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VARIATIONS IN GLUCURONIDE FORMATION BY PERINATAL LIVER

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Abstract—Glucuronide synthesis in liver can be important neonatally in preventing toxicity from accumulation either of drugs themselves or of endogenous compounds resulting from their action. The synthetic enzyme responsible, UDP-glucuronyltransferase (UDP-GT) appears perinatally. Clear demonstration for or against wide differences among substrates and species in its pattern of appearance is highly desirable.

In this work the pattern has been followed in detail for three substrates through the perinatal period in two species. Evidence obtained warrants these conclusions: (a) perinatal liver UDP-GT activity towards different substrates can differ widely in any one species; (b) development of UDP-GT activity towards one substrate can differ widely between two species; (c) these differences are important enough to affect many investigations in perinatal biochemical pharmacology, both *in vivo* and *in vitro* and (d) there are very probably several UDP-glucuronyltransferases in any one species.

GLUCURONIDE formation is an important pathway of drug "detoxication" and the microsomal enzyme UDP-glucuronyltransferase (E.C. 2.4.1.17) is responsible for glucuronyl transfer from UDPglucuronic acid to various drugs.[1] This enzyme appears in the liver around the time of birth, and in perinatal drug-toxicity studies it is obviously essential to know how widely applicable are developmental patterns obtained with any one drug in any one species.

Earlier, well-documented, evidence suggested a broadly similar gradual appearance of UDP-glucuronyltransferase towards several substrates, reaching adult levels sooner or later after birth.[2, 3] However, exceptions to this pattern have been briefly suggested,[4–9] and in some instances enzyme levels could be achieved at birth which actually exceeded adult values.

The present work describes clear evidence of gross variation in developmental rates of UDP-glucuronyltransferase towards different phenolic substrates, these rates themselves differing greatly between two species. Preliminary accounts of this work have been submitted.[7, 10]

MATERIALS AND METHODS

Mice were mixed laboratory stock, rats of Wistar strain. Ages of mouse fetuses were derived from Gruneberg;[11] rat fetuses were measured from nose to rump. The acceptor substrates, *o*-aminophenol, *p*-nitrophenol and phenolphthalein, were commercial samples purified before use; donor substrate was 98% purity UDP-glucuronic acid, ammonium salt, from Sigma Chemical Co., St. Louis, Miss. Liver homogenates were prepared by the method of Dutton and Storey[12] and estimations

of UDP-glucuronyltransferase activity towards the three substrates followed respectively the procedures of Dutton and Storey,[12] Isselbacher[13] and a modification of the method used by Levvy and Marsh.[14] Sliced tissue was treated as by Dutton and Storey.[12]

Conjugates measured in homogenates were those arising only in the presence of UDP-glucuronic acid. Such conjugates were hydrolysed by rat-preputial gland β-glucuronidase;[15] if its inhibitor, glucaro-1,4-lactone were present, hydrolysis did not occur. The conjugates were therefore presumably β-glucuronides. Maternal tissues, which exhibited no consistent change in UDP-glucuronyltransferase activity, served as controls. Protein N was the basis of measurement.[16] No sex difference occurred in mice and had not yet appeared in the young rats used. Two substrates were usually compared with a tissue preparation from the same animal; when necessary, litters were pooled to yield sufficient material. UDP-glucuronic acid was added to "practical saturation" (see Stevenson and Dutton[17]) and at the concentrations employed was checked not to be a limiting factor. Concentrations of acceptor substrates used were also not limiting.

RESULTS

These are best illustrated by the graphs. Figure 1 shows how, in late-foetal mice, liver UDP-glucuronyltransferase activity developed more rapidly towards phenolphthalein than towards *o*-aminophenol. After birth, the first activity levelled off, the

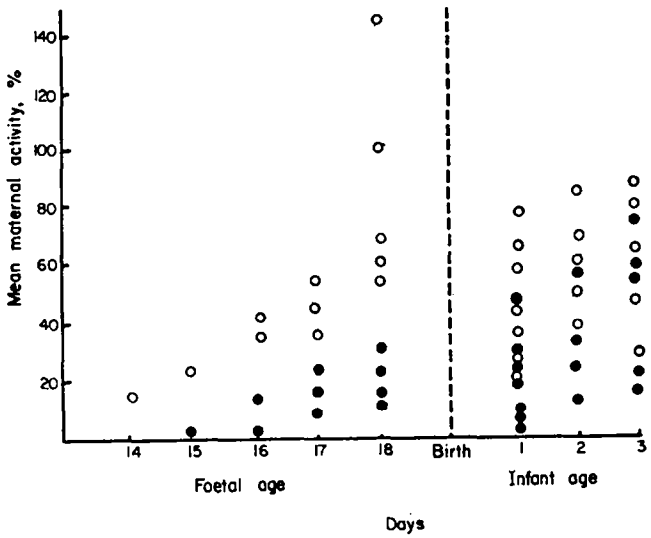

FIG. 1. Activity of UDP-glucuronyltransferase in perinatal-mouse liver homogenates measured (on a protein-*N* basis) as UDPglucuronic acid-dependent synthesis of *o*-aminophenyl (●) and phenolphthalein (○) glucuronides, and expressed above as a percentage of the mean maternal synthesis under the same conditions. Flasks contained 0·5 M-tris buffer with 0·15M-$MgCl_2$, either 0·15mM *o*-aminophenol or 0·03mM phenolphthalein. Tissue present was 10 mg wet weight for the first, and 5 mg for the second substrate. When added, UDPglucuronic acid was 0·56mM. Final volume was 0·6 ml, and incubation was at 37° for 30 min under N_2. For estimation procedures and comments on substrate concentrations, see text.

second rose, so that the distinction was lost. The molar ratio of the amounts of the two substrates conjugated confirmed this (Table 1).

