

## HYPERPYREXIA AS A CONTRIBUTORY FACTOR IN THE TOXICITY OF AMPHETAMINE TO AGGREGATED MICE

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A rise of body temperature into a range lethal to mice preceded death in groups of mice injected with amphetamine sulphate. At dose levels from 8.8 to 66.7 mg/kg, mortality was associated with the extent of rise in body temperature of the mice, irrespective of the actual dose administered. Isolated mice given comparable doses of amphetamine also showed a marked increase in body temperature. However, except in a very few cases, it did not rise into the range found to be lethal. Amphetamine was more toxic to isolated mice subjected to foot-shock than to isolated mice housed under normal conditions. This has also been shown to be related to the extent of rise in body temperature of the mice. The effect of a number of substances on grouped amphetamine toxicity was investigated. Chlorpromazine and phenoxybenzamine partially antagonized the sharp rise in temperature following the administration of amphetamine, and also significantly reduced mortality. Calcium acetylsalicylate was without effect on the rise of body temperature or on mortality. Both the hypothermic compound 4-methyl-5-( $\beta$ -chloroethyl)-thiazole (S.C.T.Z.) and L-thyroxine sodium potentiated the rise in temperature and caused a significant increase in mortality.

Since the findings of Chance (1946) that amphetamine is considerably more toxic to grouped mice than to mice housed singly in individual cages, several workers have investigated the factors influencing this toxicity. Although it has been shown that the LD<sub>50</sub> of amphetamine to grouped mice is dependent both on the environmental temperature and the degree of aggregation of the mice (Chance, 1946; Hogn & Lasagna, 1960), the underlying cause for the greater toxicity of amphetamine to grouped mice is not fully understood. Swinyard, Clark, Miyahara & Wolf (1961) have suggested that anxiety or fear is a critical factor in the enhanced lethality of amphetamine, whilst Burn & Hobbs (1958) concluded that the mice died as a result of the increased excitement observed with grouped animals. Both Lasagna & McCann (1957) and Burn & Hobbs (1958) found that chlorpromazine and reserpine antagonized grouped amphetamine toxicity at dose levels which had little effect on the toxicity of amphetamine to isolated mice. Protection against the lethal effect of amphetamine to grouped mice has therefore been suggested as a suitable test for determining the tranquillizing action of a substance.

During the course of testing compounds for their action against grouped amphetamine toxicity in this laboratory, the importance of room temperature became very apparent. At 26.7° C (80° F), following the intraperitoneal injection of

amphetamine sulphate to grouped mice, the LD50 was found to correspond with that reported in the literature, being in the region of 25 mg/kg. However, a reduction in the room temperature to 21.1° C (70° F) produced approximately a 4-fold decrease in toxicity, so that the LD50 was of the order of 100 mg/kg.

Amphetamine is known to produce hyperthermia, and a significant association has been found to exist in grouped mice between increased lethality and actions on motor activity and rectal temperature (Greenblatt & Osterberg, 1961). They found no corresponding association in isolated mice. In view of the hyperactivity which occurs and since the LD50 is markedly dependent on environmental temperature, it appeared possible that death in grouped mice could follow hyperpyrexia and heat exhaustion. Experiments were therefore carried out in which the rectal temperature of mice was measured every 20 min for a period of at least 2 hr following the injection of amphetamine sulphate.

#### METHODS

Unless otherwise stated, female Schofield albino mice of weight 20 to 24 g were used. Prior to the experiments they were housed in normal stock cages at a room temperature of 20 to 22° C and were allowed free access to food and water.

*Toxicity of amphetamine to aggregated and isolated mice.* Mice were placed in groups of 5, or individually, in metal boxes 9×15×11 cm deep, which were covered by wide-mesh lids. Tests were carried out at room temperatures of 21 to 22° C or 26 to 27° C and were ended after 7 hr. At the beginning of each experiment, shortly after being placed in the metal boxes, the rectal temperature of the mice was measured using a thermocouple (Electric Thermometer Type TE 3, Sierex). The probe was inserted into the rectum to a constant depth of 2.5 cm and was removed after each reading. Racemic amphetamine sulphate was dissolved in distilled water and given by intraperitoneal injection at a constant volume of 10 ml./kg. Following the injection, the temperature of each mouse was measured every 20 min up to 2 hr and then every hour up to 6 hr. In order to keep the number of mice per box constant throughout each grouped experiment, animals which died were removed and replaced by stock animals. LD50 values were calculated by the method of moving averages (Thompson, 1947) using the tables constructed by Weil (1952). Where this was not possible the technique of probit analysis (Finney, 1952) was employed.

