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The prenatal and postnatal development of UDP-glucuronyltransferase activity towards bilirubin and the effect of premature birth on this activity in the human liver

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Liver UDP-glucuronyltransferase activity towards bilirubin was studied in a total of 88 human subjects, including foetuses, premature and full-term newborn babies, infants, children and adults. Determination of very low enzyme activity was performed by high-pressure liquid chromatography. Prenatal and postnatal changes of the activity can be divided into four developmental phases, i.e. middle foetal, late foetal, neonatal and early infantile, and mature. The activities of the first three phases corresponded to about 0.1, 0.1–1 and 1–100%, respectively, of the mature-phase values (mean ± S.D.: 1320 ± 514 μg/h per g of liver, $n = 27$).

Hepatic UDP-glucuronyltransferase (EC 2.4.1.17) is important because it is the rate-limiting enzyme in the excretion of bilirubin and various drugs in the neonatal period (Gartner *et al.*, 1977). The development of UDP-glucuronyltransferase activity has been extensively investigated, mainly in small laboratory animals (Dutton, 1966; Dutton & Burchell, 1977). The development of the transferase activity in rat liver from negligible to adult values towards any of the various substrates occurs in either of two distinct stages, one during the late-foetal period (group 1) and the other during the early-neonatal period (group 2) (Wishart *et al.*, 1978). These two groups of transferase activities may also be differentiated under a variety of experimental conditions. However, in human subjects it is believed that these two activities develop postnatally when either bilirubin or 2-aminophenol is used as substrate (Onishi *et al.*, 1979). Detailed results on the perinatal development of human hepatic UDP-glucuronyltransferase, especially towards bilirubin and its mechanism, have not been reported previously, since precise measurement of very low bilirubin glucuronide concentrations has not been possible before introduction of h.p.l.c. The present paper describes a sensitive and specific assay for UDP-glucuronyltransferase activity towards bilirubin and discusses the developmental changes of the enzyme activities in human foetal and neonatal liver and the relationship of the activities in the period after birth, i.e. the birth-dependent development.

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

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Materials and methods

Patients

The livers of only intact normal-appearing foetuses obtained from cases of elective abortion were selected for the study. In addition, the livers were obtained from premature and full-term infants who lived for less than 28 days of life.

Results for patients over 1 month old were identical with cases described previously (Onishi *et al.*, 1979). Patients who received phenobarbital are excluded, since this induces the enzyme. Autopsies were performed no later than 12h after death, during which cadavers were stored in a cold-room at 4°C. But foetuses were stored in −20°C until autopsy. It was confirmed previously that the enzyme activity remains almost unchanged (within 10%) until up to 12h after death (Onishi *et al.*, 1979). Informed consent was obtained in all cases. Tissues 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).

Assays of bilirubin UDP-glucuronyltransferase activity of the foetal and neonatal liver

The procedure involves the combination of the method described by Black *et al.* (1970) and the

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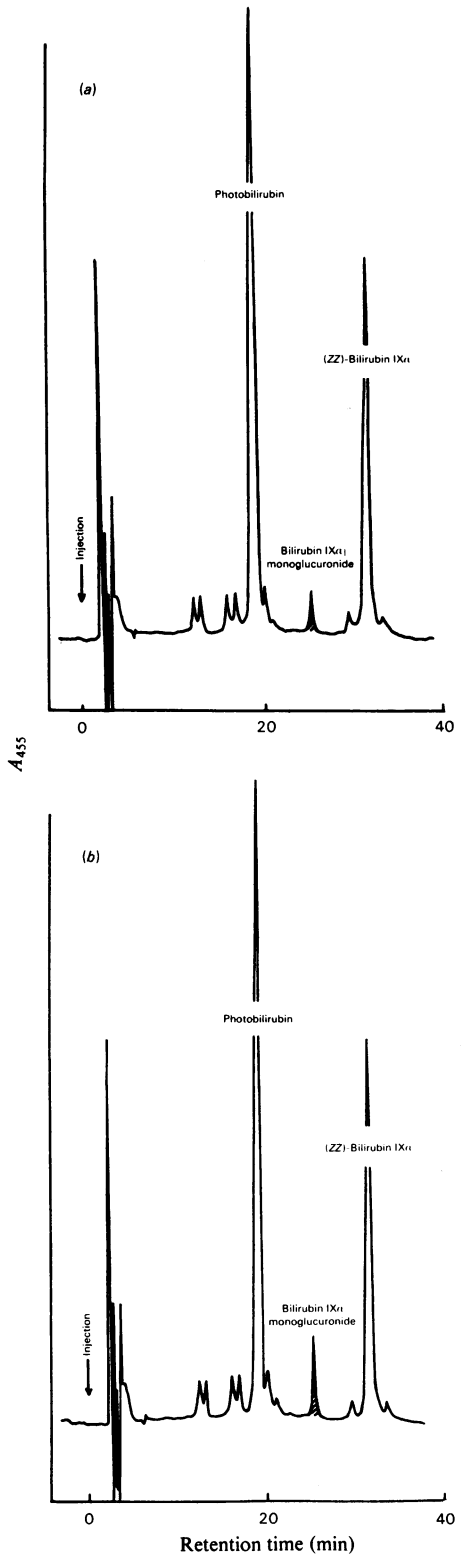

