Regulation of phenobarbital-inducible cytochrome *P*-450s in rat and mouse liver following dexamethasone administration and hypophysectomy

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Cytochrome *P*-450s are a superfamily of haem-containing proteins involved in the metabolism of foreign compounds, as well as a variety of endogenous molecules. The hepatic levels and function of this diverse group of enzymes are determined by both constitutive and xenobiotic regulators. To examine the role of constitutive factors in cytochrome *P*-450 regulation, the levels of three distinct groups of phenobarbital-inducible hepatic cytochrome *P*-450s were studied following dexamethasone-treatment or hypophysectomy. In the mouse, dexamethasone was a potent inducer of proteins within the PB₁ (subfamily IIC), PB_{2c} (family III) and PB₃ (subfamily IIB) families. These findings were strikingly different from the effects in the rat where essentially no effect on PB₃ expression and indeed suppression of proteins related to PB₁ was observed. Determination of mRNA concentration indicated that the difference was at the level of transcription. These findings indicate that synthetic glucocorticoids have the potential to be potent phenobarbital-like inducing agents. In the mouse hypophysectomy, like dexamethasone, induced hepatic mRNA of *P*-450 from families *P*-450IIB, *P*-450IIC and *P*-450III. Again a species difference was observed as this treatment had essentially no effect in the rat. These data in the mouse indicate that factors produced in the pituitary can either affect the transcription rate of phenobarbital and dexamethasone-inducible *P*-450 genes or influence the stability of their mRNAs.

INTRODUCTION

An increased ability to detoxify chemicals appears to be an important aspect of the biological evolutionary process. A major component of an organism's defence against chemical adversity is the superfamily of haem-containing proteins termed cytochrome *P*-450s. These mono-oxygenases are found in most species and organs and are characterized by their broad substrate specificity (Lu & West, 1980).

In mammals, the major organ of chemical detoxification is the liver, where a multiplicity of distinct cytochrome *P*-450s have been identified. It has been known for some time that microsomal mono-oxygenases can be distinguished by their response to induction by foreign compounds (Ernster & Orrenius, 1965; Remmer & Merker, 1965). This differential effect, together with developmental and sexual differences, has demonstrated the existence of multiple enzymes of overlapping substrate specificity. An estimate from protein purification data would suggest there are more than 13 forms of cytochrome *P*-450 in the rat liver (Waxman, 1986). This view is supported by the molecular cloning of specific cytochrome *P*-450 cDNAs (Adesnik & Atchison, 1986; Wolf, 1986).

Collectively, cytochrome *P*-450s represent a superfamily of enzymes with families of *P*-450 genes located on different chromosomes (Wolf, 1986; Nebert *et al.*,

1987). Several distinct families have been grouped on the basis of their sequence homology and regulation by xenobiotic inducers. These inducers include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Gonzalez *et al.*, 1985; Sogawa *et al.*, 1985) (*P*-450I family), phenobarbital (PB) (Atchison & Adesnik, 1983; Stupans *et al.*, 1984) (*P*-450II family), and pregnenolone-16 α -carbonitrile (PCN) (Hardwick *et al.*, 1984; Simmons *et al.*, 1985) (*P*-450III family). As recommended at the recent *P*-450 Gene Workshop we are using our previously used nomenclatures (Wolf, 1986; Wolf *et al.*, 1986). However, a new nomenclature has been proposed for the *P*-450 gene superfamily and these are listed in Nebert *et al.* (1987). PB₁ is a member of the family *P*-450IIC, PB_{2c} is a member of sub-family *P*-450III and PB₃ is a member of the sub-family *P*-450IIB. According to previous nomenclatures PB_{1a} is equivalent to PB₁ described by Wolf *et al.* (1984) and Waxman & Walsh (1983), PB_{2c} is related to *P*-450s characterized by their induction by pregnenolone-16 α -carbonitrile (Wolf *et al.*, 1986) and PB_{3a} is equivalent to a major phenobarbital-inducible *P*-450 described by Ryan *et al.* (1982) as form b. Proteins within family III can also be induced by synthetic glucocorticoids such as dexamethasone (Schuetz *et al.*, 1984). The dose of dexamethasone required to induce these enzymes is 50-times greater than the dose required to activate the glucocorticoid receptor (Schuetz & Guzelian, 1984) and therefore appears to be by some other

Abbreviation used: TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene.

