## REFERENCES

1. D. T. MASON and K. L. MELMON, *J. clin. Invest.* **45**, 1685 (1966).
2. M. L. MASHFORD and R. ZACEST, *Aust. Ann. Med.* **16**, 326 (1967).
3. R. ZACEST and M. L. MASHFORD, *Aust. J. exp. Biol. med. Sci.* **45**, 89 (1967).
4. J. H. GADDUM and E. W. HORTON, *Br. J. Pharmac.* **14**, 117 (1959).
5. M. L. MASHFORD and R. ZACEST, *Aust. J. exp. Biol. med. Sci.* **45**, 661 (1967).
6. T. L. GOODFRIEND and D. L. BALL, *J. Lab. clin. Med.* **73**, 501 (1969).
7. J. SPRAGG, K. F. AUSTEN and E. HABER, *J. Immunol.* **96**, 865 (1966).
8. H. RINDERKNECHT, B. J. HAVERBACK and F. ALADJEM, *Nature, Lond.* **213**, 1130 (1967).
9. T. C. TALAMO, E. HABER and K. F. AUSTEN, *J. Lab. clin. Med.* **74**, 816 (1969).
10. T. L. GOODFRIEND, L. LEVINE and G. D. FASMAN, *Science* **144**, 1344 (1964).
11. J. M. STEWART and J. D. YOUNG, *Solid State Peptide Synthesis*. W. H. Freeman, San Francisco (1969).
12. H. G. KWA and F. VERHOFSTAD, *Biochim. biophys. Acta* **133**, 186 (1967).
13. V. HERBERT, K. S. LAU, C. W. GOTTLIEB and S. J. BLEICHER, *J. clin. Endocrinol.* **25**, 1375 (1965).
14. J. D. HOROWITZ and M. L. MASHFORD, *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.* **263**, 332 (1969).
15. V. EISEN, *Br. J. Pharmac. Chemother.* **22**, 87 (1964).
16. I. MIWA, E. G. ERDÖS and T. SEKI, *Life Sci.* **7**, 1339 (1968).


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## Inhibition of tryptophan oxygenase *in vitro* by steroid compounds

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LIVER tryptophan oxygenase (TPO) is subject to induction both by its substrate and by adrenal glucocorticoids.[1] Although TPO activity has been shown to be elevated in the livers of pregnant rats[2,3] the administration of oestrone and progesterone in combination to non-pregnant females failed to produce an increase in enzyme activity.[3] Further, it was subsequently found that the subcutaneous injection of progesterone alone, in a dose of 4 or 8 mg daily for 28 days, caused a significant reduction of TPO levels in rat liver.[4]

In an attempt to investigate this problem further we have studied the effect of *in vitro* additions of progesterone and various other steroids on TPO.

### *Methods*

Livers were obtained from adult female Sprague–Dawley rats killed by decapitation. A particle-free supernatant was prepared by centrifugation of a 10% (w/v) homogenate at 105,000 *g* for 60 min at 4°, and the TPO activity determined by the method of Leklem *et al.*[5]

The steroids were dissolved in 100% ethanol such that the required amount for addition to the enzyme assay medium was present in 0·1 ml. Control assays were carried out with each experiment, both in the presence and absence of ethanol, so that every inhibition study had its own control, prepared from the same liver as was used in flasks containing the steroid. The ethanol did not affect the activity of TPO. The mean control value for TPO obtained in 22 assays was 3·4 ± 0·8 μmoles kynurenine produced/g wet liver tissue/hr.

*Results and discussion*

Inhibition of TPO activity was obtained with a number of steroids (Table 1). Progesterone and deoxycorticosterone, and to a lesser degree testosterone propionate, were effective inhibitors, but corticosterone and cortisol did not inhibit the enzyme, even when present at a 1 mM concentration. All five steroids are $\Delta^4$-3-oxosteroids, but the latter two compounds have an 11$\beta$ hydroxyl group and it seems likely that the presence of this substituent was responsible for the absence of an inhibitory effect.

TABLE 1. *In vitro* INHIBITION OF TRYPTOPHAN OXYGENASE BY STEROIDS AND DIETHYLSTILBOESTROL

| | Steroid concentration | | | |
|---|---|---|---|---|
| | 50 $\mu$M | | 100 $\mu$M | |
| Compound | Enzyme activity* | Inhibition (%) | Enzyme activity* | Inhibition (%) |
| Progesterone | 2·7 ± 0·3 | 16–22 | 2·2 ± 0·5 | 25–36 |
| Deoxycorticosterone | 2·8 ± 0·3 | 18–21 | 2·5 ± 0·2 | 18–26 |
| Testosterone propionate | 3·4 ± 0·1 | 0 | 3·0 ± 0·1 | 9–13 |
| Oestradiol-17$\beta$ | 3·1 ± 0·2 | 7–11 | 2·3 ± 0·1 | 16–21 |
| Ethinyl oestradiol | 3·0 ± 0·1 | 11–19 | 2·8 ± 0·3 | 15–25 |
| Oestrone 3-sulphate | 3·4 ± 0·1 | 0 | 3·1 ± 0·2 | 9–11 |
| Diethylstilboestrol | 3·1 ± 0·2 | 9–11 | 2·6 ± 0·3 | 21–26 |

* $\mu$mole kynurenine/g wet tissue/hr (mean ± S.D.). The percentage inhibition is given as the range of results obtained from three to four separate experiments.