Fig. 1. *H.p.l.c. of the supernatants obtained from the incubation mixtures*

Experimental details are given in the text. (*a*) shows the scan for the control sample, and (*b*) shows the scan for the test sample. The difference in the two peak areas for bilirubin glucuronide between control and test samples represents the rate of glucuronidation during incubation.

h.p.l.c. method described by Onishi *et al.* (1980). Homogenates of 20mg of liver/100μl suspension were prepared in ice-cold 0.25M-sucrose containing 1mM-disodium EDTA. Bilirubin UDP-glucuronyltransferase activities were assayed in digitonin-activated homogenates in the presence of $MgCl_2$ and UDP-glucuronic acid as the sugar donor. An incubation mixture of the same composition, except for the omission of UDP-glucuronic acid and $MgCl_2$, served as control. We have developed a new bilirubin UDP-glucuronyltransferase assay procedure that permits measurement of very low activity. The electronically integrated counts ($\mu V \cdot s$) of peak area of bilirubin glucuronide separated by h.p.l.c. were measured by a Chromatopac R1A instrument and the differences in the integrated counts for test and control samples were calculated (Figs. 1*a* and 1*b*). This procedure is certainly necessary to detect low glucuronidation after incubation of poorly active preparations, since chromatographic analysis of the control samples shows the small amount of bilirubin glucuronide present (Fig. 1*a*). The concentration of bilirubin glucuronide in the Figure is expressed as μg by using the conversion factor previously determined by the h.p.l.c. system, i.e. $4.2 \times 10^5 \mu V \cdot s$ (i.e. integrated counts) are equivalent to 1.0μg of bilirubin. The enzyme activities of each patient were plotted against either gestational weeks for foetuses and premature infants or weeks after birth for infants and patients over the neonatal period.

Sample preparation of the incubation mixtures for h.p.l.c.

To remove the remaining bilirubin in the sample, 1vol. of incubation mixture was vortex-mixed with 9vol. of chloroform for 15min and then centrifuged at 1000rev./min for 10min. Since 27% of bilirubin glucuronide in the sample was lost by the extraction, a correction was made. Supernatant (1vol.) was also vortex-mixed with 1vol. of the eluent, which consisted of 60% acetonitrile in 0.01M-phosphate buffer (pH7.4) containing 0.1% tetra-n-butylammonium hydroxide (Wako, Osaka, Japan), and then centrifuged at 1000rev./min for 5min.

The amount of bilirubin glucuronide in the supernatant aqueous phase (100–200μl) was analysed by the h.p.l.c. method described by Onishi *et al.* (1980).

Results and discussion

The developmental changes of hepatic UDP-glucuronyltransferase activity towards bilirubin during the perinatal period are shown in Fig. 2.

In foetal liver from 17 to 30 weeks of gestation, the presence of some UDP-glucuronyltransferase activities towards bilirubin, which correspond to about 0.1% of adult values, was demonstrated by the h.p.l.c. method, and bilirubin glucuronide was detected in the control samples of foetal liver, proving that glucuronyltransferase is indeed active *in vivo*.

No increase in the activity was observed until 30 weeks of gestation. However, between 30 and 40 weeks of gestation, the activities in foetuses, and premature and full-term infants who survived less than 7 days of life, gradually but significantly increased from 0.1% to 1.0% of the values found in the adult liver. After birth, the activities began to increase at an exponential rate and reached values similar to those found in adult liver by 14 weeks of age, after which the activity remained constant. Thus it was demonstrated that the prenatal and postnatal development of the hepatic UDP-glucuronyltransferase activities towards bilirubin could be divided into four immature phases (i.e. middle foetal, late foetal, neonatal and early infant) and the mature phase.