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mechanism. The actual mechanism of cytochrome *P*-450 regulation within families II and III however remains elusive.

The use of mouse genetics has played an important role in identifying the mechanism of regulation of *P*-450s within family I (Nebert & Jensen, 1979) and represents a powerful approach for the study of *P*-450s within other families (Simmons *et al.*, 1985; Noshira & Negishi, 1986). We are at present developing this strategy to elucidate the constitutive factors involved in *P*-450 regulation. In this work we describe the effects of a synthetic glucocorticoid, dexamethasone, and hypophysectomy on mRNA and protein levels and activities of three *P*-450 subfamilies (*P*-450IIB, *P*-450IIC and *P*-450III) in the mouse and rat liver.

MATERIALS AND METHODS

Male Wistar rats (200 g) or male CBA/Ca, BALB/c, C3H/He or C57BL/6 mice (25 g) were used. Animals were treated with either dexamethasone (20, 100 or 200 mg/kg, intraperitoneally in corn oil) or phenobarbital (80 mg/kg intraperitoneally in 0.9% saline) on three consecutive days before use. Control animals received vehicle only. Animals were sacrificed by cervical dislocation and a sample of liver was rapidly excised and homogenized with guanidinium hydrochloride for the preparation of mRNA (see below). The remaining liver samples were homogenized in 1.15% KCl, buffered with 10 mM-phosphate, pH 7.4. The preparation of microsomal fractions then followed conventional procedures. Microsomes were stored in 0.25 M-sucrose buffered with 10 mM phosphate, pH 7.4, at a protein concentration of 10–20 mg/ml, at -70°C before use. Protein determinations were made using the method of Lowry *et al.* (1951) and cytochrome *P*-450 reductase was determined

as described previously (Adams *et al.*, 1985). The metabolism of the mono-oxygenase substrates, benzphetamine and pentoxy- and benzyloxy-resorufin was determined colorimetrically (Nash, 1953) or fluorimetrically (Burke *et al.*, 1985). Antibodies to the purified cytochrome *P*-450 isoenzymes (PB_{1a}, PB_{2c} and PB_{3a}) were prepared as described previously (Wolf *et al.*, 1986). Western blots were carried out using a modified version of the method reported by Towbin *et al.* (1979) and as described by Adams *et al.* (1985) with the following modifications: nitrocellulose filters were blocked with 3% bovine serum albumin and exposed to antiserum (diluted 1:500) for 1 h. Bound antibody was visualized using either the peroxidase-antiperoxidase method using 4-chloro-1-naphthol as substrate or ^{125}I -protein A. Immunohistochemistry was carried out on frozen liver sections using the method described by Buchmann *et al.* (1985) and Wolf *et al.* (1984).

Total liver RNA was extracted by the guanidine hydrochloride method (Cox, 1968). RNA was subsequently fractionated on denaturing formaldehyde gels and analysed as described by Meehan *et al.* (1984). cDNA probes were labelled either by the nick-translation or the random-priming method (Rigby *et al.*, 1977; Feinberg & Vogelstein, 1983, 1984). Hybridization and washing conditions were as described previously (Meehan *et al.*, 1984).

Materials

Resorufin analogues were purchased from Molecular Probes Inc., Junction City, OR, U.S.A. Emulgen 911 was a generous gift from the Kao-Atlas Company, Tokyo, Japan. Nitrocellulose filters were purchased from Schleicher and Schull, Dassel, Germany, and reagents for nick translations were purchased from BRL Ltd., Bethesda, MD, U.S.A. Other reagents were of high

Table 1. Effect of dexamethasone and phenobarbital on levels and activities of the microsomal mono-oxygenase system in rat and mouse liver

1 unit of reductase activity = 1 nmol of cytochrome c. reduced/min at 37°C . Values in parentheses represent fold increase over control values. (—), Value could not be determined as control value was zero. The values for mono-oxygenase substrate metabolism are the means of six to nine determinations. Male Wistar rats or CBA mice were used. The *P*-450 and reductase values were obtained from pooled samples from three animals.

