3. R. SULSER and J. V. DINGELL, *Biochem. Pharmac.* **17,** 634 (1968).
4. J. V. DINGELL and A. D. BASS, *Biochem. Pharmac.* **18,** 1535 (1969).
5. J. R. MITCHELL, J. H. CAVANAUGH, J. V. DINGELL and J. A. OATES, *J. Pharm. exp. Ther.* **172,** 108 (1970).
6. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmac.* **12,** 357 (1963).
7. R. SJOQVIST, W. HAMMER, H. SCHUMACHER and J. R. GILLETTE, *Biochem. Pharmac.* **17,** 915 (1968).

Biochemical Pharmacology, Vol. 20, pp. 1723–1725. Pergamon Press, 1971. Printed in Great Britain

Role of steroid hormones in hepatic microsomal enzyme induction*

(*Received* 11 *June* 1970; *accepted* 2 *January* 1971)

THE HYDROXYLATING enzymes associated with hepatic microsomes are active against a wide variety of substrates, including steroids, polycyclic hydrocarbons and many drugs.[1] They are adaptive enzymes; pretreatment of rats and other species with many substrates leads to the induction of increased levels of enzyme activity.[1] These are associated with an increase in the concentration of hepatic microsomal cytochrome P-450,[2] the terminal oxidase of the hydroxylating enzymes.

It seems likely that the principal endogenous substrates of the enzymes are steroids[3–5] and from observations on the effect of castration, adrenalectomy and steroid hormones on enzyme activity,[6] it has been suggested that the levels of microsomal enzyme activity are controlled by the concentration of circulating steroid hormones.[1,7,8] *In vitro* studies have shown that competition occurs when a drug substrate and a steroid hormone are incubated together with liver microsomes.[5] Such competition could occur also *in vivo*, and it has been postulated that the induction of the enzymes by foreign substances, for example, phenobarbitone, is mediated through changes taking place in an endogenous, steroid hormone controlled, regulatory system as a result of interference with endogenous steroid metabolism.[7,8]

There is some controversy as to the role of the steroid hormones in microsomal enzyme induction.[7–9] The experiments reported here represent an attempt to clarify the position, and the results suggest that enzyme induction is independent of these hormones.

A preliminary report of this work has already appeared.[10]

For these experiments castrated, adrenalectomized, castrated and adrenalectomized, sham-operated and intact male rats of the Carworth CFE strain (Sprague–Dawley derived) initially weighing about 140 g were purchased from Carworth Europe (Alconbury, Herts.). The animals were housed in mesh-floored cages in a conventional animal house. Their sole source of drinking water was 1% NaCl solution, available *ad lib.* Although 1% saline was given to all the rats in these experiments, subsequent investigation showed that giving saline to non-adrenalectomized rats had no effect on the enzyme activities measured. Food (modified diet 41B, Oxoid Ltd., Southwark Bridge Road, London, S.E.1) was available *ad lib.* also. All animals grew at a mean rate of between 3 and 6 g/day throughout the period of the experiments. Rats given phenobarbitone received daily i.p. injections of Na phenobarbitone in 0·15 M saline at a dose of 100 mg/kg. Controls were injected with an equivalent volume of saline. Regimes of drug administration were begun on the second day following operation and continued for 7 days, the rats being killed 24 hr after the last injection.

The efficacy of the adrenalectomies was checked by taking a group of four adrenalectomized rats at random from the same batch as those used in the experiments, and giving them water instead of saline. These rats all became moribund within 24 hr. On histological examination of tissue taken post mortem from the vicinity of the adrenals, no adrenal tissue was seen in any of the adrenalectomized animals. Neither was any ectopic adrenal tissue apparent on gross examination. The control rats used in the experiments described were not sham-operated, but in a separate series of experiments no significant differences were seen in any of the quantitites measured between groups of sham-operated and non-operated rats.

* This work was supported by a grant from the Medical Research Council.