The effects of birth on perinatal development of hepatic UDP-glucuronyltransferase activity towards bilirubin in premature infants is shown in Fig. 3. It was proved that marked postnatal development of the transferase activities in premature infants who survived for 8–28 days of life occurs irrespective of gestational age, in contrast with the slow development *in utero*. The values were equivalent to those for full-term infants who lived for 8–28 days of age. Thus we found that, in liver, the activities towards bilirubin showed slow maturation *in utero* during late normal gestation and a marked increase after normal or premature birth. This indicates that birth-related, rather than age-related, factors are of

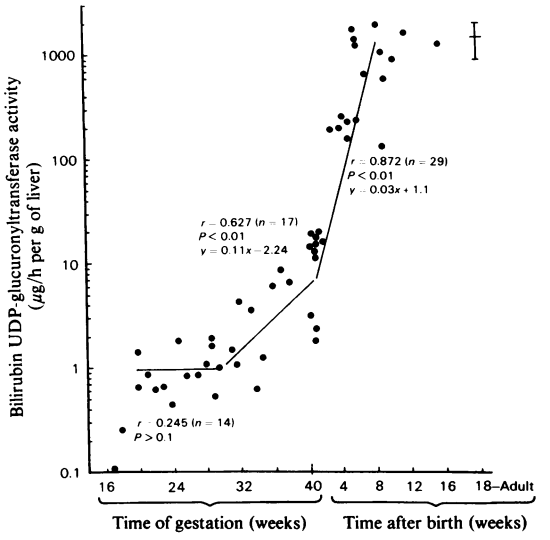

Fig. 2. *Developmental pattern of hepatic UDP-glucuronyltransferase activities towards bilirubin in the human*

The enzyme activities were plotted against weeks of gestation and weeks after birth on a semi-logarithmic scale. The line of best fit was determined by computer, by the method of least squares. y = enzyme activities, x = age in days of gestation or days after birth. Each point represents the activity of the liver homogenate of a single patient, but the results of the cases over 18 weeks after birth are means ± S.D. (1320 ± 514 μg/h per g of liver; $n = 27$).

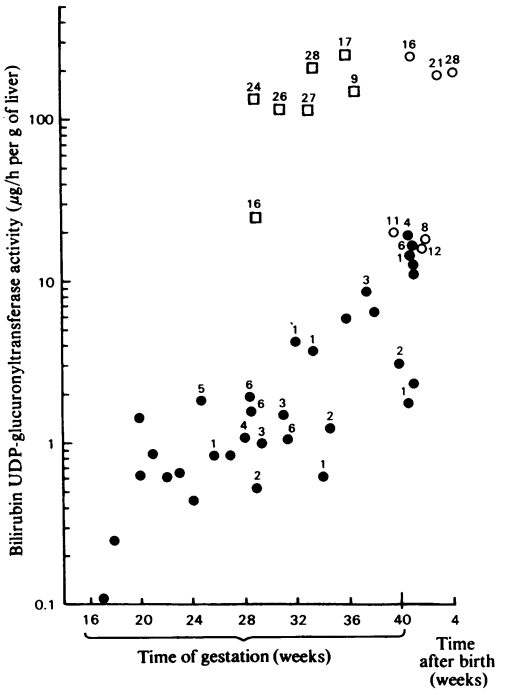

Fig. 3. *Effect of premature birth on the development of hepatic UDP-glucuronyltransferase activities towards bilirubin*

Premature delivery, irrespective of gestational weeks, evoked a precocious increase in transferase activities, equal in rate to the normal postnatal increase. The numbers shown beside the symbols represent the age (days) at which death occurred. Transferase activities are also shown for premature (□) and full-term infants (○) who lived over 8 days after birth. ●, Transferase activities for foetuses, and premature and full-term infants who died within 7 days of birth.

importance in the postnatal development of human liver UDP-glucuronyltransferase activity towards bilirubin. Up to now, it has been generally assumed that human neonatal icterus is a result of slow conjugation of bilirubin, owing to decreased activity of bilirubin UDP-glucuronyltransferase in the neonatal liver. Detailed information on this problem has hitherto not been available. Premature or delayed delivery of rats indicated that rapid onset of liver transferase activity towards bilirubin depends on birth, not age (Campbell & Wishart, 1980). This rapid onset is superimposed on a slow development *in utero* during the last stages of gestation (Campbell & Wishart, 1980). More limited data were obtained with rhesus monkey (Gartner *et al.*, 1977), suggesting a similar effect there. Our results indicate that these facts are also applicable to the bilirubin UDP-glucuronyltransferase activity of human perinatal liver.

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