Functional Heterogeneity of UDP-Glucuronosyltransferase as Indicated by its Differential Development and Inducibility by Glucocorticoids

DEMONSTRATION OF TWO GROUPS WITHIN THE ENZYME'S ACTIVITY TOWARDS TWELVE SUBSTRATES

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1. UDP-glucuronosyltransferase activity towards 12 substrates has been assessed in rat liver during the perinatal period. 2. Between days 16 and 20 of gestation, enzyme activities towards the substrates 2-aminophenol, 2-aminobenzoate, 4-nitrophenol, 1-naphthol, 4-methylumbelliferone and 5-hydroxytryptamine (the 'late foetal' group) surge to reach adult values, while activities towards bilirubin, testosterone, β -oestradiol, morphine, phenolphthalein, and chloramphenicol (the 'neonatal' group) remain negligible or at less than 10% of adult values. 3. By the second postnatal day, enzyme activities towards the neonatal group have attained, or approached adult values. 4. Dexamethasone precociously stimulates in 17-day foetal liver *in utero* transferase activities in the late foetal, but not the neonatal group. A similar inductive pattern is found for 15-day foetal liver in organ culture. 5. It is suggested that foetal glucocorticoids, whose synthesis markedly increases between days 16 and 20 of gestation, are responsible for triggering the simultaneous surge of all the hepatic UDP-glucuronosyltransferase activities in the late foetal group. The neonatal group of activities apparently require a different or additional stimulus for their appearance. 6. The relationship of these two groups of transferase activities to other similar groups observed during induction by xenobiotics and enzyme purification is discussed.

That the diminished capacity of foetal and early neonatal liver to synthesize glucuronides (Karunaratnam *et al.*, 1949) is due partly, or predominantly, to their low hepatic UDP-glucuronosyltransferase (EC 2.4.1.17) activity has been known for some 20 years (see Dutton, 1966a, for references). However, the time of attainment of adult transferase function during the perinatal period in laboratory animals varies depending on substrate (see Dutton, 1971; Lucier & McDaniel, 1977) or species (Dutton, 1966b).

At least some of these variations could have arisen from artefactual or age-dependent activations of the transferase *in vitro* (Dutton & Burchell, 1974; Leakey & Donald, 1976). Recently, overcoming this difficulty by optimally activating the enzyme *in vitro*, we have reported that glucocorticoids precociously stimulate UDP-glucuronosyltransferase activity in rat liver towards 2-aminophenol, suggesting that glucocorticoid hormones trigger the normal appearance of this activity (Wishart *et al.*, 1977a; Wishart & Dutton, 1977a).

The present work compares, during the last foetal and first postnatal weeks, the development of rat liver transferase activity towards 2-aminophenol and

bilirubin. The former activity develops to adult values between days 16 and 20 of gestation, and the latter during the first two postnatal days. Activities towards a further ten substrates were also studied and found to resemble either the activity towards 2-aminophenol or the activity towards bilirubin, both in developmental pattern and in their inducibility by glucocorticoids.

Preliminary accounts of parts of this work have already been published (Wishart *et al.*, 1977b; Wishart & Dutton, 1977b).

Materials and Methods

Rats were Wistar strain, time mated and (where appropriate) injected with dexamethasone (9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione) as described previously (Wishart & Dutton, 1977b). Organ culture was as described for chick-embryo liver (Burchell *et al.*, 1972).

Chemicals were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., or BDH Chemicals, Poole, Dorset, U.K. Morphine sulphate (B.P.) was purchased locally. 1-[1- 14 C]Naphthol, [*N*-methyl- 14 C]morphine hydrochloride, [4- 14 C]-

testosterone (17 β -hydroxyandrost-4-en-3-one) and [4-¹⁴C]oestradiol (oestra-1,3,5(10)-triene-3,17 β -diol) were from The Radiochemical Centre, Amersham, Bucks., U.K.

UDP-glucuronosyltransferase activity towards bilirubin was assayed by the method of Heirwegh *et al.* (1972) and towards 2-aminophenol as before (Wishart *et al.*, 1977b).

