was also seen with the calcium free Krebs-bicarbonate. It seems that glyceryl trinitrate relaxation response on vascular muscle may be influenced by the ionic media in isolated tissue studies. Possibly muscular relaxation induced by glyceryl trinitrate is accompanied by ion fluxes.

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Effect of adrenal demedullation and adrenalectomy on amphetamine toxicity in isolated and aggregated mice

SIR,—Amphetamine toxicity is greater in aggregated than in isolated mice (Thiessen, 1964), and heightened adrenal secretory activity (medullary or cortical, or both) in aggregated mice has been implicated as a causative factor.

D'Arcy & Spurling (1961) found that amphetamine toxicity in isolated mice was increased by pretreatment with cortisol or corticotrophin. Moreover, Weiss, Laties & Blanton (1961) reported that adrenalectomy reduced the enhanced toxicity of amphetamine in isolated mice subjected to unavoidable foot-shock. Foot-shock also increases amphetamine toxicity in individual as well as aggregated mice; moreover, the events leading to death in amphetamine-treated, shocked, isolated mice appeared to be the same as those observed in aggregated mice treated with this drug (Weiss & others, 1961; Askew, 1962). Most recently, Richards, Nicol & Young (1966) reported that adrenalectomy reduced the enhanced toxicity of desoxyephedrine in aggregated mice. In contrast, Mennear & Rudzik (1965) observed that amphetamine toxicity in aggregated mice was not altered by adrenalectomy. We have now made some experiments designed to determine the significance of the adrenal in the "amphetamine aggregation effect".

Novice, male, albino mice of a random bred Swiss strain (Maxfield; Cincinnati, Ohio) were injected intraperitoneally with an aqueous solution of (+)-amphetamine sulphate (1 ml/100 g body wt), and were either isolated or aggregated (3 per cage) in metal cages (7 × 7 × 7·5 cm), one side of which was wire mesh to permit observation. Aggregated mice that died during the 3 hr observation period were replaced by untreated mice to maintain aggregation. Ambient temperature was 24 ± 1°.

To evaluate the significance of the adrenal medulla in the amphetamine aggregation effect, (+)-amphetamine toxicity was measured in demedullated, sham-operated and non-operated mice. Enucleation was done under ether anaesthesia via bilateral incisions in the lumbar musculature. Each adrenal capsule was incised and the medulla with most of the attached cortical parenchyma gently squeezed out with small forceps. Histology showed regeneration of the cortex, but not the medulla, to take place within 21 days. The adrenal capsule was not incised in sham-operated mice; non-operated mice remained caged throughout the operative period. Post-operatively, mice were housed in their home cages (45 × 24 × 12 cm) in groups of 15 for not less

than 30 days with Purina laboratory chow and water available *ad libitum*. At the time of drug administration, mice ranged in weight from 25 to 35 g and were 9 to 12 weeks of age. The results obtained from this study are presented in Table 1.

In a second series of experiments, (+)-amphetamine toxicity was measured in mice kept in their home cages in groups of 15 for 16–29 days and then bilaterally adrenalectomized, sham-operated, or non-operated. Post-operatively, these mice were maintained under conditions described above except that the adrenalectomized mice had 1% saline as drinking water. At the time of drug administration (48 hr post-operatively), mice weighed between 20 and 30 g and were 7 to 9 weeks of age. The results from this study are in Table 1.

It is evident from the results that the susceptibility of mice (isolated or aggregated) to (+)-amphetamine- (30 or 100 mg/kg) induced lethality was not significantly altered by prior demedullation. Similarly, the lethal effects of (+)-amphetamine were not antagonized by adrenalectomy (Table 1). In fact, at the 30 mg/kg dose level, adrenalectomy slightly increased the incidence of

TABLE 1. EFFECT OF ADRENAL DEMEDULLATION AND ADRENALECTOMY ON (+)-AMPHETAMINE LETHALITY IN ISOLATED AND AGGREGATED MICE

Treatment	% Dead 3 hr after (+)-amphetamine			
	30 mg/kg		100 mg/kg	
	Isolated	Aggregated	Isolated	Aggregated
Adrenal demedullation				
Non-operated	6·7 (30)*	51·7 (60)	31·4 (118)	66·7 (63)
Sham-operated	3·3 (30)	52·1 (48)	31·4 (102)	61·9 (63)
Demedullated	6·7 (30)	59·5 (42)	25·2 (115)	69·3 (75)
Adrenalectomy				
Non-operated	8·3 (12)	30 (30)	25 (12)	70 (30)
Sham-operated	0 (12)	26·6 (30)	16·6 (12)	70 (30)
Adrenalectomized	8·3 (12)	46·6 (30)	33·3 (12)	63·3 (30)

* Number of mice tested in parentheses

lethality in aggregated mice (sham-operated compared to adrenalectomized, $P = 0{\cdot}10$). The fact that aggregation enhanced (+)-amphetamine toxicity in the absence of the adrenal medulla, and also in the absence of the entire gland, does not prove conclusively that the adrenal does not participate in the amphetamine aggregation effect. It does, however, prove that the adrenal is not essential for this phenomenon. It seems reasonable to conclude that the enhanced toxicity of (+)-amphetamine in aggregated mice is mediated through some mechanism which does not involve adrenal activity.

The ability of adrenalectomy to protect mice against the enhanced toxicity of amphetamine produced by foot-shock (Weiss & others, 1961), but not by aggregation, suggests that the increased toxicity observed in these situations is mediated through different mechanisms. However, this discrepancy in results may also be due to differences of method. As to the protection provided by adrenalectomy against desoxyephedrine toxicity in aggregated mice (Richards & others, 1966), initial consideration must be given to possible pharmacological differences between these two agents. However, the results reported by these authors might very well reflect the fact that LD50 values were employed for quantitation of desoxyephedrine toxicity. Dose-lethality relationships, described by George & Wolf (1966) and Gardocki & others (1966a,b) raise serious questions concerning the validity of employing LD50 values to quantitate amphetamine toxicity in mice. Perhaps similar limitations apply to the use of this expression as a measure of desoxyephedrine toxicity.

In view of recent evidence which indicates that death resulting from amphetamine at high doses follows a different physiological train of events than death from lower doses (George & Wolf, 1966; Gardocki, Schuler & Goldstein, 1966a, b), two dose levels of (+)-amphetamine were used by us, the lower, 30 mg/kg, being intended to reflect the actions of low doses and the higher, 100 mg/kg, to be representative of higher doses. Although aggregation significantly increased the toxicity of (+)-amphetamine at both doses ($P < 0.05$), the effect was more marked at the lower dose. Furthermore, symptoms preceding death and the time of death of the animals depended not on the environmental conditions imposed on the mice or their prior surgical treatment but on the dose of (+)-amphetamine they received. Almost all deaths resulting from the higher dose occurred within 60 min of drug administration and were associated with convulsions. In contrast, deaths from 30 mg/kg dose were preceded by lethargy and coma, and nearly always occurred within 90 to 180 min of injection. These observations again suggest different causes of death at the two dose levels studied.

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