*Determination of the upper range of body temperature lethal to mice.* Untreated mice were exposed to a high environmental temperature in cages lined with corrugated cardboard which were partially immersed in a thermostatically controlled water bath. The air temperature in these cages in the absence of mice was varied between 33 and 38° C. Groups of 5 mice were kept in this environment for 2 hr, their rectal temperature being measured every 20 min, after which they were placed in a stock cage and kept at a room temperature of 21° C. Mortality was determined 7 hr after commencement of the exposure.

*Toxicity of amphetamine to isolated mice subjected to foot-shock.* The method employed was based on that described by Weiss, Laties & Blanton (1961). Experiments were made at room temperatures of 21° C and 26° C, in a box divided into 10 chambers 15×7.5×15 cm deep. The floor consisted of brass rods, 0.3 cm diameter, with a space of 0.5 cm between each rod. A shock of 185 V was delivered to the rods, which were of alternate polarity, through a series resistance of 68,000 ohms. Each shock pulse lasted for 0.5 sec and was repeated once every 10 sec using a programming device (Cambridge & Haines, 1962). Immediately following the intraperitoneal injection of amphetamine, mice were placed singly in the chambers and their rectal temperature was recorded every 20 min throughout the 2 hr period of the test. The mice were then placed in a stock cage at a room temperature of 21° C and mortality was determined 7 hr from the commencement of the experiment.

*Effect of various compounds on grouped amphetamine toxicity in mice.* The method used was similar to that described above for the toxicity of amphetamine to grouped mice. For each experiment, 3 groups of 5 mice were given the compound under investigation usually either at the same time or 1 hr before the injection of amphetamine. Except where otherwise stated, all injections were made by the intraperitoneal route at a constant volume of 5 ml./kg. The mean peak temperature (the mean of the maximum temperature recorded for each mouse over the 2 hr period following the injection of amphetamine) and the 7 hr mortality were compared with those obtained for 3 similar groups of control mice given the solvent only, prior to the amphetamine. The tables of Mainland, Herrera & Sutcliffe (1956) were used to determine significance between the mortality of control and experimental groups. All experiments were carried out at a room temperature of 26° C.

*Compounds.* The following compounds were dissolved in distilled water prior to use: chlorpromazine, phenoxybenzamine hydrochloride and 4-methyl-5-( $\beta$ -chloroethyl)-thiazole (S.C.T.Z.). The calcium salt of acetylsalicylic acid was prepared following the method of Collier & Shorley (1960). L-Thyroxine sodium was dissolved in 0.9% sodium chloride solution containing 0.01 N sodium hydroxide.

## RESULTS

*Toxicity of amphetamine to aggregated and isolated mice.* At a room temperature of 26° C, the LD<sub>50</sub> with 95% fiducial limits for amphetamine to grouped mice was 30.6 (21.9 to 41.8) mg/kg, whereas at 21° C it was 100 (84.5 to 118.4) mg/kg. The corresponding LD<sub>50</sub> values for mice housed singly were 96.4 (83.3 to 111.5) mg/kg at 26° C and 122.4 (99.4 to 150.8) mg/kg at 21° C. When both grouped and isolated mice were given amphetamine at dose levels in the region of 100 mg/kg and above, death followed a period of severe convulsions and almost always occurred within 30 min of the injection. The injection of amphetamine to grouped mice at dose levels below 100 mg/kg gave rise to a period of marked hyperactivity, especially at the higher room temperature. Death in these mice was preceded by a stage of apparent exhaustion and did not normally occur less than 1.5 hr from the commencement of the experiment. Convulsions rarely occurred. Hogn & Lasagna (1960) noted a similar difference in the time to death and also in the appearance of mice before death between mice given amphetamine up to 100 mg/kg and mice given higher doses.

*Effect of amphetamine on the rectal temperature of aggregated mice.* Amphetamine was given to groups of 5 mice at 6 dose levels from 8.8 to 66.7 mg/kg, the ratio between successive doses being 1.5. When the experiments were carried out at 26° C, the temperature of the mice rose 1.5 to 4.0° C in the first 20 min. The corresponding rise in temperature at 21° C was 0.5 to 3.0° C. In some mice, after the initial sharp increase, the temperature gradually levelled out and returned to, or somewhat below, the control value, normally reaching this point 2 to 4 hr following the injection. In this case the animals did not show signs of exhaustion and always survived for the duration of the experiment. In other animals, however, the rectal temperature continued to rise, sometimes to a point above 43° C, before dropping sharply at a time when the mice appeared exhausted. During this latter phase, even when respiration appeared fairly normal, the mice all showed signs of cyanosis. These mice almost always died. An example of both types of effect is shown in Fig. 1.

