hour. The other mice showed no obvious ill effects.

Table 1. *Effect of temperature on amphetamine toxicity in single and grouped mice* (% cumulative mortality)

	3rd hour, 25° C			3rd hour, 10° C		
	1/c*	3/c**	5/c***	1/c	3/c	5/c
10 mg/kg	0	4.2	0	0	4.2	0
25 mg/kg	12.5	50.0	43.3	0	4.2	0
50 mg/kg	8.3	43.8	60.0	4.2	6.2	10.0
100 mg/kg	12.5	29.2	33.3	8.3	4.2	4.4
150 mg/kg	70.8	72.9	85.0	37.5	29.2	48.3
200 mg/kg	—	95.8	96.7	—	87.5	86.7
	4th hour, 25° C			4th hour, 10° C		
	1/c	3/c	5/c	1/c	3/c	5/c
10 mg/kg	0	4.2	0	8.3	4.2	0
25 mg/kg	12.5	50.0	43.3	12.5	8.3	3.3
50 mg/kg	25.0	62.5	66.7	12.5	22.9	30.0
100 mg/kg	25.0	54.2	40.0	33.3	41,7	13.3
150 mg/kg	79.2	75.0	88.3	62.5	41,7	51.7
200 mg/kg	—	95.8	96.7	—	91.7	93.3
	5th hour, 25° C			5th hour, 10° C		
	1/c	3/c	5/c	1/c	3/c	5/c
10 mg/kg	0	4.2	0	16.7	8.3	10.0
25 mg/kg	12.5	50.0	43.3	33.3	29.2	10.0
50 mg/kg	29.2	62.5	73.3	50.0	50.0	63.3
100 mg/kg	29.2	62.5	50.0	62.5	58.3	40.0
150 mg/kg	79.2	77.1	88.3	79.2	45.8	55.0
200 mg/kg	—	95.8	96.7	—	91.7	93.3

* 1/c: Single mice. For each dose level, 24 mice were employed.

** 3/c: 3 mice per cage. For each dose level, 24 mice were employed except 50 and 150 mg/kg, where 48 were used.

*** 5/c: 5 mice per cage. For each dose level 30 mice were employed except 150 mg/kg where 60 were used.

Table 2. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

Single mice

	I*	II**	III+	IV++
Temperature 25° C				
Hour 4	20.0	36.7	26.7	30.0
Hour 5	23.3	36.7	30.0	30.0
Temperature 10° C				
Hour 4	33.3	23.3	20.0	26.7
Hour 5	66.7	53.3	40.0	33.3

Pooled data for all doses (amphetamine 10—150 mg/kg i.p.) (n = 30 per group).

* I: confining floor area of 25 cm².

** II: confining floor area of 56 cm².

+ III: confining floor area of 100 cm².

++ IV: confining floor area of 185 cm².

Table 3. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

3 mice per cage

	I*	II**	III+	IV++
Temperature 25° C				
Hour 4	68.7	58.3	64.6	47.9
Hour 5	70.8	62.5	66.7	47.9
Temperature 10° C				
Hour 4	45.8	33.3	31.3	27.1
Hour 5	60.4	39.6	43.7	45.8

Pooled data for all doses (amphetamine 10—200 mg/kg i.p.) (n = 48 per cage size).

* I: confining floor area of 25 cm².

** II: confining floor area of 56 cm².

+ III: confining floor area of 100 cm².

++ IV: confining floor area of 185 cm².

For *single* amphetamine-treated animals, it appeared to make little difference whether the ambient temperature was 25° C or 10° C, for the first three hours. Over this period of time, the only impressive difference was at the 150 mg/kg dose, where the mice kept at room temperature showed a higher mortality than those at 10° C. By five hours, however, most doses studied produced a higher mortality in the cold (see Table 1).

For *grouped* animals, on the other hand, a temperature of 10° C provided substantial protection against most dose levels of drug over the first 3 hours, but by the end of five hours, the difference in mortality between the mice kept in the cold and those kept at room temperature had narrowed appreciably (see Table 1).

Table 4. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

5 mice per cage

	III*	IV**	V***
Temperature 25° C			
Hour 4	67.1	62.8	51.4
Hour 5	68.6	65.7	54.3
Temperature 10° C			
Hour 4	33.3	32.0	34.7
Hour 5	52.0	37.3	49.3

Pooled data for all doses (amphetamine 10—200 mg/kg i.p.) (n = 70 per cage size at 25° C and 75 per cage size at 10° C).

* III: confining floor area of 100 cm². ** IV: confining floor area of 185 cm². *** V: confining floor area of 500 cm².

