The Effect of Premature and Delayed Birth on the Development of UDP-glucuronosyltransferase Activities towards Bilirubin, Morphine and Testosterone in the Rat*

Mary T. CAMPBELL† and Graham J. WISHART‡

Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

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In the rat, UDP-glucuronosyltransferase activities towards bilirubin, morphine and testosterone increase markedly after normal or premature birth. This rapid development is superimposed upon a much slower maturation of activity which occurs *in utero* during the last 2 days of normal gestation and gestation when birth is delayed. Development of all three activities is similar under these different conditions, suggesting a common developmental regulatory mechanism.

Adult activities of UDP-glucuronosyltransferase (EC 2.4.1.17), the rate-limiting enzyme of glucuronidation, are achieved perinatally. Since a delay in this maturation can render the newborn susceptible to the adverse effects of lipophiles normally detoxicated by glucuronidation, e.g. neonatal jaundice from excess unconjugated bilirubin (see Yaffe & Stern, 1976), its mechanism and timing are of considerable interest.

We have shown that the rat possesses two groups of hepatic transferase activities which differ in their substrate specificities: one group develops to adult values prenatally, whereas the other reaches them between day 20 of gestation and the second postnatal day (Wishart, 1978). Thus the newborn rat is most likely to suffer from adverse effects of substrates glucuronidated by the latter 'neonatal' group of transferase activities.

In the present work we have therefore considered in detail the perinatal development of transferase activities towards three substrates of this group, i.e. bilirubin, testosterone and morphine. We find that, in liver, activities towards all three substrates develop similarly: they show a marked surge after normal or premature birth, but a slower maturation occurs *in utero* during normal gestation or when birth is delayed by maternal progesterone treatment.

* Reprint requests should be addressed to: Professor G. J. Dutton, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

† Present address: Department of Biochemistry, University of Sydney, Sydney, N.S.W. 2154, Australia.

‡ Present address: Department of Reproductive Physiology, Agricultural Research Council's Poultry Research Centre, West Mains Road, Edinburgh EH9 3JS, Scotland, U.K.

Materials and Methods

Rats were Wistar strain, time-mated as required. Full-term foetuses were delivered by the dam and remained with her until required. Neonatal age was accurate to within 1 h of birth. Premature foetuses were delivered by Caesarean section of a dam previously killed by cervical dislocation, and the newborns were placed in a humid environment at 37°C until introduction to a foster mother at about 4 h after delivery.

Birth was delayed by maternal intraperitoneal injection of 5 mg of progesterone in arachis oil daily from day 20 of gestation. Controls were injected only with arachis oil. Subcutaneous injection of foetuses was performed through the uterine wall, exposed by laparotomy under ether anaesthesia.

Assay of maximally activated UDP-glucuronosyltransferase activities towards bilirubin, testosterone and morphine was performed on liver homogenates as described by Wishart (1978). Results are means $\pm$ S.E.M. of a given number of observations.

Results

Perinatal development of UDP-glucuronosyltransferase activities

Between days 20 and 22 of gestation, foetal hepatic UDP-glucuronosyltransferase activities towards bilirubin, testosterone and morphine increased from 2, 4 and 8% respectively to 12, 17 and 24% of the 2-day neonatal values. These activities surged within 8–12 h of birth on day 22 of gestation, reaching adult values by 48 h (Fig. 1). Adult male values for the transferase activities towards bili-

Fig. 1. Perinatal development of UDP-glucuronosyltransferase activities towards (a) bilirubin, (b) testosterone and (c) morphine under conditions of normal, premature and delayed birth in the rat

Each observation represents transferase activity from at least six pooled foetal livers, or neonatal livers from a minimum of two litters. Results are shown as means $\pm$ S.E.M. for the numbers of observations shown in parentheses. Represented in the three graphs of transferase activities towards bilirubin, testosterone and morphine are: normal development (O); development after Caesarean delivery on day 21 of gestation ($\bullet$); and development *in utero* when birth was delayed by maternal progesterone treatment (see the Materials and Methods section) ($\blacktriangle$).

rubin, testosterone and morphine were respectively 10.4 ± 0.4 (6), 18.2 ± 1.1 (5) and 140 ± 15 (5) nmol of glucuronide formed/h per mg of protein.