Post-mitochondrial supernatants (for enzyme determinations) and microsomes (for cytochrome P-450) were prepared from liver homogenates, and cytochrome P-450 and Pyramidon demethylation activity were measured by the methods that we have described previously.[11] For the measurement of aniline hydroxylation, 1 ml of 10% post-mitochondrial supernatant was added to 2 ml of a medium containing 300 μmole Na_2HPO_4 buffer (made to pH 7·4 with HCl); 10 μmole Na isocitrate; 25 μmole $MgCl_2$; 1·5 μmole NADP; and substrate, 15 μmoles aniline. (This is the incubation mixture used, with the appropriate substrate, for Pyramidon demethylation.) The reaction mixtures were incubated in air at 37° for 20 min in a metabolic shaker and then quenched by pouring into chilled tubes containing 2 g of crystalline NaCl. The product, *p*-aminophenol, was extracted and estimated by the method of Kato and Gillette.[6]

The effects of adrenalectomy, castration and combined adrenalectomy and castration on cytochrome P-450, Pyramidon demethylation and aniline hydroxylation are shown in Table 1. P-450 concentrations are similar in each group to control values. Aniline hydroxylation activity is similarly unaffected by the single or combined operations but Pyramidon demethylation activity is halved in the castrated and in the adrenalectomized rats. This is not an additive effect, however, since enzyme activity is not further reduced in the rats subjected to the combined operation.

TABLE 1. THE EFFECT OF ADRENALECTOMY AND CASTRATION ON HEPATIC MICROSOMAL ENZYME ACTIVITIES AND CYTOCHROME P-450 IN THE MALE RAT

Treatment	No. of rats	Cytochrome P-450 (nmoles/g liver)	Pyramidon demethylation (μmoles/g liver/hr)	Aniline hydroxylation (μmoles/g liver/hr)
None	4	17 ± 2	0·44 ± 0·11	0·94 ± 0·33
Adrenalectomized	4	16 ± 3	*0·20 ± 0·07	1·11 ± 0·17
Castrated	4	18 ± 3	*0·19 ± 0·10	0·86 ± 0·34
Adrenalectomized and Castrated	4	16 ± 4	*0·19 ± 0·06	0·94 ± 0·35

Values are given as means ± standard deviations.

* Indicates a value significantly different (0·05 > P) from the control. Animals were killed on the 10th day following operation. 1% saline was given as drinking water to all animals. Daily saline injections were given for 7 days before sacrifice.

The effects of adrenalectomy and castration of male rats on the activity of their hepatic microsomal drug metabolizing enzymes are well known.[1,6] The activity of the enzymes towards some substrates, e.g. Pyramidon, is decreased while that towards others, e.g. aniline, is unaltered. The results reported here are in accord with this and also indicate that the decreases in enzyme activity are not related to changes in the concentration of cytochrome P-450, the terminal oxidase of these enzymes. Castro *et al.*[12] have also found P-450 levels little affected in adrenalectomized rats, although an isolated report by Wada *et al.*[13] who observed a decrease in P-450 after adrenalectomy which could be prevented by cortisone treatment, is at variance with this.

From the results appearing in Table 2, it is clear that cytochrome P-450 induction is not impaired in rats which have been either adrenalectomized or castrated or both; there are no significant differences between the phenobarbitone-induced levels of cytochrome P-450 in the controls or any of the experimental groups. Aniline hydroxylation activity is induced to the same extent in castrated rats as in controls although in the adrenalectomized rats, whether castrated or not, somewhat lower levels are obtained. Although in the non-induced rat, Pyramidon demethylation is lower following adrenals ectomy or castration (see Table 1), phenobarbitone induces to the same extent in the operated groups as in the controls except in the rats which were castrated and adrenalectomized, where the induced level of enzyme activity is slightly, but significantly, less.

