LETTERS TO THE EDITOR

Amphetamine Toxicity in Aggregated Mice

SIR,—Amphetamine sulphate is known to be much more toxic to grouped mice than to mice housed singly in individual cages (Gunn and Gurd, 1940; Chance, 1946). It has been shown that environmental temperature and the degree of aggregation are two important factors influencing toxicity (Chance, 1946; Hogn and Lasagna, 1960) and most of the work reported in the literature has been carried out at room temperatures of 23·9–26·7° (75°–80° F.).

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° the LD50 for amphetamine in grouped mice was found to correspond with that reported in the literature. However, a reduction in the environmental temperature to 21·1° (70° F.) produced approximately a 4-fold decrease in the toxicity.

With aggregated mice, especially at the higher room temperature the injection of amphetamine at dose levels below 100 mg./kg. gave rise to a period of marked hyperactivity. Death was preceded by a stage of apparent exhaustion and did not normally occur less than 1½ hr. from the commencement of the experiment. It was seldom accompanied by convulsions. In isolated mice death followed a period of severe convulsions and normally occurred within 30 min. of the injection. Thus as found by Hogn and Lasagna (1960) there was a distinct difference in time to death and also in the appearance of mice before death between grouped and isolated mice.

Amphetamine is hyperthermic and a significant association has been found to exist in grouped mice between increased lethality and actions on motor activity and rectal temperature (Greenblatt and 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 made in which the body temperature of mice was measured every 20 min. for a period of 2 hr. after the injection of amphetamine sulphate.

Female Schofield albino mice of weight 20–22 g. were used. They were placed in groups of 5 in metal boxes 9 × 15 × 11 cm. deep, covered by a wide-mesh lid. Before the experiment they were housed in normal stock cages and allowed free access to food and water. The tests were made at room temperatures of 21–21·5° and 26–26·5°, and were terminated after 7 hr. At the commencement of the experiment, shortly after being placed in the metal boxes, the temperature of each mouse was measured using a thermocouple. The probe was inserted in the rectum to a constant depth of 3 cm. and was removed after each reading. After the injection of amphetamine the temperature of each mouse was recorded every 20 min. for a period of 160 min. and then every hr. up to 6 hr. Amphetamine sulphate was dissolved in distilled water and given by intraperitoneal injection at a constant volume of 10 ml./kg. Seven dose levels were used ranging from 8·8 mg./kg. to 100 mg./kg., the ratio between successive doses being 1·5.

When the test was carried out at 26° the body temperature of grouped mice given amphetamine at dose levels below 100 mg./kg. was found to rise 1·5–4·5° in the first 20 min. The maximum temperature rise over the same period at

21° was 3°. In some mice, after the initial sharp rise, the temperature gradually levelled out and returned slowly to or slightly below the control value, normally reaching this point 120–240 min. following the injection. In this case, the animals did not show signs of exhaustion and always survived the duration of the experiment. In other animals, however, the body temperature continued to rise, frequently to a point above 42°, before dropping sharply at a time when the animal showed signs of exhaustion. Most of these animals died. An example of both types of effect of amphetamine on body temperature is given in Fig. 1, where the dose of amphetamine was 13·2 mg./kg. and the room temperature 26°. Here the mean control temperature of the 5 mice was 38·4° and rose to 41·0° at 20 min., remaining at this level for a further 60 min.

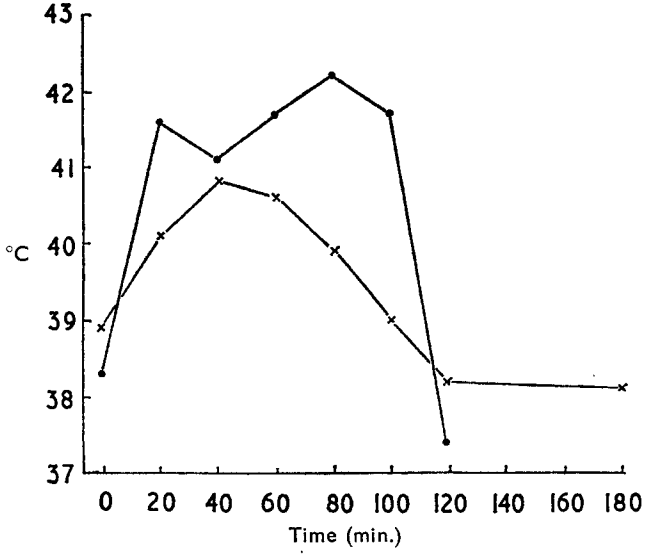

FIG. 1. Temperatures of 2 individual mice from a group of 5 given amphetamine sulphate 13·2 mg./kg., i.p. at a room temperature of 26° C. ●—● Dead at 126 min. X—X Survived for 7 hr.

After the administration of 100 mg./kg. of amphetamine to grouped mice at 26° a total mortality occurred within 34 min. of injection and was preceded by a period of violent convulsions similar to that found with isolated mice. At this room temperature the LD50 for amphetamine to grouped mice was about 25–30 mg./kg. whilst at 21° it was greater than 100 mg./kg.

At the conclusion of the experiments both at 21° and 26° it was found that, at dose levels below 100 mg./kg., all those mice whose recorded temperature had remained below 41·8° survived for the duration of the experiment. With one exception, all mice whose temperature had risen above 42·4° died. The one mouse which survived, following a rise in temperature to 43·1°, was in an exhausted state at the end of the experiment and had a body temperature of 32·2° at 6 hr. Thus from the temperature rise in individual mice it became possible to forecast with reasonable accuracy whether or not a particular animal would die. These results indicate that hyperpyrexia may be an important factor contributing to the increased toxicity of amphetamine to aggregated

mice, for although with isolated mice at dose levels in the region of the LD50 there was a marked increase in body temperature in no case did it rise above 41·8°.

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Neuromuscular Blocking Action of Succinyldicholine Stereoisomers

SIR,—An interesting fact has come to light during an investigation of the neuromuscular blocking action of a series of succinyldicholine compounds. On the cat gastrocnemius preparation, the isomers of succinyldi-(β-methyl)-choline had very little activity compared with that of suxamethonium; the L(+)-isomer was 1·35 times as effective as the D(−)-isomer on a molar basis, and the DL meso-mixture occupied an intermediate position (Table I).

TABLE I

COMPARISON OF POTENCY ON CAT GASTROCNEMIUS PREPARATION

Substance	Isomer	No. of mols of substance equivalent to 1 mol. of suxamethonium
Succinyldi-(β-methyl)choline	L(+)- D(−)- DL-meso-	887 1,200 913

The nature of this neuromuscular blocking action was further investigated, using the isolated innervated biventer cervicis muscle of the chick (Ginsborg and Warriner, 1960). Two points of note emerged. By contrast with all isomers of both the methiodide and the ethiodide series of succinyldi-(α-methyl)choline, both the L(+)- and the D(−)-isomers of succinyldi-(β-methyl)-choline were curare-like (Fig. 1), that is, they produced a reduction in twitch height without causing contracture. On a molar basis (+)-tubocurarine was 175 and 400 times as potent as the L(+)- and D(−)-isomers respectively. On the other hand the DL-meso-mixture produced a typical suxamethonium-like response, that is, it caused a contracture, which was found to be referable entirely to the meso content of the mixture. (Suxamethonium was 270 times as potent as the mixture in this action.)

The chick nerve-muscle preparation was selected since both the reduction of twitch height and the contracture are simultaneously available for comparison. In fact it appears that for suxamethonium-like neuromuscular blocking agents reduction of twitch height always runs parallel to contracture. Occasionally, however, a reduction of twitch height is observed shortly after the application