Ambient temperatures colder than 10° C (see Tables 5 and 6) increased the toxicity of amphetamine for grouped animals over that observed at 25° C.

It would appear that the grouped animals do not need to stay in the cold to obtain protection against amphetamine, since even a 10 to 15 minute exposure to 10° C *after* drug injection (see Table 7) or a 30 minute exposure to 10° C *before* drug injection (see Table 8) produced mortality rates lower than those seen in mice kept at 25° C throughout.

Behavior of mice. *Non-medicated grouped mice.* a) At 25° C mice placed into the cages appeared excited at first, ran around and made attempts to escape from the cage. Occasionally some animals fought but usually they quieted down quickly, sat, cleaned themsleves or ran around sporadically. Their activity was not as abrupt or spontaneously violent and "purposeless" as that seen in medicated animals.

b) In the cold the mice quieted down quickly, manifested tremor and piloerection. They crowded together and seemed thus perhaps to protect themselves mutually from the cold. After 5 to 6 hours a few animals manifested ataxia and marked slowing down of movements, but they recovered within 20 to 30 minutes after being returned to room temperature.

Medicated grouped mice. a) At 25° C mice became extremely restless about 5 minutes after injection; they ran all over the cage, squealed, ran into each other and frequently took the "apprehensive position", although fights were not too frequent and lasted for only brief periods. The ani-

mals showed gross tremor. Movements were very abrupt and sudden violent outbursts of motor activity occurred frequently. Salivation occurred, and the respirations deepened. Some animals had a general flaccidity of musculature and paresis of the hind legs. Occasionally mice would chew their thoracic skin until bleeding occurred. Chewing of the ears and skin of the head of other animals was seen in only a few instances. With increase of dosage, behavior became more disorganized. Up to 50 mg/kg some cooperation between animals was observable, such as for instance mutual cleaning. At high doses the animals ran into each other as if they could not see, and jumped against the walls of the cage. After 5 hours, the surviving animals had a tremor, appeared exhausted and had a marked increase in sensitivity to external stimuli such as noise, although, if not disturbed, they were not particularly overactive. When a larger group of such animals was put together in one containe, they became overtly excited again.

Table 5. *Effect of 7° C temperature on mice injected with amphetamine* (% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Temperature 25° C for 5 hours					
50 mg/kg	0	0	12.5	16.7	16.7
100 mg/kg	0	12.5	16.7	20.8	29.2
Temperature 7° C for 5 hours					
50 mg/kg	0	0	0	33.3	66.7
100 mg/kg	4.2	8.3	33.3	83.3	100.0

Comparison between the mortality of aggregated mice (3 mice per cage) injected with amphetamine 50 mg/kg and 100 mg/kg i.p. and exposed to 25° C and 7° C (n = 24 per group).

Table 6. *Effect of 3° C temperature on mice injected with amphetamine* (% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
25° C	0	0	29.2	52.4	62.5
3° C	50.0	91.7	95.8	95.8	95.8

Amphetamine 100 mg/kg i.p., 3 mice per cage. Animals remained at given temperatures for 5 hours (n = 24 per group).

b) In the cold, aggregated mice were much quieter than at room temperature and, except at the highest doses, activity ceased almost completely. The lower the dose, the more pronounced was the tendency to crowd together. Marked tremor and piloerection were present. At high doses hyperactivity prevented any effective huddling. Chewing of the wire of the cage walls, which was also observable at room temperature, was so strong in the cold, that some mice, who pushed their snouts through the wire mesh, developed edema of the snout and could not retract it. Rather frequently stronger animals chewed the ears and skin of the head of weaker animals.

Behavoir of single animals. Single animals remained much quieter than the grouped ones. The difference in behavior was more a quantita-

Table 7. *Effect of amphetamine on mice kept at 10° C for less than 5 hours* (% cumulative mortality)

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Expt. I	5 hr. 25° C (n = 48)	70.8	70.8	72.9	75.0	77.0
	5 hr., 10° C (n = 48)	27.0	27.0	29.1	41.6	45.8
Expt. II	5 hr. 25° C (n = 24)	83.3	83.3	87.5	87.5	87.5
	45 min, 10° C (n = 24)	54.1	54.1	54.1	54.1	66.6
Expt. III	5 hr. 25° C (n = 72)	75.0	75.0	77.7	81.9	86.1
	10—15 min, 10° C (n = 72)	30.5	31.9	36.1	44.4	45.8
Expt. IV	5 hr. 25° C (n = 96)	44.7	45.8	51.0	52.0	54.1
	5 min, 10° C (n = 96)	42.7	42.7	43.7	44.7	47.9

For every experiment 3 mice per cage were used and amphetamine 150 mg/kg i.p. given. Mice injected at 10° were kept at that temperature for the length of time indicated, and then moved to 25° C. Mice at 25° C remained at this temperature for 5 hours.