Activities towards the following substrates were assayed in the presence of 75 mM-tris(hydroxymethyl)methylamine (pH 7.4), 75 mM-maleic acid, 10 mM-MgCl₂ and 4 mM-UDP-glucuronic acid. The final molarity of each substrate and the source of its assay method were as follows: testosterone and oestradiol (0.80 mM; Rao *et al.*, 1976); 4-nitrophenol (0.17 M; Isselbacher *et al.*, 1962); 5-hydroxytryptamine (1.00 mM; Leakey, 1976); morphine (2.4 mM; Sanchez & Tephly, 1974); phenolphthalein (0.16 mM; see Dutton, 1966a); 4-methylumbelliferone (0.10 mM; Arias, 1962); 2-aminobenzoate (0.50 mM; Zakim & Vessey, 1973); naphthol (0.50 mM; Otani *et al.*, 1976); chloramphenicol (1.5 mM; Bock *et al.*, 1973).

The only significant deviation from the quoted method was that steroids (Rao *et al.*, 1976) were added directly to the assay mixture in ethanol (final concn. 8%, v/v), not in propylene glycol, and the reaction was stopped by addition of the extracting solvent, not by heating to 100°C. Assays were performed under conditions of maximal velocity, except those with 4-nitrophenol, 4-methylumbelliferone and phenolphthalein as substrates: assay of these transferase activities is dependent on substrate disappearance, which was measured at a linear initial rate.

Transferase activity towards each substrate was assayed with a 2% (w/v) whole-liver homogenate in the presence of a series of digitonin concentrations as described previously for 2-aminophenol (Wishart *et al.*, 1977a). Optimum activation of enzyme occurred at 0.08–0.16% (w/v) digitonin for all ages and for all substrates apart from oestradiol and chloramphenicol. Enzyme activity towards these substrates was activated with Lubrol 12 A9 since digitonin inhibited the former activity (presumably by precipitation of a digitonin–oestradiol complex) and interfered with the diazotization of chloramphenicol glucuronide. Optimum activation of these activities required 0.2–0.4 mg/ml of Lubrol.

Assays involving radioactive substrates, i.e. testosterone, oestradiol, 1-naphthol and morphine, were performed with 100 000 c.p.m. of ¹⁴C in an incubation volume of 250, 250, 125 and 125 μ l respectively. To 125 μ l of the final aqueous preparation, 4 ml of Aquasol (New England Nuclear Corp., Boston, MA, U.S.A.) was added before counting for radioactivity for 1–10 min in a Nuclear–Chicago Unilux 11 liquid-scintillation counter. Values of 1000–10 000 d.p.m. were counted against a background of 30–50 d.p.m.

Counting efficiency was 50–60%, measured from channel ratios.

Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Optimally activated transferase activities are expressed as means $\pm$ S.E.M. to 95% confidence limits.

Results

Normal perinatal development of UDP-glucuronosyltransferase activity towards twelve different substrates

As examples of two UDP-glucuronosyltransferase activities known to show different patterns of development in laboratory animals (Dutton & Burchell, 1974), activities towards bilirubin and 2-aminophenol were assayed during the last foetal and first postnatal week (Fig. 1). The main surge of these activities from low to near-adult values clearly occurs at different times. Transferase activity towards 2-aminophenol shows a surge of activity from virtually zero to 200% of adult values between days 17 and 21 of gestation, at which time activity toward bilirubin remains negligible. During the first two postnatal days, although transferase activity towards 2-aminophenol remains high and unchanged, activity towards bilirubin increases markedly from low to near adult values.

The development of activity towards a further ten substrates was then studied. Four selected developmental ages were examined (Table 1) to determine whether the surge of transferase activity for a particular substrate occurred during the late foetal period, resembling that towards 2-aminophenol, or during the early neonatal period, resembling that towards bilirubin (see Fig. 1). From evidence presented in Table 1, transferase activities towards the substrates 2-aminophenol, 2-aminobenzoate, 4-nitrophenol, 1-naphthol, 4-methylumbelliferone and 5-hydroxytryptamine all show a significant surge of activity between days 16 and 20 of gestation and are thus designated the 'late foetal' group of activities. During the same gestational period, transferase activities towards bilirubin, testosterone, β -oestradiol, morphine, phenolphthalein and chloramphenicol remain relatively unchanged, but show their significant surge of activity between day 20 of foetal age and day 2 of neonatal age. They are therefore designated the 'neonatal' group of activities. Although in 20-day foetal livers some activities for substrates in the neonatal group are reported, these are negligible or within the background interference of the assay. In contrast, the activities described as the late-foetal group exhibit values, at this age, within the adult range.

