

Postnatal Development of Uridine Diphosphate Glucuronyltransferase Activity towards Bilirubin and 2-Aminophenol in Human Liver

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UDP-glucuronyltransferase activities towards 2-aminophenol and bilirubin were studied in a total of 70 human subjects, including premature and full-term newborn babies, infants, children and adults. These two activities have been reported in rat to develop late-foetally and neonatally respectively, but in man they both develop neonatally. There is a linear relationship between the logarithm of each liver transferase activity and the logarithm of the number of days after birth during the first 3 months of life, after which each activity remains constant.

Activities of UDP-glucuronyltransferase (EC 2.4.1.17) in rat liver can be divided into two groups, judged from the developmental pattern in rat liver (Wishart *et al.*, 1978; Lucier & McDaniel, 1977; Wishart & Campbell, 1979), perinatal glucocorticoid-inducibility (Wishart *et al.*, 1978; Wishart, 1978a) and preferential inducibility by xenobiotics (Bock *et al.*, 1973; Lucier & McDaniel, 1977; Wishart, 1978b). However, studies on UDP-glucuronyltransferase in foetal or neonatal human liver have been few and have not investigated the possibility of developmental groups. The present paper describes a more extensive developmental study of the enzyme in human liver; it reports the activities in the same specimen towards 2-aminophenol and bilirubin, representative activities in rat liver of group 1 ('late-foetal') and group 2 ('neonatal') (Wishart *et al.*, 1978) respectively.

Materials and Methods

Patients

The patients included in this study are shown in Table 1. Excluded are patients with Crigler-Najjar syndrome and those who received inducers such as phenobarbital. Liver samples used were from patients who underwent upper-abdominal laparotomy and were biopsied for histological examination or from autopsied cases. Informed consent was obtained in all cases, including autopsies. The tissues for enzyme analysis were stored at -70°C until analysis. Such storage was previously found not to affect significantly the results for bilirubin UDP-glucuronyltransferase activity (Black *et al.*, 1970). A 3-month-old female infant with Crigler-Najjar syndrome was studied who had shown severe jaundice since birth.

Table 1. *Patients studied and sources of liver specimens assayed*

Measurements of UDP-glucuronyltransferase activity towards both substrates were performed on the same liver specimen, but, where insufficient amount of liver specimen was available, measurement of activity towards bilirubin was carried out as a rule except for two cases (*).

Stage of development	For bilirubin UDP-glucuronyltransferase			For 2-aminophenol UDP-glucuronyltransferase		
	No. of patients	Source of specimen		No. of patients	Source of specimen	
		Laparotomy	Autopsy		Laparotomy	Autopsy
Newborn	30	3	27	17	0	17
Full-term	10	3	7	9	0	9*
Premature	20	0	20	8	0	8
Infants	21	7	14	7	1	6
Children	5	1	4	3	0	3
Adults	14	14	0	6	6	0
Totals	70	25	45	33	7	26

At 5 months of age, total serum bilirubin was 30mg/100ml, with 0.5 mg of direct-reacting bilirubin/100ml. Hyperbilirubinaemia was unaffected by phenobarbital administration.

Chemicals

Chemicals were obtained from commercial sources and were of the highest purity available.

Assay of bilirubin UDP-glucuronyltransferase activity

This activity of 10% (w/v) liver homogenates in 250mM-sucrose/1mM-EDTA medium, pH7.4, with activation by 0.4% digitonin was determined by the method of Black *et al.* (1970). Determination of low UDP-glucuronyltransferase activity was performed by high-pressure liquid chromatography (Onishi *et al.*, 1980). The column used was a Shimadzu PCH apparatus (25cm × 4mm). The variable-wavelength detector was set at 455nm. A 150 μ l volume of acetonitrile was added to 250 μ l of the sample, which was vortex-mixed for 30s and then centrifuged at 4500g for 5min. The chromatograph was injected with 100 μ l of the supernatant. Complete separation of bilirubin and its mono- (endo-vinyl and exo-vinyl isomers) and di-glucuronides was achieved by using a linear gradient of acetonitrile [20–48% (v/v) in 70min and then 48–60% (v/v) in 3min] in 0.1M-acetate buffer, pH4.0, containing 5mM-sodium pentanesulphonate (Waters Associates, Milford, MA, U.S.A.) to supply the counter-ion.

Assay of 2-aminophenol UDP-glucuronyltransferase activity

This assay with activation by 0.2% digitonin on the same liver specimen was performed by the method of Winsnes (1969).

Results

Development of human hepatic UDP-glucuronyltransferase activities

The developmental change of hepatic UDP-glucuronyltransferase activity towards bilirubin, a group-2 substrate in the rat, is shown in Fig. 1, where the activity is less than 1% of adult values during the foetal and early neonatal period and then begins to increase at an exponential rate until it reaches the adult value by 3 months of age, after which it remains constant.

Thus it was demonstrated that there is a linear relationship between the logarithm of liver UDP-glucuronyltransferase activity and the logarithm of the number of days after birth during the first 3 months of life.