Table 8. *Effect of "pre-treatment" with cold on the toxicity of amphetamine* (% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Amphetamine 100 mg/kg i.p.					
Not "pre-treated"	23.3	36.7	53.3	66.7	66.7
"Pre-treated"	0	13.3	46.7	66.7	66.7
Amphetamine 150 mg/kg i.p.					
Not "pre-treated"	63.3	70.0	73.3	76.7	76.7
'Pre-treated"	40.0	40.0	40.0	50.0	56.7

In all instances 5 mice per cage were employed. "Pre-treatment" with cold had a duration of 30 minutes at 10° C; then the mice were moved to 25° C and injected immediately (N = 30 per group).

tive than a qualitative one except for those activities possible only in the social situation. Great excitement was easily provoked by external stimulation.

Death of mice. The pattern of death was distinctly different in cold and room temperature and for large and small doses of amphetamine. In room temperature, at 150 mg/kg and 200 mg/kg, most mice that died did so within the first 20 minutes with a median survival time of 8 minutes. A few minutes after the injection the animals became almost immobile, then took some ataxic steps and suddenly began to jump against

the cage walls. In most instances tonic and clonic convulsions followed and they could be provoked by loud noises or by touching the animal. A few mice died without having had convulsions and they manifested slow gasping breathing before death. Up to 100 mg/kg most animals died between the second and fourth hours. At these lower doses, the animals went through a period of "exhaustion", sometimes showing a marked weakness of the hind legs, and finally demonstrating gasping breathing shortly before death. Most animals "faded away", and convulsions were rare.

In the cold, some of the animals on very high doses (150 to 200 mg/kg) died within the first hour in convulsions similar to those of mice at room temperature. The remaining mice, and animals at lower doses, died much later. The movements became slow, their reactions to external stimuli were markedly decreased and finally nearly absent, they displayed ataxia, lost the righting reflex and showed muscular weakness. Eventually they lay immobile with gasping breathing of low frequency. They remained in this condition sometimes for 1 or 2 hours until they died. Only by close examination was one able to see whether such an animal was dead. When such an animal, appearing almost dead, was moved into room temperature it began to walk with an ataxic gait after a short time; such mice had a tendency to crowd together in a corner and remain there quietly. Gradually they became more active and finally hyperactive. Some of those animals died later on the same day, but it was impossible to predict the outcome in an individual animal. Other mice, which were practically immobile in the cold, but not moribund, became excited and hyperactive when placed at 25° C and exhibited a pattern of behavior similar to that of animals which had been at room temperature throughout.

Discussion

CHANCE's data (1946, 1947) have been in large part confirmed by our studies, but certain differences emerge, andthe whole problem of the toxicity of amphetamine in regard to aggregation, confinement, and temperature seems more complex than suggested by CHANCE. In the study of LASAGNA and MCCANN, it would appear that the toxicity of amphetamine for single animals was similar to that observed for single animals in the present study, despite the fact that the average temperature in the earlier study was somewhat higher than the temperature employed as "room temperature" here. This latter fact (taken in conjunction with the inability of body heat to dissipate in the metal cans used in the earlier study) probably explains the somewhat higher mortality seen in the grouped animals by LASAGNA and MCCANN.

Certainly the presence of more than one mouse per container increases the toxicity of amphetamine at room temperature whereas it

does not so increase mortality at certain colder temperatures. Furthermore, it would appear that the use of any "LD 50" values to express the drug effects for grouped animals versus single animals is misleading, since the dose-response curves are more regular and different in shape for single animals than for grouped animals. This latter fact is not surprising, since the failure of grouped animals to die simultaneously from a given dose of drug means that for early periods of observation one actually has several animals interacting in a container, whereas at later times there may be only one live animal remaining.

The question of confinement, i.e., restriction of space per animal, is also far from simple, except for experiments where all of the space in a container is available for one animal. With two or more animals, it is almost impossible to define how much space each animal has. Obviously, with a large "container" (an experimental room, for example) ten mice would really have essentially unlimited space and one could hardly talk of each mouse having "one-tenth of a room." There probably must be a certain minimal confinement for any given number of animals before one can talk sensibly of any degree of „crowding." Attempts to analyze for area effects by dividing the number of animals into total area available become extremely difficult, and the problems are compounded when animals in a group begin to die off. Our data lend little support to the notion that extreme confinement of *single* animals increases amphetamine toxicity, and only slightly more to the importance of confinement for grouped animals.