Inducibility of transferase by dexamethasone in utero

During days 17–20 of gestation there is an increase in foetal adrenal activity and, as a result, in foetal

Fig. 1. Normal development of UDP-glucuronosyltransferase activity towards (a) 2-aminophenol and (b) bilirubin in foetal and neonatal rat liver

Animals were time-mated. The first 24 h after conception was deemed 'day 0', the next 24 h, 'day 1' and so on for foetal-age determinations. Neonatal age was similarly determined postnatally. Foetal samples were pooled livers from at least two dams. Postnatal samples were from single animals from at least two litters. Each result shows the mean $\pm$ S.E.M. of the indicated number of pooled foetal or postnatal rat liver samples.

plasma glucocorticoid concentrations (see Wishart & Dutton, 1977a, for references). Because these glucocorticoid increases occur concurrently with the surge of hepatic transferase activity towards 2-aminophenol, and because they can precociously stimulate transferase activity towards 2-aminophenol, they have been considered the trigger for the normal appearance of this activity (Wishart & Dutton, 1977a). By similar reasoning, transferase activities

which develop concurrently with that towards 2-aminophenol may also be triggered by glucocorticoid hormones, although the activities which develop later, like that towards bilirubin, may require a different trigger.

After maternal injection of the synthetic glucocorticoid dexamethasone, which will pass into the foetus (Zarrow *et al.*, 1970), 17-day foetal liver transferase activities in the late foetal group are precociously stimulated severalfold, whereas the activities in the neonatal group remain negligible (Table 2).

Inducibility by dexamethasone in culture

As well as being precociously induced by dexamethasone *in utero*, UDP-glucuronosyltransferase activity towards 2-aminophenol is similarly induced in 15-day foetal liver in organ culture, showing that the glucocorticoid affects the transferase at the hepatic level (Wishart *et al.*, 1977a). When transferase activity towards a selection of the 12 substrates was assayed in foetal liver, cultured in the presence and absence of dexamethasone, it was found that activities in the late foetal group (towards 2-aminophenol, 2-aminobenzoate, 4-nitrophenol, 1-naphthol and 5-hydroxytryptamine) were stimulated severalfold by dexamethasone, whereas activities in the neonatal group (towards bilirubin, testosterone, oestradiol and morphine) remained unaffected (Table 3).

Discussion

The results presented above clearly demonstrate the functional heterogeneity of UDP-glucuronosyltransferase in rat liver. For the 12 substrates studied, transferase activities may be divided into two developmental groups: one surging to adult activities between days 16 and 20 of gestation; the other surging to adult or near-adult values between day 20 of foetal and day 2 of postnatal life. Substrates associated with the former 'late foetal' group of activities are 2-aminophenol, 2-aminobenzoate, 4-nitrophenol, 1-naphthol, 4-methylumbelliferone and 5-hydroxytryptamine. Those associated with the latter 'neonatal' group are bilirubin, chloramphenicol, morphine, oestradiol, phenolphthalein and testosterone.

Further, the synthetic glucocorticoid dexamethasone elicits, in foetal liver *in utero*, a precocious surge of transferase activity towards all those substrates in the late foetal group, whereas activities in the neonatal group remain unaffected. A similar pattern of response to dexamethasone is found in foetal liver in culture.

We therefore propose that the increased foetal glucocorticoid activity, which occurs between days 17 and 20 of gestation (see Wishart & Dutton, 1977a, for references), triggers the normal surge to adult

Table 1. *Perinatal development of UDP-glucuronosyltransferase activities towards 12 substrates*

Determination of gestational age, sampling and statistical representation are as for Fig. 1. Assays were performed as described in the Materials and Methods section.