	Treatment					
	Mouse			Rat		
	Control	Phenobarbital	Dexamethasone	Control	Phenobarbital	Dexamethasone
Cytochrome <i>P</i> -450 (nmol/mg of protein)	0.19	0.44 (2.3)	0.35 (1.8)	0.64	1.63 (2.5)	0.69 (1.1)
<i>P</i> -450 reductase (units/mg of protein)	56	131 (2.3)	150 (2.7)	160	441 (2.6)	257 (1.6)
Benzphetamine (nmol/min·mg of protein)	3.03	4.62 (1.5)	5.06 (1.7)	8.03	11.68 (1.4)	7.53 (0.9)
Benzyloxyresorufin (nmol/min·mg of protein)	0.012	1.08 (90)	1.50 (125)	2.04	11.94 (5.8)	2.01 (1.0)
Pentoxyresorufin (nmol/min·mg of protein)	0	0.12 (—)	0.20 (—)	0.18	2.88 (16)	0.23 (1.3)

Table 2. Effect of antibodies to cytochrome *P*-450 PB_{3a} on the metabolism of benzyloxyresorufin in rat and mouse liver microsomal fractions

	Microsomal sample	IgG added (mg/mg of protein)	Activity (nmol/min · mg of protein)		Control activity (%)*
			+ PI	+ PB _{3a}	
Mouse	Control	0	0.02	0.02	100
		2	0.018	0.013	72
		20	0.018	0.013	72
	Phenobarbital	0	3.95	3.95	100
		2	3.14	1.78	57
		20	2.84	0.12	4
	Dexamethasone	0	3.20	3.20	100
		0.2	3.26	1.78	54
		2	2.01	0.08	4
		40	1.74	0.02	1
Rat	Control	0	2.20	2.20	100
		20	1.80	0.00	0
		200	1.80	0.00	0
	Phenobarbital	0	9.28	9.28	100
		1	6.30	3.68	58
		10	6.85	0.35	5
		200	6.30	0.00	0
	Dexamethasone	0	2.50	2.50	100
		2	1.25	0.10	8
		20	1.25	0.07	6

* Percentage of rate with preimmune serum (PI).

purity and were obtained from the usual commercial sources.

cDNA clones

The rat liver cDNA clones, pR17 and pTF-1 were a generous gift from Drs. T. Friedberg and M. Adesnik. The clone pTF-1 corresponds to the constitutive *P*-450 form f, a member of the PB₁ (*P*-450IIC) subfamily, and pR17 to Levin form b, a member of the PB_{3a} (*P*-450IIB) subfamily (Friedberg *et al.*, 1986). The clone pPCN₁₂ was a cDNA of identical sequence to that described for the nifedipine oxidase cDNA (Beaune *et al.*, 1986) isolated in this laboratory (unpublished results). Clone M_{1p}EH4 corresponds to the mouse metallothionein cDNA (Durnam *et al.*, 1980), and was a generous gift from Dr. R. D. Palmiter.

RESULTS

Activity analysis

The administration of dexamethasone to mice had a significant effect on the hepatic content of cytochrome *P*-450 and cytochrome *P*-450 reductase (Table 1). In mice, dexamethasone (200 mg/kg) was almost as potent as phenobarbital in inducing both cytochrome *P*-450 and cytochrome *P*-450 reductase levels. In contrast, the levels of cytochrome *P*-450 were unchanged in rats following the same dexamethasone dose. However, the activity of cytochrome *P*-450 reductase was increased 1.6-fold.

Changes in mono-oxygenase activity following drug treatment are shown in Table 1. All three substrates shown have been used as marker substrates for the major rat liver phenobarbital-inducible form of *P*-450 PB_{3a} (Ryan *et al.*, 1982; Wolf *et al.*, 1986). In untreated mice the metabolism of the resorufin analogues was very low.

Fig. 1. Hepatic levels of cytochrome *P*-450 isoenzymes in rat and CBA mice following treatment with dexamethasone and phenobarbital

Proteins were quantified following transfer to nitrocellulose as described in the Materials and methods section. Bands were visualized using 4-chloro-1-naphthol as peroxidase substrate followed by labelling with ¹²⁵I-protein A. Microsomal protein (10 µg) was taken per track. The microsomal samples shown were from C, control; P, phenobarbital (80 mg/kg for 3 days)-treated animals; and D, dexamethasone (200 mg/kg for 3 days)-treated animals. The protein standards (St) were isolated from rat liver as described in the Materials and methods section.