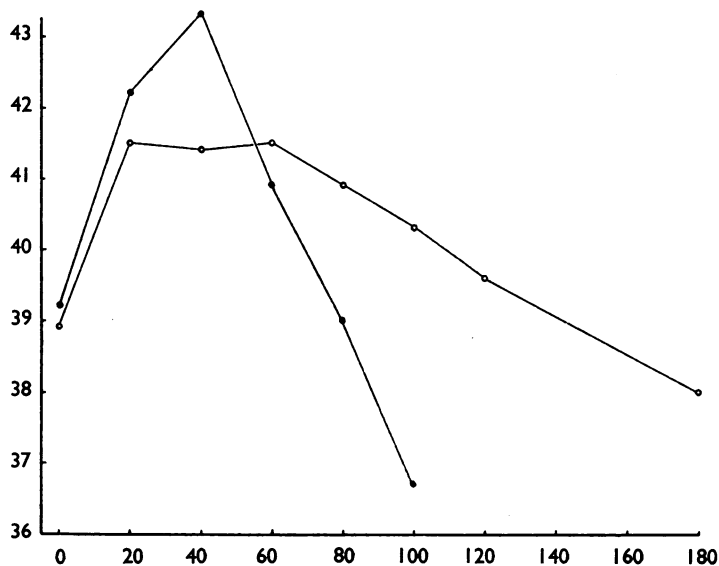


Fig. 1. Rectal temperatures of two individual mice from a group of five given amphetamine sulphate, 19.8 mg/kg, by intraperitoneal injection at a room temperature of 26°C. ●—●, dead at 116 min; ○—○, survived for 7 hr. Ordinate: rectal temp.°C. Abscissa: time (min).

Four experiments were carried out, 2 at 21° C and 2 at 26° C, involving a total of 120 mice. At the conclusion of these tests it became apparent that, irrespective of the dose of amphetamine given, all those mice whose rectal temperature had remained below 41.7° C survived, whilst, with one exception, all those whose temperature had risen above 42.4° C died.

The peak temperature attained by the mice and also the time at which this temperature was reached increased with increasing dose levels of amphetamine. In general the greatest rise in temperature followed a dose of 44.4 mg/kg (Table 1). At dose levels below 19.8 mg/kg, the maximum temperature recorded for the mice usually occurred 20 to 40 min following the injection. At dose levels of 19.8 mg/kg and above, there was a gradual increase in the time taken to reach the maximal

TABLE 1

THE MEAN PEAK TEMPERATURE AND PERCENTAGE MORTALITY RECORDED FOR GROUPED MICE GIVEN DIFFERENT DOSE LEVELS OF AMPHETAMINE

The mean peak temperature was obtained by taking the mean of the maximum rectal temperature recorded for each of 10 mice over the 2 hr period immediately following the intraperitoneal injection of amphetamine. The percentage mortality was determined 7 hr after the injection of amphetamine

Mice	Room temp. °C	Amphetamine (mg/kg)							
		5.9	8.8	13.2	19.8	29.6	44.4	66.7	
1st strain	21	Mean temp.	—	40.3	41.0	41.0	41.4	41.4	41.1
		% mortality	—	0	0	0	30	20	0
1st strain	26	Mean temp.	—	41.3	41.3	41.8	41.9	42.2	41.9
		% mortality	—	0	20	30	50	80	60
2nd strain	26	Mean temp.	41.1	41.9	42.1	42.2	42.2	42.5	42.4
		% mortality	0	40	70	70	100	90	100

temperature, so that, following 66.7 mg/kg, the peak usually occurred 80 to 100 min from the time of injection.

Similar experiments were carried out using a second strain of mice in which the LD<sub>50</sub> with 95% fiducial limits for amphetamine at 26° C was 11.6 (7.0 to 16.1) mg/kg. Death was again associated with the extent of rise in the rectal temperature of individual mice. In this strain, a more marked rise in temperature was apparent following lower dose levels of amphetamine (Table 1).

*Effect of amphetamine on the rectal temperature of isolated mice.* In view of the marked rise in temperature observed with grouped mice given amphetamine, the effect of comparable dose levels on the rectal temperature of isolated mice was investigated (Fig. 2). The increase in temperature produced by amphetamine was greater when the room temperature was 26° C than at 21° C. The most marked