Figure 2 illustrates, in perinatal rats, the strikingly more rapid development of liver UDP-glucuronyltransferase towards *p*-nitrophenol than towards phenolphthalein. Only by some 5 days after birth did the activities towards the two substrates

TABLE 1. MEAN MOLAR RATIO OF TWO SUBSTRATES CONJUGATED BY LIVER UDP-GLUCURONYLTRANSFERASE AT DIFFERENT AGES IN TWO SPECIES

Age (No. of experiments)	Substrate A	Substrate B	$\frac{\mu\text{moles A conjugated/50 mg*}}{\mu\text{moles B wet weight liver}}$
Mouse			
Adult ♀ (20)	*o*-Aminophenol	Phenolphthalein	1·0
2–4 day infants (10)	*o*-Aminophenol	Phenolphthalein	0·8
15–18 day fetuses (8)	*o*-Aminophenol	Phenolphthalein	0·3
Rat			
Adult ♀ (25)	*p*-Nitrophenol	Phenolphthalein	2·2
1–3 day infants (9)	*p*-Nitrophenol	Phenolphthalein	14·6

* Tissue preparation from the same animals was employed.
Methods as in Fig. 1 and 2 and in text.

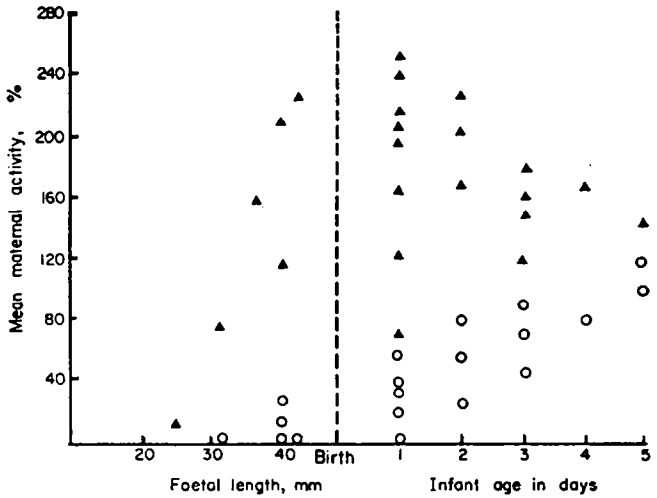

FIG. 2. Activity of UDP-glucuronyltransferase in perinatal-rat liver homogenates expressed as for Fig. 1, with *p*-nitrophenol (▲) and phenolphthalein (○) as substrates. Conditions as for Fig. 1, but with 0·14mM *p*-nitrophenol present, where indicated, with 10 mg wet weight tissue.

approach each other, one falling, one rising, towards maternal levels. The wide individual scatter previously noted[8] with *p*-nitrophenol in perinatal rat did not obscure the marked difference in treatment of the two substrates in this series of experiments. Molar ratios of substrates conjugated emphasises this difference (Table 1).

The above investigations compared two substrates in any one species. When each of the three substrates is compared in the two species, then variations are again obvious (Table 2).

No inhibitors were evident: newborn rat-liver homogenate did not significantly reduce synthesis of phenolphthalein glucuronide in foetal mouse-liver homogenates, providing that UDP-glucuronic acid was kept in sufficient excess. Artefacts of homogenization were unlikely, for similar differences in developmental patterns were evident when sliced preparations were used: as satisfactory identification of glucuronide formation with phenolphthalein and *p*-nitrophenol was difficult, fewer experiments were performed with sliced tissue.

TABLE 2. MEAN PERCENTAGE OF MATERNAL LIVER UDP-GLUCURONYLTRANSFERASE ACTIVITY EXHIBITED BY PERINATAL LIVER WITHIN 24 HR OF BIRTH (ALL VALUES APPROXIMATE)

Species	*o*-Aminophenol	*p*-Nitrophenol	Phenolphthalein
Mouse	20	70*	86
Rat	100–200*	200	20

* Values assessed from a previous set of experiments.
Methods as in Figs. 1 and 2 and in text.

DISCUSSION

The above results clearly confirm what has been increasingly suspected: that although UDP-glucuronyltransferase is absent from all early-foetal livers investigated, its time of appearance and rate of development can vary markedly from substrate to substrate and species to species. This finding has obvious significance in those perinatal toxicity-trials and therapeutic investigations which involve glucuronidation of drugs or which interfere with glucuronidation of endogenous substrates.[8, 18]

An immediate explanation is that several UDP-glucuronyltransferases exist in each species.[19,20] However, "microsomes" alter as liver develops[18, 21, 22] and altering accessibility to them of various substrates must therefore be considered, even though the radical species differences encountered above make this unlikely. Work is needed with a more stable form of solubilised UDP-glucuronyltransferase[23] than has yet been prepared.

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