However, on dexamethasone treatment the metabolism of benzyloxy- and pentoxyresorufin was significantly increased to levels higher than those observed on phenobarbital induction. The fold increase could not be calculated for pentoxyresorufin as no control activity could be detected. In marked contrast, in the rat,

dexamethasone did not affect these activities, but they were all significantly induced by phenobarbital. This species difference could be explained by differences in the enzymatic activity of orthologous rat and mouse *P*-450s towards these substrates, by the differential induction of rat and mouse cytochrome *P*-450 isoenzymes, or by

Fig. 2. Immunohistochemical localization of cytochrome *P*-450 isoenzymes in (a) mouse and (b) rat liver following treatment with dexamethasone and phenobarbital

Male C57BL/6 mice or Wistar rats were treated with phenobarbital (500 p.p.m. in the diet) or dexamethasone (20 mg/kg, intraperitoneally in corn oil) on 3 consecutive days prior to sacrifice. Controls received corn oil only. *P*-450 isoenzymes were visualized in frozen liver sections using diaminobenzidine as peroxidase substrate (Wolf *et al.*, 1986; Towbin *et al.*, 1979). The letters c and p show the centrilobular and periportal regions respectively. Magnification: 56-fold.

Fig. 3. Immunohistochemical localization of cytochrome *P*-450 PB_1 in mouse and rat liver following dexamethasone treatment

The dexamethasone dose was 100 mg/kg intraperitoneally for 3 days. Co, control; Dex, dexamethasone-treated animals; c, centrilobular; p, periportal regions.

different effects of dexamethasone on protein turnover. To investigate whether the activities measured were mediated by cytochrome *P*-450s within the equivalent families in rat and mouse, polyclonal antibodies raised to purified rat cytochrome PB_{3a} were used to inhibit the microsomal metabolism of benzyloxyresorufin. At the highest concentrations this antibody almost completely inhibited (95%) the metabolism of this substrate in all the rat and mouse samples (Table 2). The antibody to PB_{2c} had no inhibitory effect on this activity (results not shown). These results suggest that the increased metabolism of this substrate following dexamethasone in the mouse is due to the induction of members of the PB_{3a} family. To investigate this further the levels of specific isoenzymes were determined by Western-blot analysis.

Protein expression and immunohistochemistry

In mice, dexamethasone induced all three cytochrome *P*-450 isoenzymes examined to the same extent as phenobarbital. In marked contrast this compound caused a suppression of *P*-450 PB_1 levels in the rat (Fig. 1). The effect on PB_{3a} levels in the rat was variable, ranging from a suppression (Fig. 1) to slight elevation in different experiments. This was probably due to variation in the constitutive expression of this protein between animals (results not shown). PB_{2c} -related proteins were induced in the liver of both species by dexamethasone treatment. The regulation of homologues of PB_1 and

PB_{2c} in the mouse by phenobarbital, which has not been previously reported, was very similar to the rat.

Studies were then carried out to establish whether the changes in cytochrome *P*-450 expression following administration of the two inducers could also be observed at the histochemical level. In addition, the localization of these cytochrome *P*-450s has not been reported for the mouse. In general, the changes in staining intensity mirrored those observed in Western-blot analysis. The localization of the cytochrome *P*-450 isoenzymes related to PB_{1a} , PB_{2c} and PB_{3a} within livers of control animals was similar for mice and rats in that all isoenzymes were found preferentially in the centrilobular region (Fig. 2). The assignment of centrilobular or periportal regions was based on the vessel structure observed at high magnification and was unequivocal. In the mouse the administration of either dexamethasone or phenobarbital led to a strong induction of all three *P*-450 isoenzymes. In the case of phenobarbital the induction was in the cells of central and mid-zonal regions of the liver acinus. The localization was not so clearly defined in the dexamethasone-treated animals (3×20 mg/kg). Although PB_{2c} and PB_{1a} were predominantly localized in the centrilobular and mid-zonal regions, PB_{3a} however, was more diffusely distributed through the liver lobule. In rats, only the staining intensity of PB_{2c} was significantly elevated following dexamethasone administration (3×20 mg/kg). Indeed, the level of PB_{3a} was reduced

Fig. 4. Hepatic cytochrome *P*-450 mRNA levels in rats following phenobarbital or dexamethasone treatment

Total hepatic RNA (10 μ g), obtained 24 h after administration of the drug, was used per track. Samples were probed with rat pTF-1 (subfamily IIC) or pR17 (subfamily IIB) or pPCN₁₂ (subfamily III) cDNAs as described in the Materials and methods section. C, control; D, dexamethasone; P, phenobarbital-treated animals.