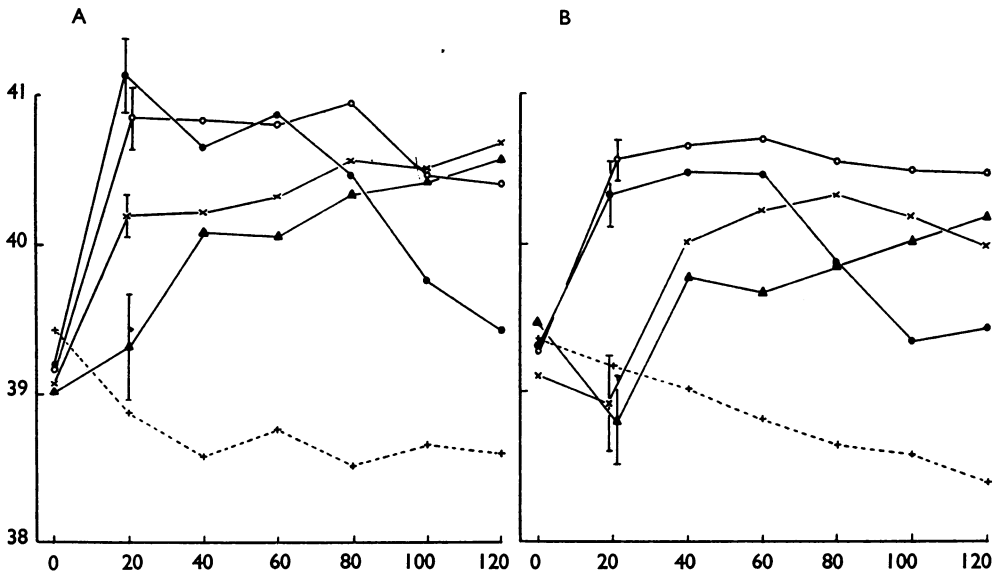


Fig. 2. Mean rectal temperature of isolated mice given different dose levels of amphetamine sulphate by intraperitoneal injection. I denotes standard error of mean. A: Room temperature, 26°-27° C. ●—●, 20 mg/kg (10); ○—○, 40 mg/kg (10); ×—×, 60 mg/kg (10); ▲—▲, 80 mg/kg (10); +---+, control H<sub>2</sub>O (5). B: Room temperature 21°-22° C. ●—●, 25 mg/kg (10); ○—○, 50 mg/kg (15); ×—×, 75 mg/kg (15); ▲—▲, 100 mg/kg (15); +---+, control (water) (5). (Figure in brackets shows number of mice used at each dose level.) Ordinate: rectal temp. °C. Abscissa: time (min).

rise, at both room temperatures, followed the injection of 20 to 50 mg/kg amphetamine, where the mean rectal temperature of the mice rose 1 to 2° C in the first 20 min. As the dose level was increased above 50 mg/kg, the hyperthermic action of amphetamine became less marked, so that there was no significant change in temperature over the first 20 min following dose levels of 75 to 80 mg/kg. A significant hypothermia was apparent at this time in mice given 100 mg/kg amphetamine at 21° C ( $P < 0.05$ ).

Of the 95 mice used in the tests, 4 died, following amphetamine at dose levels of 20 to 50 mg/kg. These deaths, which occurred 2 to 5 hr from the time of injection, followed a period of exhaustion, and convulsions did not occur. The maximum temperatures recorded for these mice were 42.3 to 43.0° C. Thus their temperatures had risen into the range which had previously been found to precede death in grouped mice.

*Determination of the upper range of body temperature lethal to the mice.* Since, following the injection of amphetamine to aggregated mice, those animals whose rectal temperature had remained below 41.7° C survived whilst those whose temperature had risen above 42.4° C died, experiments were carried out to determine the upper range of temperature which would prove lethal to the mice. The maximum rectal temperature recorded for mice exposed to environmental temperatures of 33 to 38° C for 2 hr varied between 40.8 and 43.4° C. Out of 160 mice, 69 died within 7 hr whilst 91 survived. The results are given in Table 2, where mortality is compared with the extent of rise in the rectal temperature of the mice.

TABLE 2  
RELATIONSHIP BETWEEN MAXIMUM RECTAL TEMPERATURE AND MORTALITY RECORDED FOR UNTREATED MICE EXPOSED TO A HIGH ENVIRONMENTAL TEMPERATURE

Untreated mice, in groups of 5, were exposed to an environmental temperature varying from 33° to 38° C, for a period of 2 hr. The rectal temperature was measured every 20 min. Mortality was determined 7 hr after the commencement of the exposure

Maximum rectal temp., °C	Total no. mice	No. deaths	No. survivors
<41.7	74	0	74
41.7-42.4	39	25	14
>42.4	47	44	3

From Table 2 it can be seen that in these mice, when the rectal temperature remained below 41.7° C, there were no deaths, whilst when the temperature rose above 42.4° C the mortality was 94%. Death was preceded by a period of exhaustion.

*Toxicity of amphetamine to isolated mice subjected to foot-shock.* Weiss *et al.* (1961) found that amphetamine was considerably more toxic to isolated mice subjected to foot-shock at regular intervals than to non-shocked isolated mice. Thus the LD50 for shocked mice was 27.0 mg/kg whilst for the non-shocked mice it was 128.0 mg/kg. In view of these results, the effect of amphetamine at dose levels of 20 and 40 mg/kg on the rectal temperature of isolated foot-shocked mice was determined (Table 3).

The maximum temperature recorded for control mice injected with water only was 40.3° C. There were no deaths. In the amphetamine-treated mice the rectal temperature rose to a maximum of 40.6 to 43.5° C. Death was preceded by a period of exhaustion and was related to the rise in temperature of the individual mice.