Surgical delivery of foetuses 24 h before the normal time of birth evoked a precocious surge in transferase activities, equal in rate to the normal postnatal surge (Fig. 1). This precocious appearance of activity was dependent on the newborns being suckled by a foster mother. When left unfed, transferase activity towards bilirubin increased from 0.58 ± 0.09 (6) nmol of glucuronide formed/h per mg of protein on delivery to 0.98 ± 0.09 (6) nmol/h per mg at 8 h, but no further development occurred by 14 and 20 h after delivery.

Gestation was prolonged for up to 2 days by progesterone administration to the dam. Although some placental deterioration occurred, the foetuses remained attached to the maternal system and foetal transferase activities continued to rise, apparently at much the same rate as during the last 2 days of normal gestation (Fig. 1). Progesterone, at the dosage given, did not elicit any precocious rise in foetal transferase activities *in utero* before day 22 of gestation, nor did it decrease transferase activities in neonatal rats 48 h after intraperitoneal injection of the maximum sublethal dose of 5 μ g per animal.

Discussion

Activities towards the substrates bilirubin, testosterone and morphine all show a markedly accelerated development after normal birth, and a

similar, but precocious, rise after Caesarean delivery 24 h before normal birth. Both normal and premature postnatal development of transferase activities occurs against a background of slower development *in utero* apparent during the last 2 days of normal gestation. When birth is delayed by treatment of the dam with progesterone, this postnatal surge in activities is prevented, but the basal increase of activities *in utero* continues unchanged. For these neonatal-group (Wishart, 1978) substrates the surge therefore requires the process of birth; birth presumably either supplies stimulatory, or removes inhibitory, factors.

Whether maternal milk contributes such factors remains unclear. Undoubtedly suckling is necessary for normal postnatal development of these transferase activities in the rat, but because this development occurs slowly, it was not possible to ascertain whether unfed newborns were primarily deprived of nourishment or of more specific transferase-stimulating factors. Attempts to resolve this by hand-rearing animals with natural and artificial diets proved impractical. Likely hormonal regulators were also investigated. Injection of 0.15 mg of dibutyryl cyclic 3':5'-AMP into foetuses *in utero* precociously stimulated the normally birth-related (see Greengard, 1971) surge of tyrosine aminotransferase activity within 6 h, but up to 5 mg per foetus did not affect UDP-glucuronosyltransferase activity towards these three substrates within 6–24 h. Nor did the following, added in amounts shown per foetus: adrenaline (30 μ g), tri-iodothyronine or thy-

roxine (50 μ g), somatotropin (growth hormone; 300 μ g), glucagon (80 μ g), insulin (50 μ g) or bilirubin (100 μ g) (M. T. Campbell & G. J. Wishart, unpublished work); continuous infusion of hormones, shown to be necessary for precocious stimulation of transferase activity in the chick embryo (see Leakey *et al.*, 1976), may be required.

Glucocorticoid injection accelerates, but does not initiate, postnatal development of the activity to bilirubin in CD-strain rats (Leakey *et al.*, 1979), but dexamethasone (125 μ g per foetus) was not useful in Wistar rats, where the natural surge is somewhat more rapid; adrenalectomy of newborns was also not helpful, because they appear to ingest glucocorticoid with maternal milk (M. T. Campbell & G. J. Wishart, unpublished work) and did not thrive on an artificial diet.

Whatever the regulatory mechanism associated with birth may be, from observations on their similar perinatal behaviour, we suggest that it is shared by

transferase activities towards the substrates bilirubin, morphine and testosterone.

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