Ambient temperature, too, presents complex problems. Within certain limits, cold appears to have a protective effect against amphetamine toxicity, most marked in the grouped animals. But if the temperature is low enough, or exposure to cold maintained long enough, the "stress" of cold appears to augment the toxicity of the drug.

CHANCE (1947) assumed that all mice dying of the effects of amphetamine did so in convulsions and that external stimuli increased the excitability and thus lowered the convulsive threshold of the animals. In the literature two different kinds of death are described in various animal species. EHRICH et al. (1937, 1939) reported that rats "faded away" without convulsions and that some rabbits and monkeys "faded away" while others died in convulsions. SCHULZ and DECKNER gave metamphetamine to white mice and observed death in exhaustion due to respiratory arrest. In our study, mice injected with amphetamine up to 100 mg/kg died predominantly a slower death with signs of exhaustion, and convulsions were rare. Doses of 150 mg/kg produced in most animals death within the first 20 minutes and predominantly in violent convulsions. This suggests that high doses of amphetamine provoke convulsions, but that the mode of death at lower dosages and in mice which do not have convulsions is due to other toxic effects of the drug, effects

which appear considerably increased by the excitatory effects of aggregation. An interesting toxic reaction is the paresis of the hind legs in many animals, particularly at high dosages, a phenomenon described in rabbits by EHRICH et al. Self-mutilation, such as chewing of the thoracic skin, has been reported previously after amphetamine in rats (EHRICH and KRUMBHAAR) and rabbits (EHRICH et al.) and after metamphetamine in mice (SCHULZ and DECKNER).

There is a considerable variation in mortality from amphetamine, particularly in grouped animals, even when mice appear to be of similar weight and age and ambient temperature and other conditions are kept reasonably constant. This fact is well demonstrated in the results on protection of animals by exposure to cold for varying periods of time. It will be seen in the experiment in which animals were kept in the cold for five minutes and then transferred to room temperature, that the control animals, i.e., those kept et room temperature for the entire period, showed a mortality after amphetamine that was considerably lower than the mortality in other control groups in the same series of experiments (see Table 7). We consider the relationship between the mortality of simultaneously compared groups more meaningful than the absolute mortality of the mice in any given experiment. Reasons for the variability in grouped animals have been discussed above.

Cold would appear to have a sedating and quieting effect on animals, as evident both in the behavior of unmedicated animals put in the cold and in the behavior of medicated animals transferred from cold to room temperature. Death from amphetamine at high doses at room temperature appears to result from a different physiological train of events than does death from lower doses at room temperature. In addition, death from amphetamine in the cold is by and large a slower phenomenon and probably a different one from either of the types of death seen at room temperature. The protective influence of cold against the early death with convulsions in single animals does not allow any definite conclusions to be drawn because of the multiple changes which occur simultaneously in an organism under such conditions. The complexity of the problem has been reviewed by FUHRMAN.

Summary

1. White mice were injected with amphetamine sulfate in doses ranging between 10 and 200 mg/kg, and placed into wire mesh cages of various sizes. Identical groups were kept in a temperature of 25° C and in a cold room of 10° C for a period of 5 hours. The number of dead animals was registered at hourly intervals.

2. At room temperature aggregation increased the toxic effects of amphetamine. Confinement had less influence on mortality. In-

creasing the number of animals from three to five per container did not cause a significant difference in the results.

3. The influence of cold on the toxicity of amphetamine was complex. In the first few hours, a temperature of 10° C had a definite protective influence in grouped animals and a slight one in single animals. Later, this protection was lost in varying degrees. Temperatures lower than 10° C increased the toxicity of amphetamine. A short exposure of the mice to 10° C, either before or after the injection of amphetamine, had a protective influence.

4. The behavior of the animals is described. Cold had a "sedating" and "quieting" effect on non-medicated mice and counteracted the excitation caused by amphetamine. Death from lower dosages of amphetamine was distinctly different from that at high dosages, the latter type of death being preceded by convulsions. In the cold, death was preceded by a long period of moribundity.

5. Death from amphetamine is a complicated phenomenon, and the toxic effects of the drug affected by many variables. Interactions become more complex in studies of grouped animals, where greater variability in response can be expected. It would appear important for investigators reporting results with drugs of this class to describe in sufficient detail the conditions obtaining in their experiments.

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