*Effect of various compounds on grouped amphetamine toxicity.* The effect of chlorpromazine 1 mg/kg and 2 mg/kg on the rectal temperature of grouped mice given amphetamine 20 mg/kg and 40 mg/kg was investigated. The chlorpromazine

TABLE 3

EFFECT OF FOOT-SHOCK ON THE TOXICITY OF AMPHETAMINE TO ISOLATED MICE  
 Single mice, housed in compartments  $7.5 \times 15 \times 15$  cm deep, were exposed to foot-shock once every 10 sec for 2 hr following the intraperitoneal injection of amphetamine. The rectal temperature was measured every 20 min. Mortality was determined 7 hr from the commencement of the test. The numerals in brackets show the no. of deaths

Room temp., °C	Amphetamine (mg/kg)	Total no. mice	No. mice with maximum rectal temp.		
			<41.7°	41.7°-42.4°	>42.4°
21	—	5	5 (0)	—	—
	20	10	6 (0)	3 (2)	1 (1)
	40	15	9 (0)	5 (2)	1 (1)
26	—	5	5 (0)	—	—
	20	10	—	9 (7)	1 (1)
	40	5	—	4 (4)	1 (1)
	Total	50	25 (0)	21(15)	4 (4)

was given 1 hr before the injection of amphetamine. In each experiment both the mean peak temperature and also the mortality were significantly lower in the chlorpromazine-treated mice than in similar groups of control mice which had received amphetamine only (Table 4). The mean rectal temperature of the control mice reached a maximum 40 to 60 min after the injection of amphetamine, and was, in all cases, at least  $1^\circ$  C higher than that for the chlorpromazine-treated mice. This is demonstrated in Fig. 3, where the dose of chlorpromazine was 2 mg/kg and amphetamine 40 mg/kg.

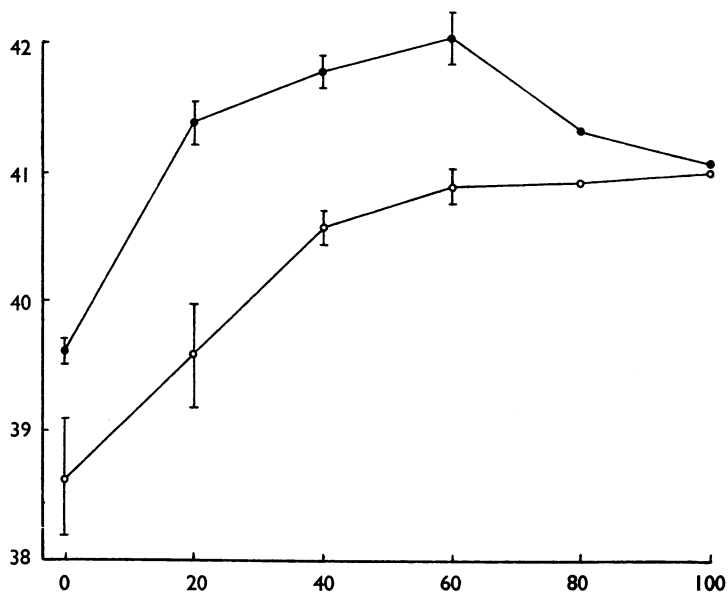


Fig. 3. Mean rectal temperature of grouped mice given amphetamine sulphate, 40 mg/kg, 1 hr after the intraperitoneal injection of chlorpromazine, 2 mg/kg (○ — ○), or water (● — ●), at a room temperature of  $26^\circ$  C. I denotes standard error of mean. Mortality for the mice given chlorpromazine was 3/15 and for the control mice 14/15. Ordinate: rectal temp.  $^\circ$  C. Abscissa: time (min).

TABLE 4  
 THE MEAN PEAK TEMPERATURE AND MORTALITY RECORDED FOR GROUPED MICE GIVEN DIFFERENT COMPOUNDS PRIOR  
 TO OR AT THE SAME TIME AS AMPHETAMINE AT A ROOM TEMPERATURE OF 26° C

The mean peak temperature was obtained by taking the mean of the maximum rectal temperature recorded for the mice over the 2 hr period immediately following the intraperitoneal injection of amphetamine. i.p. = intraperitoneal; s.c. = subcutaneous; S.C.T.Z. = 4-methyl-5-( $\beta$ -chloroethyl)-thiazole. The numerals in brackets give the standard error of the mean