Fig. 5. Induction of hepatic cytochrome *P*-450 mRNA in mice following administration of dexamethasone and hypophysectomy

Total RNA (10 μ g) was loaded per track. Samples were probed with the following cDNAs: pR17, subfamily IIB; pTF-1, subfamily IIC; pPCN₁₂, subfamily III. Composites were from the same blots, irrelevant bands being omitted. The animal treatments were: C, control; P, phenobarbital; Dex, dexamethasone; Hx, hypophysectomy.

Fig. 6. Induction of hepatic cytochrome *P*-450 and metallothionein mRNA in mice treated with dexamethasone

Total RNA (10 μ g) was run per track. The cDNA probes used were as described in Fig. 5. M₁pEH₄ is a probe for metallothionein.

and that of PB_{1a} was unchanged. In both cases these proteins were more diffusely distributed through the liver lobule. When higher dexamethasone doses were used (3 $\times$ 100 mg/kg), it was interesting that the cells containing the highest PB_{1a} concentration were changed from the centrilobular to the periportal region (Fig. 3).

The staining intensity in the experiment shown was elevated. However, this effect was variable and complicated by a change in liver structure (some necrosis and loss of cell structure) observed at high dexamethasone doses.

RNA analysis

The effect of dexamethasone on the levels of *P*-450 mRNA from subfamilies *P*-450IIB, IIC and III were studied by Northern-blot analysis (Figs. 4 and 5). Dexamethasone induced PB_{2c} -related mRNA in both rats and mice (5- to 10-fold). Control mice had very low basal levels of PB_3 -related mRNA (pR17) which were significantly induced by dexamethasone (10- to 20-fold). This induction was as great as that seen after phenobarbital treatment. In rats, the basal level of PB_3 mRNA was higher, and variable, which made it difficult to assess the effects of dexamethasone treatment although there was an overall very slight induction. In both rats and mice phenobarbital and dexamethasone induced the levels of PB_1 mRNA. The slight induction in mRNA levels in the rat is in contrast to the Western-blot data where dexamethasone apparently suppressed PB_1 levels.

The induction of mouse liver mRNA by dexamethasone was time-dependent and required 24 h for maximal effect (Fig. 6). It was much slower than the glucocorticoid receptor-mediated induction of metallothionein which was maximally induced after 4 h, indicating that different mechanisms are involved (Fig. 6).

The effect of hypophysectomy on the regulation of phenobarbital-inducible *P*-450 mRNA levels was also investigated, as this tissue has been reported to be involved in the regulation of a variety of sex-specific *P*-450 genes and is known to be affected by dexamethasone (Fig. 5). A striking increase in mRNAs hybridizing to all three cDNA probes was observed on hypophysectomy in the mouse, the effect being as large, if not larger than phenobarbital or dexamethasone induction. This was particularly the case for the *P*-450IIC and *P*-450III probes where mRNA levels were induced 10- to 20-fold. These effects were independent of the mouse strains used, i.e. CBA/Ca, BALB/c or C3H/He. In all samples, contrapsin mRNA level, which is known to be suppressed by hypophysectomy, was reduced (Hill *et al.*, 1985) (results not shown). Hypophysectomy had essentially no effect on the levels of the *P*-450 mRNAs in the rat (results not shown). This difference between rats and mice was therefore similar to that observed on dexamethasone treatment.

DISCUSSION

Dexamethasone has profound effects on cytochrome *P*-450 expression as a single agent and in combination with other inducers. In our present study a number of observations on the effect of this compound on cytochrome *P*-450 levels can be made. (1) Dexamethasone is a potent phenobarbital-like inducer in the mouse. (2) There is a profound difference between the rat and mouse in their response to this compound. (3) This species difference appears to occur either at the transcriptional level or in the stabilization of mRNA. (4) Hypophysectomy essentially mimicked the effects of dexamethasone on *P*-450 mRNA levels indicating that

factors produced in the pituitary have the potential to regulate phenobarbital and dexamethasone-inducible *P*-450 genes. (5) A species difference is observed in the effects of dexamethasone on the lobular distribution of proteins related to cytochrome PB_1 .