Substrate	UDP-glucuronosyltransferase activity (nmol of glucuronide formed/h per mg of protein)				20-Day foetal liver activity (% of adult male activity)
	16-Day foetus	20-Day foetus	2-Day neonate	Adult male	
2-Aminophenol	1.1 ± 0.7 (3)	53.4 ± 7.2 (6)	60.6 ± 7.3 (3)	37.2 ± 2.0 (3)	144
2-Aminobenzoate	0.8 ± 0.1 (3)	11.2 ± 0.8 (3)	14.1 ± 0.3 (3)	10.9 ± 0.7 (3)	103
4-Nitrophenol	52.2 ± 3.0 (4)	235 ± 52 (6)	364 ± 20 (3)	208 ± 19 (3)	113
1-Naphthol	13.0 ± 2.1 (3)	127 ± 11 (4)	187 ± 9.0 (3)	132 ± 7.0 (3)	96
4-Methylumbelliferone	42 ± 14 (4)	252 ± 38 (4)	288 ± 12 (4)	254 ± 14 (3)	99
5-Hydroxytryptamine	18, 22	115 ± 14 (3)	151 ± 30 (3)	131 ± 18 (4)	88
Bilirubin	0.4 ± 0.3 (4)	0.8 ± 0.3 (3)	8.9 ± 1.0 (3)	11.8 ± 0.6 (4)	7
Testosterone	0.8 ± 0.3 (4)	0.9 ± 0.3 (3)	12.0 ± 0.7 (3)	9.3 ± 1.0 (3)	10
Oestradiol	2.2 ± 0.3 (3)	1.5 ± 0.4 (5)	9.1 ± 0.4 (3)	13.7 ± 1.5 (3)	11
Morphine	0 (4)	21 ± 14 (3)	183 ± 25 (3)	132 ± 7 (3)	16
Phenolphthalein	5.7 ± 2.8 (4)	10.7 ± 3.1 (5)	108 ± 90 (4)	120 ± 15 (3)	9
Chloramphenicol	3.0 ± 1.0 (3)	4.0 ± 1.0 (3)	26.5 ± 2.0 (3)	22.0 ± 3.0 (3)	18

Table 2. *Effect of dexamethasone on 17-day rat foetal UDP-glucuronosyltransferase activity towards 12 substrates in utero*

Pregnant rats were injected intraperitoneally with 300 µg of dexamethasone in 0.1 ml of arachis oil on day 14, 15 and 16 of gestation. Control rats were injected similarly with arachis oil only. Animals were killed on day 17. Transferase activities, expressed as the mean ± S.E.M., are from a number of samples, that number being shown in parenthesis. Each sample comprises a pool of foetal livers, from at least two mothers.

Substrate	UDP-glucuronosyltransferase activity (nmol of glucuronide formed/h per mg of homogenate protein)	
	Control	+ Dexamethasone
2-Aminophenol	2.6 ± 0.1 (3)	32.7 ± 2.0 (4)
2-Aminobenzoate	0.9 ± 0.2 (3)	7.0 ± 0.4 (3)
4-Nitrophenol	73.0 ± 6 (4)	231 ± 20 (3)
1-Naphthol	27.0 ± 4.6 (8)	120 ± 13 (10)
4-Methylumbelliferone	25.0 ± 7.0 (3)	195 ± 3 (4)
5-Hydroxytryptamine	15.4 ± 3.0 (7)	72.7 ± 4.1 (8)
Bilirubin	0.9 ± 0.1 (3)	0.7 ± 0.2 (3)
Testosterone	0.5 ± 0.1 (5)	0.3 ± 0.1 (6)
Oestradiol	1.9 ± 0.3 (3)	2.4 ± 0.2 (3)
Morphine	0.3 ± 0.3 (3)	4.4 ± 3.2 (3)
Phenolphthalein	10.8 ± 6.7 (4)	14.8 ± 6.0 (4)
Chloramphenicol	3.5 ± 1.0 (4)	4.0 ± 1.0 (4)

values of all those transferase activities described above as the late foetal group.