Compound	Dose (mg/kg) and route	Time before amphetamine (min)	Amphetamine (mg/kg)	Rectal temp °C		Mortality		Difference	
				Controls	Treated	Controls	Treated	Rectal temp.	Mortality
Chlorpromazine	1.0 i.p.	60 min	20	41.4 ( $\pm 0.2$ )	40.5 ( $\pm 0.2$ )	9/15	2/15	$P < 0.01$	$P < 0.05$
	2.0 i.p.	60 min	20	41.5 ( $\pm 0.2$ )	40.2 ( $\pm 0.2$ )	10/15	1/15	$P < 0.001$	$P < 0.01$
	2.0 i.p.	60 min	40	41.8 ( $\pm 0.1$ )	40.6 ( $\pm 0.2$ )	14/15	3/15	$P < 0.001$	$P < 0.001$
Phenoxybenzamine	10.0 i.p.	60 min	20	41.9 ( $\pm 0.1$ )	41.4 ( $\pm 0.1$ )	47/75	21/75	$P < 0.001$	$P < 0.01$
	Calcium acetylsalicylate	30 min	20	42.4 ( $\pm 0.1$ )	42.2 ( $\pm 0.2$ )	13/15	12/15	$P > 0.30$	$P > 0.50$
		At same time	15	41.6 ( $\pm 0.2$ )	42.3 ( $\pm 0.2$ )	6/15	11/15	$P < 0.01$	$P > 0.10$
S.C.T.Z.	75.0 i.p.	At same time	20	41.6 ( $\pm 0.2$ )	42.3 ( $\pm 0.2$ )	7/15	14/15	$P < 0.01$	$P < 0.05$
	1.0 s.c.	For 4 days preceding test	15	41.7 ( $\pm 0.2$ )	42.7 ( $\pm 0.1$ )	7/15	15/15	$P < 0.001$	$P < 0.01$
L-Thyroxine sodium	2.5 s.c.	For 4 days preceding test	15	42.0 ( $\pm 0.3$ )	42.8 ( $\pm 0.1$ )	9/15	15/15	$P < 0.01$	$P < 0.05$



In general, the mice given chlorpromazine appeared somewhat less hyperactive than the controls. Of a total of 45 mice pretreated with chlorpromazine 6 died, following a rise in rectal temperature to a maximum of 41.7 to 42.7° C.

*Phenoxybenzamine* has been shown to antagonize the toxicity of amphetamine to grouped mice (Maxwell, 1959; Weiss *et al.*, 1961). The rectal temperature of mice given phenoxybenzamine, 10 mg/kg, 1 hr before amphetamine, 20 mg/kg, was therefore compared with that for control mice given amphetamine only. A total of 5 experiments was made, involving 75 control mice and 75 mice pretreated with phenoxybenzamine. In each experiment the mortality and also the mean peak temperature of the pretreated groups were lower than in the control groups, although the difference was not significant. However, when the results of the 5 experiments were combined and a comparison then made, both the mean peak temperature and the mortality were significantly lower in the phenoxybenzamine treated mice (Table 4). The mice which had received phenoxybenzamine appeared slightly less hyperactive than the controls.

In order to test the effect of an antipyretic agent on grouped amphetamine toxicity, *calcium acetylsalicylate*, 100 mg/kg, was used. This dose antagonized the rise in temperature produced in mice by the subcutaneous injection of a 15% suspension of brewer's yeast in 0.9% sodium chloride solution given 16 hr previously, but was without effect on the temperature of mice pretreated with 0.9% sodium chloride solution only. When calcium acetylsalicylate was given to mice 30 min before the injection of 20 mg/kg amphetamine, there was no significant difference in the mortality between the treated and control groups, nor was there a significant difference in the mean peak temperature recorded for the 2 groups over the 2 hr period following the injection of amphetamine (Table 4).

*4-Methyl-5-( $\beta$ -chloroethyl)-thiazole* (S.C.T.Z.) is a hypnotic agent which at sub-hypnotic doses produces marked hypothermia in rats and mice (Charonnat, Lechat & Chareton, 1958; Jarman, personal communication). Thus following the intraperitoneal injection of 75 mg/kg to mice at a room temperature of 26° C, the rectal temperature fell to a minimum of 35.6° C at 40 min. The temperature of control mice injected with water only was 38.6° C at this time. Tests were therefore carried out in which grouped mice were given 75 mg/kg S.C.T.Z. at the same time as amphetamine, 20 mg/kg. The results are shown in Table 4. The temperature of the mice given S.C.T.Z. rose to a significantly higher level than that of the controls and there was also a significantly greater mortality. When a similar test was made using 15 mg/kg amphetamine, there was again a significantly higher mean peak temperature in the group given S.C.T.Z., although in this case, whilst the mortality was also greater, the difference was not significant. Hyperactivity was considerably more marked in the mice given S.C.T.Z. than in the control mice which had received amphetamine only.