Lineweaver–Burk plots for TPO activity in the presence of 100 $\mu$M progesterone or ethinyl oestradiol and various concentrations of haematin were consistent with the general form of competitive inhibition (Fig. 1).

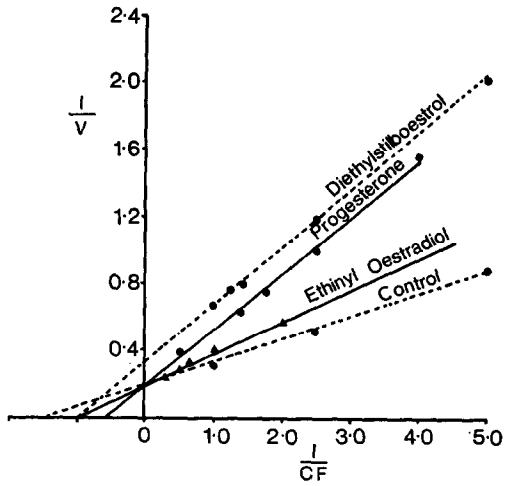


FIG. 1. Lineweaver–Burk plots for progesterone, ethinyl oestradiol and diethylstilboestrol. The steroid concentrations were 100 $\mu$M. The ordinate is the reciprocal of the rate of TPO activity ($1/V$), and the abscissa is the reciprocal of the haem concentration ($1/CF$).

Ethinyl oestradiol inhibited TPO, but its 3-methyl ether derivative, mestranol, was without effect. Oelkers and Nolten[6] reported that the inhibition of TPO by the unconjugated natural oestrogens oestradiol-17$\beta$, oestrone and oestriol was greater than that obtained with the corresponding 3-sulphate esters, and this is confirmed by our results. It appears, then, that the nature of the substituent at position 3 on the steroid molecule is an important factor in determining the occurrence or not of enzyme inhibition. The 3-oxosteroids progesterone, deoxycorticosterone and testosterone propionate were TPO inhibitors, and it may be that enolation of the group at position 3 must take place to allow the steroid molecule to interact either with the haem cofactor directly, or to compete with the haem for the cofactor binding site on the apoprotein. The necessary enolation may be restricted by sulphate esterification, and prevented by the presence of an inert group such as the ether substituent of mestranol.

Diethylstilboestrol inhibited TPO, but in this instance the Lineweaver–Burk plot had the features of a non-competitive inhibition. A number of other non-steroidal phenolic compounds are also known to be non-competitive inhibitors of TPO.[7]

The levels of steroids that were found to inhibit TPO activity in this and other studies[6,8] were well above the physiological range. However, such levels may be reached when large doses of steroids are used for *in vivo* studies of the effect of hormones upon the activity of this and other enzymes. Greengard *et al.*,[3] in an attempt to define a hormonal basis for the elevated levels of liver TPO in pregnant rats, administered to non-pregnant animals a combination of 1 $\mu$g oestrone and 4 mg progesterone, or three or 10 times these amounts of steroids daily for 13 days, and failed to obtain any significant increase in enzyme activity. Our results suggest that such large doses of progesterone may inhibit TPO by direct competition with the haem cofactor, and mask any stimulation due to the oestrogenic component. It is of interest, therefore, that we obtained significant increases in liver TPO when female rats were given 10 $\mu$g oestradiol benzoate alone daily for 14 days,[9] whereas a combination of 20 $\mu$g oestradiol benzoate and 4 mg progesterone produced a reduction in enzyme activity.[4]

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## REFERENCES

1. W. E. KNOX, *Br. J. exp. Path.* **32**, 462 (1951).
2. S. AURICCHIO, N. RIGILLO and R. DITORO, *Minerva Pediat.* **12**, 1463 (1960).
3. P. GREENGARD, H. J. KALINSKY and T. J. MANNING, *Biochim. biophys. Acta* **156**, 198 (1968).
4. D. P. ROSE and R. R. BROWN, Unpublished work.
5. J. E. LEKLEM, J. WOODFORD and R. R. BROWN, *Comp. Biochem. Physiol.* **31**, 95 (1969).
6. W. OELKERS and W. NOLTEN, *Hoppe-Seyler's Z. Physiol. Chem.* **338**, 105 (1964).
7. E. FRIEDEN, G. W. WESTMARK and J. M. SCHOR, *Archs Biochem. Biophys.* **92**, 176 (1961).
8. W. OELKERS and H. J. DULCE, *Hoppe-Seyler's Z. Physiol. Chem.* **337**, 150 (1964).
9. D. P. ROSE and I. P. BRAIDMAN, *Am. J. clin. Nutr.* in press.

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### Effect of cysteine on inducible synthesis of $\beta$-galactosidase in *Escherichia coli*

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EXOGENOUS cysteine inhibits the growth of *Escherichia coli* when the cells are cultured on mineral salts–glucose medium.[1–3] This inhibitory effect of cysteine is strongly antagonized by the simultaneous addition of leu-ileu-threo-val* to the culture medium.[3] It was suggested[3] that cysteine inhibits the synthesis of these amino acids. This assumption is supported by the findings of other authors, according to which cysteine, *in vitro*, inhibits the activities of two enzymes (i.e. homoserine dehydrogenase[4] and acetohydroxy acid synthetase[5]) in the biosynthetic pathways of these four amino acids.