Effects of (+)-amphetamine on lactic acid concentrations in the tissues of aggregated mice

SIR,—Aggregation and other stresses markedly enhance the toxicity of amphetamine to mice. In aggregated mice, (+)-amphetamine induces tissue glycogen depletion, hypoglycaemia and tissue noradrenaline depletion (Moore, 1963; Moore, Sawdy & Shaul, 1965). The present report concerns the effects of (+)-amphetamine on lactic acid levels in the tissues of aggregated mice.

Male albino mice (Charles River Mouse Farms), 24–30 g, were injected intraperitoneally with saline or with (+)-amphetamine sulphate (10 mg/kg) and placed in groups of 4 in 9 cm square wire mesh cages. When killed, 60–90 min after injection, the saline-treated mice were quiet and often sleeping. Mice injected with (+)-amphetamine could be divided into 2 distinct groups. One group, termed "excited," exhibited constant motor activity. Mice in the other group, termed "depressed," lay quietly in their cages in an exhausted condition. They exhibited laboured breathing and some loss of motor control. It is during this depressed state that deaths occur; approximately 25% of the aggregated mice die within 4 hr of injecting 10 mg/kg of (+)-amphetamine (Moore, 1963).

Mice were killed by decapitation, and at the same time one hind leg was excised. The first few drops of blood from the trunk were collected in small heparinised beakers. In the "depressed" group blood from 2 or 3 mice had to be pooled to obtain a sufficient quantity for analysis. Glucose was determined in 0·2 ml aliquots of blood by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N.J., U.S.A.). For lactic acid analysis a 0·1 ml aliquot of blood was added to 1·9 ml of cold water. Samples of skeletal muscle, liver and renal cortex, weighing 80–120 mg, were quickly dissected, weighed and homogenised in 1·9 ml cold water. Perchloric acid (2 ml, 0·8N) was added to all samples and the mixture centrifuged at 9,000 × *g* for 5 min. The resulting supernatant was neutralised with 10N potassium hydroxide, and after copper-calcium hydroxide precipitation lactic acid was determined in the supernatant (Barker & Summerson, 1941). These results are summarised in Table 1.

TABLE 1. BLOOD GLUCOSE AND BLOOD AND TISSUE LACTATE CONCENTRATIONS IN AGGREGATED MICE TREATED WITH (+)-AMPHETAMINE SULPHATE

Treatment	Lactic acid (μmoles/g wet weight)				Glucose (mg %)
	Skeletal muscle	Liver	Kidney	Blood*	Blood
Saline	8·49 ± ·63	3·61 ± ·55	3·76 ± ·31	1·62 ± ·03	136·6 ± 6·7
Amphetamine (excited)	10·07 ± ·58	3·43 ± ·54	4·24 ± ·43	1·85 ± ·32	116·3 ± 9·3
Amphetamine (depressed) ..	7·45 ± ·94	2·51 ± ·75	4·98 ± ·63	1·73 ± ·30	27·2 ± 2·9

Each value represents the mean (±1 standard error) of 10 determinations.
* The values for blood lactate represent μmoles/ml of whole blood.

The tissue and blood lactate concentrations in the control or saline treated mice were similar to those reported in the rat by Schön (1965) and by Gey & Pletscher (1961). Lactic acid levels in the tissues and blood of both excited and depressed (+)-amphetamine-treated mice were not significantly different ($P > 0·05$) from those of the saline treated controls. It should be noted that the blood glucose concentration showed the same pattern as previously reported (Moore & others, 1965). That is, following the injection of amphetamine, the depressed but not the excited mice exhibited marked hypoglycaemia.

Peterson, Hardinge & Tilton (1964) suggested that death from amphetamine may follow neuromuscular blockade. With *in vitro* studies they showed that the neuromuscular blocking action of amphetamine was enhanced by the addition of lactic acid to the bath. The concentration of lactate used by these investigators was based upon the report by Fletcher & Hopkins (1917) that, in fatigued muscle, lactate may reach a level of 0·25% (approximately equivalent to 28 μmoles/g). The relevance of these *in vitro* findings to the death of aggregated mice is questionable since, as shown here by direct measurement, the lactic acid content of skeletal muscle does not increase in the exhausted mice and is only one quarter of that used in the *in vitro* studies. However, as reported previously and substantiated here, the (+)-amphetamine treated mice which become depressed also develop a marked hypoglycaemia. This hypoglycaemia may be an important factor leading to the death of the aggregated mice (Moore & others, 1965).

Acknowledgement. This work was supported by Grant AM 06275 from the National Institute of Arthritis and Metabolic Diseases.

Department of Pharmacology and Toxicology,
Dartmouth Medical School,
Hanover, N.H., U.S.A.
January 5, 1966

KENNETH E. MOORE

References

Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.*, **138**, 535–554.
Fletcher, W. M. & Hopkins, F. G. (1917). *Proc. R. Soc., Ser. B*, **89**, 444–467.
Gey, K. F. & Pletscher, A. (1961). *Experientia*, **17**, 25–27.
Moore, K. E. (1963). *J. Pharmac. exp. Ther.*, **142**, 6–12.
Moore, K. E., Sawdy, L. C. & Shaul, S. R. (1965). *Biochem. Pharmac.*, **14**, 197–204.
Peterson, D. I., Hardinge, M. G. & Tilton, B. E. (1964). *J. Pharmac. exp. Ther.*, **146**, 175–179.
Schön, R. (1965). *Analyt. Biochem.*, **12**, 413–420.

Blockade of the psychotic syndrome caused by nialamide in mice

SIR,—Inhibitors of monoamine oxidase, for example, nialamide, are known to cause a rise in noradrenaline and 5-hydroxytryptamine (5-HT) content of mouse brain whereas dopamine is hardly affected. This accumulation of amines is accompanied by a characteristic syndrome of stereotype movements of the animals involving restlessness, enhanced spontaneous motility, head movements, but no aggressiveness (Carlsson & Corrodi, 1964). These effects develop slowly after a high dose of nialamide (500 mg/kg i.p.) and are pronounced after 2½–3 hr.

By pretreating the animals with different inhibitors of the biosynthesis of noradrenaline, dopamine or 5-HT, or all three, the accumulation of these amines was blocked to discover whether the elevated level of noradrenaline or 5-HT, or of both, was responsible for the development of this syndrome.

Earlier work has shown that α-n-propyl-3,4-dihydroxyphenylacetamide (H22/54) and α-ethoxy-2,3-dihydroxyphenylacetamide (H33/07) could block this syndrome caused by nialamide (Carlsson, Corrodi & Waldeck, 1963; Carlsson & Corrodi, 1964). Both substances inhibit the hydroxylation of tyrosine and tryptophan, and so prevent the synthesis, in the animal, of dopamine, noradrenaline and 5-HT (Carlsson, & others, 1963; Carlsson & Corrodi, 1964). L-α-Methyldopa has been shown to inhibit the synthesis of