Daily injection of *L-thyroxine sodium* 2 mg/kg increases the metabolic rate of mice after only 2 doses (Spencer & West, 1961). Two groups of 15 mice were therefore taken, one of which received daily subcutaneous injections of thyroxine, 2.5 mg/kg, for 4 days whilst the other was given injections of the solvent alone. On the fifth day the mice were injected with amphetamine, 15 mg/kg. Following the

injection of amphetamine, the mean peak temperature of those mice which had been pretreated with thyroxine rose to a significantly higher level than that of the controls, and there was also a significantly greater mortality. In a second experiment in which the mice were pretreated with thyroxine, 1 mg/kg, a similar result was obtained (Table 4). Following the injection of amphetamine the mice pretreated with thyroxine became noticeably more hyperactive than the controls.

*Effect of circulation of the air in the mouse boxes on grouped amphetamine toxicity.* Since death in aggregated mice given amphetamine appeared to be associated with the rise in body temperature of the mice, an experiment was carried out in which circulation of the air in the cages was increased by mounting a fan immediately above them. The mortality in 4 groups of 5 mice given amphetamine, 30 mg/kg, and housed in boxes beneath the fan was compared with that for 4 similar groups of mice kept in the same room but without any artificial circulation of the air. At the end of the 7 hr test period, 17 of the 20 control mice had died compared with only 2 in the experimental groups, showing that circulation of the air in the cages caused a significant reduction in mortality.

#### DISCUSSION

Events leading to death in mice given amphetamine were dependent to a great extent upon dose. Thus both with grouped and isolated mice at dose levels of 100 mg/kg and above, death followed a period of severe convulsions and usually occurred within 30 min of the injection. Amphetamine was, however, more toxic to grouped than to isolated mice, especially at a room temperature of 26° C. Under these conditions, comparatively low doses of amphetamine gave rise to a period of marked hyperactivity, which was followed by a phase of exhaustion in those mice which died. Death seldom occurred less than 1.5 hr from the time of injection and convulsions were rarely seen. Similar results were obtained by Hogn & Lasagna (1960) at a room temperature of 25° C which led them to suggest that death from amphetamine at high doses follows a different physiological train of events than death from lower doses.

When the rectal temperature of grouped mice was measured at intervals following the injection of amphetamine at dose levels of 8.8 to 66.7 mg/kg, it was found that the period of hyperactivity was accompanied by a marked elevation in temperature, and that many of the mice subsequently died. With these mice, the maximum recorded rectal temperature frequently reached a point above 42° C. During the period of exhaustion which followed, a precipitous drop in temperature always occurred, the temperature often falling as much as 5° C within 40 min.

At the conclusion of the first experiments it became apparent that those mice whose temperature had remained below 41.7° C survived, whilst those whose temperature had risen above 42.4° C usually died. In later tests, therefore, it became possible to predict with reasonable accuracy, sometimes as early as 20 min following the injection of amphetamine, which mice would die, even though deaths seldom occurred less than 1.5 hr from the time of injection.

Death in grouped mice given amphetamine at dose levels of 66.7 mg/kg and below appeared to be directly related to the extent of rise of the body temperature,

irrespective of the actual dose of amphetamine administered. At the conclusion of the work, therefore, a comparison was made between mortality and the maximum recorded temperature for all mice given amphetamine only, at dose levels from 8.8 to 66.7 mg/kg, where the room temperature was 26° C. The results were plotted in the form of 2 histograms, one giving the maximum rectal temperature recorded for the survivors and one the maximum temperature of those mice which died (Fig. 4). The mean peak temperature for the 95 survivors was 41.22° C, whilst