A difference between rats and mice in cytochrome *P*-450 regulation, analogous to that found here, has been reported for the compound 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) (Poland *et al.*, 1981). TCPOBOP is a potent inducer of benzphetamine metabolism in mice, a marker substrate for 'phenobarbital-like' induction, but has no effect in rats even at high doses. Whether TCPOBOP acts in a similar fashion to dexamethasone and has potentially 'glucocorticoid-like' properties remains to be established.

In spite of the similarities in the effects of hypophysectomy and dexamethasone it is not clear whether they act through a common mechanism. Initial studies indicate that dexamethasone and phenobarbital will still induce hepatic *P*-450s in hypophysectomized mice (unpublished results) and also that the elevation in mRNA following hypophysectomy does not result in increased *P*-450 protein levels. However, factors produced in the pituitary can regulate phenobarbital- and dexamethasone-inducible *P*-450 mRNA in the mouse liver. This raises the intriguing question of why we have not made similar observations in the rat where no change in mRNA levels was observed on hypophysectomy.

This species difference was also found in the response to dexamethasone. In contrast to the mouse, only very slight changes in rat liver mRNA levels were observed for PB_1 and PB_{3a} following dexamethasone treatment. These findings are in contrast to those of Simmons *et al.* (1987) who found large increases in PB_{3a} -related mRNA levels but no increase in PB_{3a} protein. More recently Yamazoe *et al.* (1987) also reported slight changes in this mRNA species but, in contrast to the report of Simmons *et al.* (1987), this increase was accompanied by elevated protein levels. This issue is therefore somewhat confused and it may be that constitutive PB_{3a} levels, the dose, mode of administration, animal strain etc. may play a role in the response to this synthetic glucocorticoid in rats. In our studies PB_1 -related mRNA, however, did appear to be slightly induced in the rat by dexamethasone, but not translated. This response was, however, variable.

The finding that the PB_1 -related mRNA did not appear to be translated in the rat is intriguing in view of the change in PB_1 localization following dexamethasone treatment. A change in hepatic morphology could explain these results, but the localization of other proteins was not affected by dexamethasone which argues against this. It may be that dexamethasone specifically induces certain members of the very extensive PB_1 sub-family (Wolf, 1986; Wolf *et al.*, 1986), while suppressing the expression of others. The overall effect could be a reduction in PB_1 content as seen on a Western blot. The fact that PB_1 levels were significantly induced in the mouse would then explain why no change in localization was seen in this species.

There are many similarities in cytochrome *P*-450 isoenzyme composition between species and orthologous forms between rat, mouse, rabbit and human have been identified (Wolf, 1986). However, it is clear that this gene superfamily has evolved very rapidly and in different species to different extents. The human *P*-450 superfamily, for example, seems less complex than that in

small mammals (Meehan *et al.*, 1988; Miles *et al.*, 1988). It therefore appears that in addition to the many similarities, significant differences between species in drug-metabolizing capacity must occur. As a consequence it is difficult to establish which model may best reflect the mechanism of isoenzyme regulation in man. It is however intriguing that synthetic, and possibly endogenous glucocorticoids have the potential to regulate major families of phenobarbital-regulated *P*-450s.

