

(20 mg/kg s.c.), hyoscine (2.5 mg/kg s.c.) and L-DOPA (100 mg/kg p.o., given with the peripheral DOPA decarboxylase inhibitor, Ro-4-4602, 50 mg/kg i.p.). Dexamphetamine (10 mg/kg s.c.), L-amphetamine (20 mg/kg s.c.) and methylphenidate (20 mg/kg p.o.) have no significant effect.

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A modified motor activity test to discriminate major and minor tranquillizers

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In the screening for major and minor tranquillizers there is no single test sufficiently sensitive and flexible to distinguish between classes of tranquillizing agents, or having the potential to recognize a novel type of activity (Irwin, 1962). The test we have evaluated could well possess some of these attributes. Its presentation here is primarily to introduce its potential discriminative powers for different classes of sedative and tranquillizing compounds. The test design is a modification of Somers' motor activity experiment where animals are dosed and 30 min later placed in a 'Perspex' motor activity counting chamber and their perambulatory activity recorded every 5 min over a 1 h period. Upon this is imposed a facility so that the animals may be submitted to photic stimuli at regular time intervals throughout the test period.

This intermittent photic stimulus caused an increase in the overall activity of the control animals and a change in their pattern of behaviour over the 1 h period. Dosed animals showed different patterns of behaviour which were related to the type of drug adopted, and the effects were reproducible.

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Analysis of labile brain constituents using a technique for the instantaneous fixation of brain tissue *in vivo*

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It is well established that, in order to preserve the *in vivo* levels of many metabolites in the central nervous system, it is essential to stop metabolism as quickly as possible by means of rapid freezing. The procedure most commonly used for small animals is the total immersion of the whole animal in a liquid gas or in fluorocarbons cooled to their freezing point with liquid nitrogen. Since these techniques take several seconds to freeze the innermost regions of the brains of rats and mice (Ferrendelli, Gay, Sedgwick & Chang, 1972) it is likely that post-mortem alterations in brain metabolites are unavoidable even under these conditions.

A new apparatus has recently been devised (Veech, Harris, Veloso & Veech, 1972) with which it is possible to remove and fix brain tissue in less than one second. The forebrain of rats or mice is instantaneously expelled under pressure on to an aluminium disc previously cooled in liquid nitrogen. Using such a machine, we have confirmed the

TABLE 1

Metabolite	Method of brain fixation		
	Rapid freezer	Immersion in liquid N ₂	Decapitation and head frozen after 15 sec
Glucose (μ mole/g)	1.37 \pm 0.05	1.06 \pm 0.05*	0.37 \pm 0.02*
Glycogen (μ mole/g)	1.82 \pm 0.10	1.77 \pm 0.04	1.32 \pm 0.08*
ATP (μ mole/g)	2.17 \pm 0.03	2.07 \pm 0.03	1.63 \pm 0.05*
Phosphocreatine (μ mole/g)	3.13 \pm 0.11	2.51 \pm 0.16*	0.92 \pm 0.03*
Lactate (μ mole/g)	1.31 \pm 0.08	1.85 \pm 0.09*	3.39 \pm 0.24*
Cyclic AMP (nmole/g)	0.54 \pm 0.05	1.04 \pm 0.09*	1.94 \pm 0.14*
Acetylcholine (μ g/g)	3.85 \pm 0.31	3.11 \pm 0.21*	2.50 \pm 0.10*

Values shown are the mean of at least four results \pm S.E.M.

Asterisks indicate values that are significantly different from those obtained using the rapid freezer ($P < 0.05$).

findings in the rat of Veech *et al.* (1972) that this method of freezing results in significantly different lactate and creatine phosphate levels in mouse forebrain when compared to liquid nitrogen sacrifice. In addition, we have extended the analyses to include glucose, glycogen, adenosine 3',5'-monophosphate (cyclic AMP) and acetylcholine (Table 1). It is hoped that data on biogenic amine and amino acid levels will also be presented. It is clear that considerable caution is required in the interpretation of physiological or drug-induced biochemical alterations in the central nervous system unless instantaneous fixation of brain tissue *in vivo* is accomplished.

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Some effects of the acute and chronic administration of sex hormones on brain monoamine concentrations

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The acute administration of ethinyl oestradiol (25 μ g/kg) and progesterone (2.5 mg/animal) has been shown to affect brain monoamine metabolism in ovariectomized rats (Tonge & Greengrass, 1971). The concentrations of brain monoamines also fluctuate at times when female sex hormone levels vary outside the usual values (Greengrass & Tonge, 1971, 1972). Synthetic oestrogen/progesterone combinations used in contraception have been reported to cause depression in some women (Kane, 1968) and there is evidence that depression may be due to disturbances of monoamine metabolism (Coppen, 1967).

The effects of the acute and chronic administration of oestrogen/progesterone combinations on brain monoamine metabolism have now been examined in intact female mice. Noradrenaline, 5-hydroxytryptamine, their precursors and metabolites have been determined in the 'fore-brain' (cortex), the 'middle-brain' (hypothalamus, thalamus and striatum) and the 'hind-brain' (pons, medulla, mid-brain and cerebellum) after the administration of ethinyl oestradiol (5 μ g) and progesterone (1 mg) daily for periods of 1 to 6 oestrous cycles. Noradrenaline concentrations were reduced in the middle-brain after 1 and 8 cycles; forebrain concentrations were also reduced after 8 cycles. Dopamine concentrations were decreased in the fore- and middle-brain after 1 cycle, but fore-brain concentrations returned to dioestrus levels after subsequent cycles. 5-Hydroxytryptamine concentrations were increased in the fore- and middle-brain after