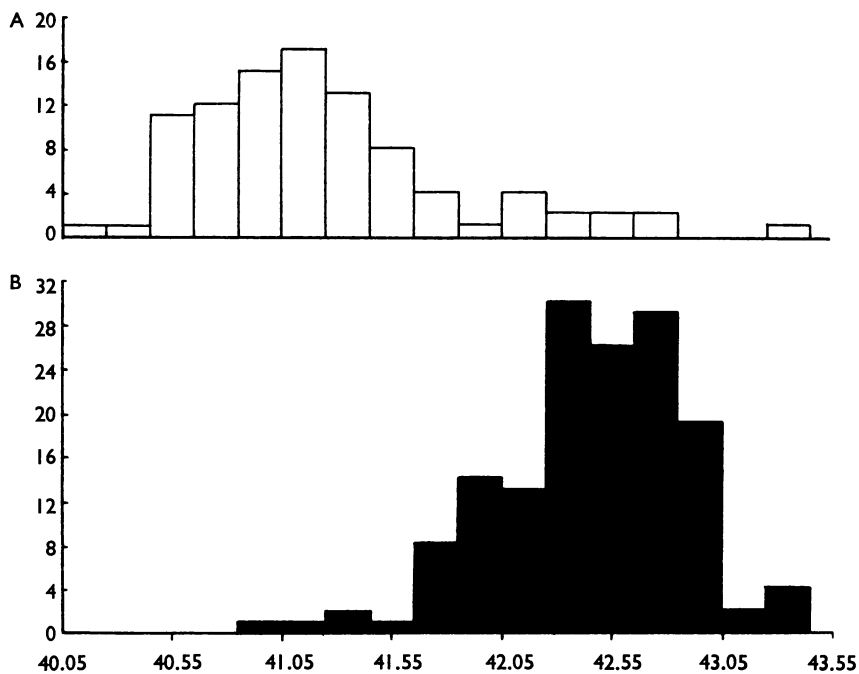


Fig. 4. Peak rectal temperature of grouped mice given amphetamine only at dose levels of 8.8–66.7 mg/kg at a room temperature of 26° C. Abscissa gives rectal temperature divided into intervals of 0.2° C. The height of each block shows the number of mice whose maximum recorded temperature was in that range. A, survivors; B, deaths. The mean peak temperature of 95 survivors was 41.22°, and that for the 150 mice which died 42.45°. Ordinates: no. mice. Abscissa: rectal temp. °C.

that for the 150 mice which died was 42.45° C. In addition, the temperature at which 50% of the mice would be expected to die (LT50) was calculated, using the maximum recorded temperature for each mouse over the 2 hr period immediately following the injection of amphetamine. The method used was based on a probit transformation of the percentages which died with given maximum temperature, and calculation of the weighted regression line against an untransformed temperature scale. The LT50 with 95% fiducial limits was 41.74° ± 0.12° C ( $b=1.98$ ).

When untreated mice were exposed to a high environmental temperature for a period of 2 hr, it was again found that when the maximum recorded rectal temperature remained below 41.7° C the mice survived, whilst, when it rose to a point above

42.4° C, they almost always died. A total of 160 mice was used in these investigations. The LT50 in terms of the rectal temperature, with 95% limits, was  $41.99^{\circ} \pm 0.12^{\circ} \text{C}$  ( $b=2.97$ ). The values obtained both for the LT50 and also for the slope of the line are just significantly greater than the corresponding values for grouped mice given amphetamine ( $P<0.05$ ). However, in addition to its hyperthermic action, amphetamine would be expected to possess other toxic properties which could account for the slight reduction in LT50. In view of this, it appears likely that, for grouped mice given amphetamine, death follows a rise in body temperature into the range which is lethal to the mice.

Although comparatively low doses of amphetamine in isolated mice produced a marked hyperthermia, the rectal temperatures of only a very few rose into the range shown to be lethal to the mice. However, with grouped mice given comparable dose levels of amphetamine, hyperactivity occurred and a more marked increase in temperature became apparent, so that the rectal temperature of a considerable number of the mice reached the lethal range. This additional increase in temperature would account for the greater toxicity of amphetamine to aggregated mice.

It had been observed, in our experiments with aggregated mice, that the greatest increase in rectal temperature and also the greatest mortality occurred following dose levels of 30 to 45 mg/kg. This corresponds to the level of amphetamine which in isolated mice had the most marked hyperthermic action. Results quoted by Hogn & Lasagna (1960) show that in their experiments, at dose levels below 150 mg/kg, the greatest mortality in grouped mice, at a room temperature of 25° C, followed the administration of 50 mg/kg amphetamine. Similarly in the experiments of Weiss *et al.* (1961) with foot-shocked mice, the dose-lethality function reached a plateau at 40 mg/kg which extended to 120 mg/kg.

The effect of a number of substances on grouped amphetamine toxicity was investigated. Two of the compounds, chlorpromazine and phenoxybenzamine, were active in antagonizing the toxicity of amphetamine in aggregated mice. This was associated with a less marked rise in temperature of the treated mice compared to those given amphetamine only. In neither case did the compounds enable the mice to withstand a rise in temperature which would be lethal to untreated mice. It is of interest to note that almost all the compounds so far reported to antagonize grouped amphetamine toxicity, and which can therefore be assumed to antagonize the hyperthermic action of amphetamine, are antagonists of adrenaline. In addition to chlorpromazine and reserpine, Maxwell (1959) tested a considerable number of compounds possessing adrenaline blocking action and showed that they reduced the toxicity of amphetamine in aggregated mice to a considerably greater extent than in isolated mice.

4-Methyl-5-( $\beta$ -chlorethyl)-thiazole was tested in grouped mice given amphetamine since, on its own, it produces a fairly pronounced fall in temperature in mice kept at 26° C. However, when given together with amphetamine it potentiated both the mortality and also the rise in temperature of the mice compared to the controls. L-Thyroxine sodium also potentiated the toxicity of amphetamine, and this was

again associated with a significantly greater rise in temperature of the pretreated animals. Calcium acetylsalicylate, which had no effect on the rise of body temperature of the mice, was without effect on mortality.

In general, therefore, it can be said that those compounds which antagonized the rise in body temperature of grouped mice given amphetamine antagonized its toxicity. Conversely, those compounds which increased the toxicity of amphetamine potentiated the rise in temperature.

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