sion (5 ml; 40 mg in 0·05M tris buffer pH 7·6) for 10 min at 37°. Trypsin (bovine crystalline 7·5−8 × 10^3 BAEE units mg^{-1}) solution (50 μl; 100 μg ml^{-1} in 10^{-3}N HCl) was added and the reaction (37°) stopped after 15 min by filtration. Absorbance (520 nm) of released dye was measured and the percentage change compared with the control calculated, giving the amount of inhibition or activation.

Concentration (mM in digest) of modifier required for 50% activation were found to be: flazalone, 2; ketoprofen, 4; flufenamate, >16. The effect of preincubating a solution of modifier and enzyme at 37° was also investigated by removing 1 ml aliquots of modifier-enzyme mixture into 5 ml substrate suspension (giving 1 mg modifier, 5 μg enzyme in 6 ml digest) and determining inhibition or activation as above.

Table 1. *Enzyme modification by NSAIs after preincubation with enzyme*

Preincubation time (min)	% change in enzyme activity		
	Flazalone	Ketoprofen	Flufenamate
0	+20	+18	−54
20	+24	+59	−75
40	+36	+156	−83

+, activation; —, inhibition

The time-dependent enzyme modification revealed by preincubating these NSAIs with trypsin (Table 1) suggests direct action on the enzyme leading to activation with flazalone and ketoprufen but inhibition with flufenamate. Activation by the latter compound when preincubated with substrate suggests substrate protection of the enzyme. Enzyme modification giving either activation or inhibition, as shown for the NSAIs here, is in accord with the existence of forms of trypsin more or less active than the native enzyme (Krieger, Kay & Stroud, 1974). The non-essential nature of the acid function shown here gives emphasis to the idea that NSAI-induced trypsin modification follows a hydrophobic modifier-enzyme interaction involving the hydrophobic pocket (Keil, 1971) near the active site of the enzyme.

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The effect of hydrocortisone on tryptophan metabolism in the rat

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Curzon (1969) and Lapin & Oxenkrug (1969) have suggested that depressive illness may be caused by the induction of liver tryptophan pyrrolase by plasma corticosteroids leading to decreased synthesis and turnover of 5-hydroxytryptamine (5-HT) in the brain.

In this investigation the effects of hydrocortisone on brain tryptophan and serum 'free' and total tryptophan concentrations have been determined in the rat. In addition, the endogenous concentrations of 5-HT, the turnover of the amine in the brain and the activity of liver tryptophan pyrrolase have been determined after hydrocortisone injection.

Tryptophan was assayed by the method of Denckla & Dewey (1967); 5-HT by a modification of the method of Snyder, Axelrod & Zweig (1965); 5-hydroxyindole-3-acetic acid (5-HIAA) by the method of Giacalone & Valzelli (1966) and liver tryptophan pyrrolase activity by the method of Knox & Auerback (1955).

The activity of liver tryptophan pyrrolase was increased by 53% 3 h after the intraperitoneal injection of hydrocortisone (15 mg kg^{-1}). However, after the same period serum

'free' tryptophan concentrations were increased by 50% (2·12 ± 0·12 μg ml^{-1} to 0·38 ± 0·38 μg ml^{-1}), total serum tryptophan concentrations were unchanged and brain tryptophan concentrations were increased by 14% (1·22 ± 0·05 to 1·39 ± 0·05 μg g^{-1}). There was no change in the endogenous concentrations of 5-HT, but brain 5-HIAA concentrations were increased by 20% (267 ± 9 ng g^{-1} to 321 ± 12 ng g^{-1}).

The activity of liver tryptophan pyrrolase was reduced by 60% 3 h after the injection of allopurinol (20 mg kg^{-1}). Total serum tryptophan concentrations were simultaneously reduced by 60% and serum 'free' tryptophan concentrations by 53%. The concentrations of tryptophan in the brain were not significantly altered.

The results confirm the findings of Curzon & Green (1969) that liver tryptohan pyrrolase activity is increased by hydrocortisone. However, instead of the predicted decrease of 5-HT turnover, there was a significant increase in serum 'free' tryptophan and brain tryptophan concentrations. The fact that these increased concentrations were associated with an increase in brain 5-HIAA concentrations suggests an increased turnover of 5-HT in the brain. Similarly, although the decrease in tryptophan pyrrolase activity produced by allopurinol confirms the findings of Curzon & Green (1969), in our experiments this decrease was associated with a decrease rather than an increase in serum tryptophan levels.

The reasons for the increase in brain 5-HT turnover following hydrocortisone injection are not known, but the results of these experiments suggest that the action of corticosteroids in increasing tryptophan pyrrolase activity does not play a significant part in the regulation of brain 5-HT metabolism.

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An examination of the possible contribution of circulating corticosterone to the changes in brain monoamine metabolism during the oestrous cycle, pregnancy and the post-partum period in mice

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Greengrass and Tonge have shown that changes in brain monoamine concentrations occur during the oestrous cycle (1971), the post-partum period (1972a) and pregnancy (in press) and have attributed these changes to fluctuations in the levels of female sex hormones at these times. They have also shown that there are interactions between female sex hormones and psychotropic drugs (1972b) and have suggested that the relatively high incidence of mental disturbances at times when oestrogen and progesterone levels are fluctuating indicates an involvement of these hormones in the regulation of the mental state. Alterations in circulating levels of corticosterone have been shown to accompany some affective disorders (Coppen, 1967) and it is therefore possible that some of the disturbances of brain monoamine metabolism that are believed to exist in the affective disorders may result from the effects of corticosterone rather than sex hormones.

Plasma corticosterone levels have been measured during the oestrous cycle, pregnancy and the post-partum period to see whether any obvious correlation between these levels and alterations in brain monoamine metabolism could be detected. Corticosterone was measured by the method of Zenker & Bernstein (1957); blood was collected from the carotid artery and jugular vein after decapitation of the mice. There were no statistically significant differences in corticosterone levels at the oestrus and dioestrus stages of the cycle, but a marked increase at proestrus; the fluctuation did not appear to correlate with changes in brain monoamine metabolism previously described (Greengrass & Tonge, 1971). There was a gradual increase in corticosterone concentrations during pregnancy, with an acceleration