In previous investigations of the role of steroid hormones in microsomal enzyme induction, both Conney *et al.*[9] and Orrenius *et al.*[7,8] found that induction of enzyme activity could still take place in steroid hormone-depleted rats, although to a lesser extent than in controls. Orrenius inferred that steroid hormones are involved in the maintenance of normal levels of microsomal enzyme activity and in enzyme induction.[7,8] But since the results from this laboratory, Conney's[9] and Orrenius's[7,8] all demonstrate that induction can still take place in the steroid hormone-depleted rat, it follows that this cannot be the fundamental control. The lack of any effect on cytochrome P-450, the concentration of

TABLE 2. THE EFFECT OF ADRENALECTOMY AND CASTRATION ON THE INDUCTION OF HEPATIC MICROSOMAL ENZYMES AND CYTOCHROME P-450 BY PHENOBARBITONE IN THE MALE RAT

Treatment	No. or rats	Cytochrome P-450 (nmoles/g liver)	Pyramidon demethylation (μmoles/g liver/hr)	Aniline hydroxylation (μmoles/g liver/hr)
None	4	110 ± 10	2·4 ± 0·3	4·5 ± 0·2
Adrenalectomized	4	120 ± 10	2·2 ± 0·5	*3·6 ± 0·5
Castrated	4	130 ± 20	2·2 ± 0·4	4·6 ± 0·5
Adrenalectomized and Castrated	4	110 ± 10	*1·9 ± 0·1	*3·7 ± 0·3

Values are given as means ± standard deviations.

* Indicates a value significantly different ($P < 0.05$) from the control. Animals were killed on the 10th day following operation. 1% saline was given as drinking water to all animals. Injections of Na phenobarbitone were given i.p. at a dose of 100 mg/kg daily for 7 days before sacrifice.

which changes in response to many of the stimuli affecting enzyme activity, supports this thesis. Possibly the selective changes in basal and induced levels of enzyme activity as a result of steroid hormone depletion are related to changes in enzyme conformation rather than enzyme synthesis. Nevertheless, the idea that induction by foreign substances is mediated through interaction with an endogenous regulatory system remains an attractive one, and results from this laboratory suggest that dietary factors may be important in this respect.[10,14,15]

Department of Experimental Pathology,
University College Hospital Medical School,
University Street, London WC1E 6JJ

W. J. MARSHALL*

REFERENCES

1. A. H. CONNEY, *Pharmac. Rev.* **19,** 317 (1967).
2. L. ERNSTER and S. ORRENIUS, *Fedn Proc. Fedn. Am. Socs exp. Biol.* **24,** 1190 (1964).
3. R. KUNTZMAN, R. WELCH and A. H. CONNEY, *Advan. Enzyme Reg.* **4,** 149 (1966).
4. S. ORRENIUS and L. ERNSTER, *Life Sci.* **6,** 1473 (1967).
5. T. R. TEPHLY and G. J. MANNERING, *Molec. Pharmac.* **4,** 10 (1968).
6. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150,** 285 (1965).
7. S. ORRENIUS, Y. GNOSSPELIUS, M. L. DAS and L. ERNSTER, in *Symposium on the Structure and Function of the Endoplasmic Reticulum in Animal Cells* (Ed. P. N. CAMPBELL) Universitetsforlaget, Oslo (1968).
8. S. ORRENIUS, M. L. DAS and Y. GNOSSPELIUS, in *Microsomes and Drug Oxidations* (Eds. J. R. GILLETTE *et al.*) Academic Press, New York (1969)
9. A. H. CONNEY, I. A. MICHAELSON and J. J. BURNS, *J. Pharmac. exp. Ther.* **132,** 202 (1961).
10. W. J. MARSHALL and A. E. M. MCLEAN, *Biochem. J.* **115,** 27P (1969).
11. W. J. MARSHALL and A. E. M. MCLEAN, *Biochem. Pharmac.* **18,** 153 (1968).
12. J. A. CASTRO, F. E. GREENE, P. GIGON, H. SASAME and J. R. GILLETTE, *Biochem. Pharmac.* **19,** 2461 (1970).
13. F. WADA, T. HIGASHI, T. UMEMOTO and Y. SAKAMOTO, *Biochim. biophys. Acta* **86,** 422 (1964).
14. W. J. MARSHALL and A. E. M. MCLEAN, *Biochem. J.* **114,** 77P (1969).
15. W. J. MARSHALL and A. E. M. MCLEAN, *Biochem. J.* in press.

* Present address: Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London S.E.5.