TABLE 1

Metabolite	Method of brain fixation: Rapid freezer	Immersion in liquid N_2	Decapitation and head frozen after 15 sec
Glucose (μmole/g)	1·37±0·05	1·06±0·05*	0·37±0·02*
Glycogen (μmole/g)	1·82±0·10	1·77±0·04	1·32±0·08*
ATP (μmole/g)	2·17±0·03	2·07±0·03	1·63±0·05*
Phosphocreatine (μmole/g)	3·13±0·11	2·51±0·16*	0·92±0·03*
Lactate (μmole/g)	1·31±0·08	1·85±0·09*	3·39±0·24*
Cyclic AMP (nmole/g)	0·54±0·05	1·04±0·09*	1·94±0·14*
Acetylcholine (μg/g)	3·85±0·31	3·11±0·21*	2·50±0·10*

Values shown are the mean of at least four results ±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 μg/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 μg) 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

3 and 8 cycles. There were also alterations in tryptophan, tyrosine, normetanephrine and 5-hydroxyindoleacetic acid concentrations.

These findings confirm our earlier observations that female sex hormones alter brain monoamine concentrations, and also suggest that the effects of the chronic administration of oestrogen/progesterone combinations may differ from those produced by acute dosage.

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The uptake of 5-hydroxytryptamine by the rabbit heart *in vitro*

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Hearts from rabbits given heparin (500 units/kg) 5 min before killing were perfused at constant pressure by the Langendorff technique with Tyrode solution at 37° C and set up for recording right ventricular tension and rate (Fozard & Muscholl, 1971). The uptake of 5-hydroxytryptamine (5-HT) was estimated indirectly by measuring the difference between the arterial and venous concentrations of 5-HT in the perfusion fluid, and directly by assaying the 5-HT accumulated by the heart.

After 30 min equilibration with drug-free Tyrode, perfusion with 5-HT (0·25, 0·5 and 1×10^{-8} g/ml) was begun and continued for 55 minutes. The whole of the coronary flow was collected in seven aliquots (0–2, 2–4, 4–6, 6–10, 10–25, 25–40, 40–45 min) and a sample from each was assayed for 5-HT along with two aliquots of the arterial solution taken from the inflow cannula at the beginning and end of the perfusion. After a further 5 min perfusion with drug-free Tyrode, to wash out the extracellular space, the heart was removed from the cannula, cut into small pieces, firmly blotted and weighed. The 5-HT content of perfusates and hearts was assayed by the method of Snyder, Axelrod & Zweig (1965).

Rabbit hearts removed 5-HT from the perfusion fluid with the magnitude of the arterio-venous difference (expressed as % of the arterial concentration) being inversely proportional to the perfusion concentration, and declining slowly over the perfusion period. Between 6 and 10 min after starting 5-HT perfusion, the mean % arterio-venous differences were $34·0\pm4·9$ (mean, S.E. of mean, $n=5$), $16·4\pm2·4$ ($n=6$) and $13·6\pm1·3$ ($n=5$) for the 0·25, 0·5 and 1×10^{-8} g/ml concentration levels respectively. Hearts from non-heparinized rabbits removed a similar quantity of 5-HT from a perfusion solution of 1×10^{-8} g/ml as hearts from animals given heparin, although their endogenous 5-HT content was significantly larger. There was no significant change in the force or rate of cardiac contraction, coronary flow or cardiac water content as a result of 5-HT perfusion. The cumulative removal of 5-HT from the perfusion fluid (expressed as ng/g heart wet weight) was essentially linear for the first 10 min, although the rate of removal declined slowly thereafter. Analysis of the cardiac 5-HT content (expressed as ng/g heart wet weight) after perfusion with 0·25, 0·5 and 1×10^{-8} g/ml gave values of $78·6\pm7·0$ ($n=5$), $121·9\pm18·0$ ($n=6$) and $152·6\pm27·3$ ($n=4$) respectively, which in each case represent an increase over the value obtained in control experiments ($67·8\pm1·9$, $n=7$) in which perfusion was with Tyrode alone. The percentage of 5-HT removed from the fluid which was retained by the heart was 5·6, 17·5 and 18·0 for the 0·25, 0·5 and 1×10^{-8} g/ml concentration levels respectively. Initial rates of removal of 5-HT were calculated from the individual removal curves during the time period 2—10 minutes. When S/v was plotted against S (where S=perfusion concentration of 5-HT and