More recent work (Campbell & Wishart, 1978) indicates that transferase activities towards morphine

Table 3. *Effect of dexamethasone on the development of UDP-glucuronosyltransferase activity towards eight substrates in 15-day foetal liver organ cultures*

Livers were removed from foetuses at 15 days of gestation and maintained in organ culture (see the Methods section) for 4 days in the presence of 2 mM-dexamethasone acetate. Control cultures contained no dexamethasone. Each result is a mean ± S.E.M. of a number of samples, shown in parenthesis. Each sample contains liver from at least four separate culture dishes.

Substrate	UDP-glucuronosyltransferase activity (nmol of glucuronide formed/h per mg of homogenate protein)	
	Control cultures	+ 2 µM-dexamethasone
2-Aminophenol	2.0 ± 0.5 (4)	34.5 ± 2.6 (4)
2-Aminobenzoate	0.7 ± 0.4 (4)	4.2 ± 0.5 (4)
4-Nitrophenol	151 ± 31 (4)	323 ± 69 (4)
1-Naphthol	44, 28	113, 169
5-Hydroxytryptamine	18.6 ± 0.8 (3)	56.8 ± 0.8 (3)
Bilirubin	0, 0.6	0.3, 0.1
Testosterone	0.5 ± 0.1 (4)	0.1 ± 0.1 (4)
Oestradiol	0.1, 0.9	1.1, 0.1
Morphine	5.2 ± 2.0 (3)	3.0 ± 1.6 (3)

and testosterone, shown in the present paper to develop between foetal day 20 and neonatal day 2, develop most markedly between birth and day 2, i.e. like that towards bilirubin. Activities towards morphine, testosterone and bilirubin may therefore share the same trigger; this trigger remains unidentified, although it is birth-related and may involve glucocorticoids (Campbell & Wishart, 1978).

Our division of transferase activities into these two

developmental groups is consistent with evidence from elsewhere. Lucier & McDaniel (1977) found that rat liver transferase activities towards 4-nitrophenol, 4-methylumbelliferone and 1-naphthol rose to higher-than-adult 'peak' values immediately after birth, while activities towards diethylstilboestrol, oestradiol, oestrone, phenolphthalein and testosterone rose postnatally; they interpreted their findings as indicative of a 'steroidal' and 'non-steroidal' group, which would appear a simplification. The results of Fuchs *et al.* (1977) also show that transferase activities towards bilirubin and testosterone develop after birth.

Development of glucuronidation in two distinct stages may have important consequences for the perinatal animal. The late foetus and early neonate should be capable of glucuronidating substrates in the first, but not the second group. This would occur not only in rat, but also in rabbit, mouse and guinea pig, where we have also found evidence of the two groups (G. J. Wishart, M. T. Campbell & A. M. Donald, unpublished work). In man, the groupings are not yet clear but the known neonatal toxicity of chloramphenicol and bilirubin, both glucuronidated by the later-developing group of activities, is noteworthy.

Our developmental groups of transferase activities also appear to coincide with those groups induced preferentially by certain xenobiotics. Thus Bock *et al.* (1973, 1977) find that activities towards morphine, bilirubin and chloramphenicol (in our neonatal group) are preferentially stimulated in adult rats by phenobarbital, whereas those towards 4-nitrophenol and 1-naphthol (in our late-foetal group) are preferentially stimulated by 3-methylcholanthrene. We have found (Wishart & Dutton, 1977b; Wishart, 1978) that in adult rats 3-methylcholanthrene stimulates selectively all six activities in the late foetal group, whereas phenobarbital preferentially stimulates those in the neonatal group. Again, according to Lucier & McDaniel (1977), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin stimulates in adult rat liver only those transferase activities in their non-steroid (i.e. our late foetal) group.

Two distinct groups of transferase activities therefore appear to exist. They may reflect two or more enzyme proteins, or a single enzyme protein whose specificity depends on a changing lipid environment. del Villar *et al.* (1975) claim to have separated two fractions from rat liver which respectively glucuronidate 4-nitrophenol and morphine, and Bock *et al.* (1977) have separated, also from rat liver, protein with transferase activity towards morphine from protein with activity towards both morphine and 1-naphthol. In each case, the proposed physical heterogeneity of the partly purified transferase activity is consistent with our division of functional heterogeneity.

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