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REFERENCES

- Adams, D. J., Seilman, S., Amelizad, Z., Oesch, F. & Wolf, C. R. (1985) *Biochem. J.* **232**, 869–876
- Adesnik, M. & Atchison, M. (1986) *CRC Crit. Rev. Biochem.* **19**, 247–309
- Atchison, M. & Adesnik, M. (1983) *J. Biol. Chem.* **258**, 11285–11295
- Beaune, P. H., Ubenhauer, D. R., Bork, R. V., Lloyd, R. S. & Guengerich, F. P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8064–8068
- Buchmann, A., Kuhlmann, W., Schwarz, M., Kunz, H. W., Wolf, C. R., Moll, E., Friedberg, T. & Oesch, F. (1985) *Carcinogenesis* **6**, 513–521
- Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., Guengerich, F. P. & Mayer, R. T. (1985) *Biochem. Pharmacol.* **34**, 3337–3345
- Cox, R. A. (1968) *Methods Enzymol.* **12**, 120–129
- Durnam, D. M., Perrin, F., Gannon, F. & Palmiter, R. D. C. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6511–6515
- Ernster, L. & Orrenius, S. (1965) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **124**, 1190–1199
- Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **136**, 6–13
- Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* (Addendum) **137**, 266–267
- Friedberg, T., Waxman, D. J., Atchison, M., Kumar, A., Haaparanta, T., Raphael, C. & Adesnik, M. (1986) *Biochemistry* **25**, 7975–7983
- Gonzalez, F. J., Kimura, S. & Nebert, D. W. (1985) *J. Biol. Chem.* **260**, 5040–5047
- Hardwick, J. P., Gonzalez, F. J. & Kasper, C. B. (1984) *J. Biol. Chem.* **258**, 10182–10186
- Hill, R. E., Shaw, P. J., Barth, P. K. & Hastie, N. D. (1985) *Mol. Cell. Biol.* **5**, 2114–2122
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lu, A. Y. H. & West, S. B. (1980) *Pharmacol. Rev.* **31**, 277–295
- Meehan, R. R., Barlow, D. P., Hill, R. E., Hogan, B. L. M. & Hastie, N. D. (1984) *EMBO J.* **3**, 1881–1885
- Meehan, R. R., Gosden, J. R., Rout, D., Hastie, N. D., Freidberg, T., Adesnik, M., Buckland, R., van Heyningen, V., Fletcher, J., Spurr, N. K., Sweeney, J. & Wolf, C. R. (1988) *Am. J. Hum. Gen.* **42**, 26–37
- Miles, J. S., Spurr, N. K., Gough, A. C., Jowett, T., McLaren, A. W., Brook, J. D. & Wolf, C. R. (1988) *Nucleic Acids Res.*, in the press
- Nash, T. (1953) *Biochem. J.* **55**, 416–421
- Nebert, D. W. & Jensen, N. M. (1979) *CRC Crit. Rev. Biochem.* **6**, 401–437
- Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1987) *DNA* **6**, 1–11
- Noshiro, M. & Negishi, M. (1986) *J. Biol. Chem.* **261**, 15923–15927
- Poland, A., Mak, I. & Glover, E. (1981) *Mol. Pharmacol.* **20**, 442–450
- Remmer, H. & Merker, H. J. (1965) *Ann. N.Y. Acad. Sci.* **173**, 79–86
- Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251
- Ryan, D. E., Thomas, P. E., Reik, L. M. & Levin, W. (1982) *Xenobiotica* **12**, 727–744
- Schuetz, E. G. & Guzelian, P. S. (1984) *J. Biol. Chem.* **259**, 2007–2012
- Schuetz, E. G., Wrighton, S. A., Barwick, J. L. & Guzelian, P. S. (1984) *J. Biol. Chem.* **259**, 1999–2006
- Schuetz, E. G., Wrighton, S. A., Safe, S. H. & Guzelian, P. S. (1986) *Biochemistry* **25**, 1124–1133
- Simmons, D. L., Lalley, P. A. & Kasper, C. B. (1985) *J. Biol. Chem.* **260**, 515–521
- Simmons, D. L., McQuiddy, P. & Kasper, C. B. (1987) *J. Biol. Chem.* **262**, 326–332
- Sogawa, K., Gotoh, O., Kawajiri, K., Harada, T. & Fujii-Kuriyama, Y. (1985) *J. Biol. Chem.* **260**, 5026–5032
- Stupans, I., Ikeda, T., Kessler, D. J. & Nebert, D. W. (1984) *DNA* **3**, 129–137
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Waxman, D. J. (1986) in *Cytochrome P-450, Structure, Function, Mechanism and Biochemistry* (Ortiz de Montellano, P. R., ed.), pp. 525–539 Plenum Press, New York and London
- Waxman, D. J. & Walsh, C. (1983) *Biochemistry* **22**, 4846–4855
- Wolf, C. R. (1986) *Trends Genet.* **2**, 209–214
- Wolf, C. R., Moll, E., Friedberg, T., Oesch, F., Buchmann, A., Kuhlmann, W. D. & Kunz, H. W. (1984) *Carcinogenesis* **5**, 993–1001
- Wolf, C. R., Seilman, S., Oesch, F., Mayer, R. T. & Burke, M. D. (1986) *Biochem. J.* **240**, 27–33
- Yamazoe, Y., Shimada, M., Murayama, N. & Kato, R. (1987) *J. Biol. Chem.* **262**, 326–332

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