As shown in Fig. 2, the developmental change of UDP-glucuronyltransferase activity with 2-aminophenol, a group-1 substrate in the rat, showed a

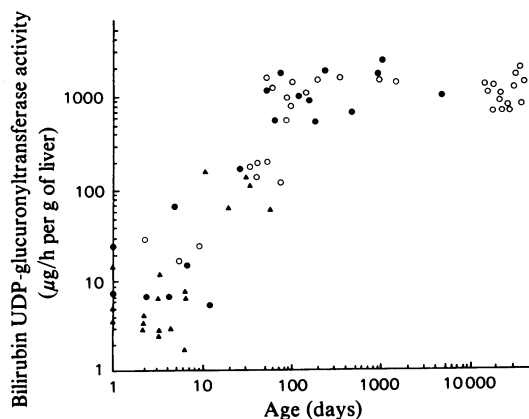


Fig. 1. Developmental change of human hepatic UDP-glucuronyltransferase activity towards bilirubin

Experimental details are given in the text. Both the ordinate and the abscissa are plotted as logarithmic scales. ●, Autopsied cases; ○, upper abdominal laparotomy; ▲, premature newborn babies.

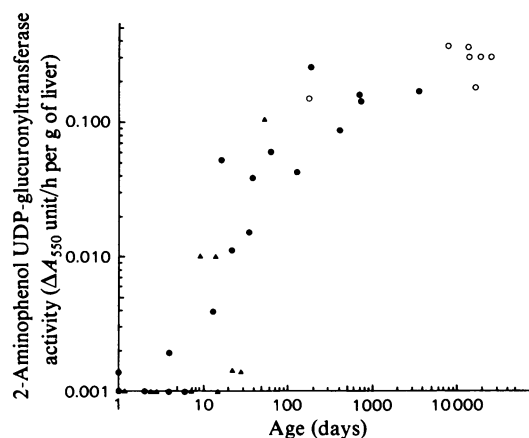


Fig. 2. Developmental change of human hepatic UDP-glucuronyltransferase activity towards 2-aminophenol

Experimental details are given in the text. Both the ordinate and the abscissa are plotted as logarithmic scales. ●, Autopsied cases; ○, upper abdominal laparotomy; ▲, premature newborn babies.

pattern quite similar to that of the activity towards bilirubin, the change being markedly different from results in the rat (Wishart, 1978a; Wishart *et al.*, 1978).

Hepatic UDP-glucuronyltransferase activities in Crigler-Najjar syndrome

In our case of Crigler-Najjar syndrome type 1 (first Japanese case), hepatic UDP-glucuronyltransferase activity was not detected by the high-

pressure-liquid-chromatography method when bilirubin was utilized as the substrate. However, hepatic UDP-glucuronyltransferase activity towards 2-aminophenol showed the same value as the control.

Discussion

Extensive investigation of the development of UDP-glucuronyltransferase activity has been performed, mainly in small laboratory animals, which show no jaundice in the neonatal period (Dutton, 1966; Dutton & Burchell, 1977). However, studies on human neonatal subjects have not been undertaken systematically. The main reasons for this may be as follows: (1) it has been widely assumed that human hepatic UDP-glucuronyltransferase began to be inactivated immediately after death; (2) since UDP-glucuronyltransferase is tightly bound to the microsomal membrane, the enzyme activity is easily affected by the storage conditions, presence of detergents, nucleotides, phospholipids and sonication conditions (Dutton & Burchell, 1977); (3) accurate measurement of the reaction products of bilirubin glucuronidation is difficult. With regard to problem (1), we observed that the enzyme activity remains almost unchanged (within 10%) until up to 12h after death. Moreover, with regard to problem (2), it was also confirmed that change of the activity in homogenate or microsomal fraction can be minimized by adding a detergent such as digitonin to the reaction mixture (Heirwegh *et al.*, 1973, 1974; Dutton & Burchell, 1977). Problem (3) was largely resolved by Heirwegh *et al.* (1973, 1974). However, they could not analyse further details in the case with very low UDP-glucuronyltransferase activity and low concentration of reaction products [e.g., liver enzyme during perinatal development, Crigler-Najjar syndrome, some cases of Gilbert syndrome (Fevry *et al.*, 1977)]. However, we have developed an excellent method involving the use of high-pressure liquid chromatography for the measurement of very low UDP-glucuronyltransferase activity towards bilirubin (Onishi *et al.*, 1980).

Our results indicate clearly that UDP-glucuronyltransferase activities towards both 2-aminophenol and bilirubin are low in human neonatal liver, and that a linear logarithmic relationship (Miller & Weil, 1963) exists between the developing transferase activities and the number of days after birth during the first 3 months of life; after this period the enzyme activities have reached adult values. These results also indicate that, if developmental groups of UDP-glucuronyltransferase exist in man as they do in rat, then the members of the groups are different; for 2-aminophenol and bilirubin, respectively members of the 'late-foetal' and 'neonatal' groups in rat

(Wishart *et al.*, 1978), both activities develop neonatally in man.

The Gunn rat is often recommended as a model for the Crigler-Najjar child (see, e.g., Cornelius & Arias, 1972), but our results suggest, contrary to earlier work (Arias *et al.*, 1969), that the Crigler-Najjar liver, unlike Gunn-rat liver, contains normal UDP-glucuronyltransferase activity towards 2-aminophenol. This, together with our developmental results, indicates that human, rather than animal, material should be used to evaluate human pathophysiological conditions.

It is of particular importance pharmacologically that the extensive study reported here clearly confirms that UDP-glucuronyltransferase activities can be quite low neonatally and increase gradually to adult values.

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