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Regulation of Onset of Development of UDP-Glucuronosyltransferase Activity towards *o*-Aminophenol by Glucocorticoids in Late-Foetal Rat Liver *in utero*

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1. A precocious development of UDP-glucuronosyltransferase activity (EC 2.4.1.17) towards *o*-aminophenol is demonstrated in 15–17-day foetal rat liver *in utero* after dexamethasone administration to the mother. 2. This stimulation of liver transferase activity *in utero* is directly proportional to the dose of dexamethasone injected. 3. Precocious development of transferase activity *in utero* can also be effected with the natural glucocorticoid cortisol by multiple injections of large amounts of this hormone into the mother. 4. Transferase activity towards *o*-aminophenol in foetal lung, kidney and upper alimentary tract can also be precociously stimulated by dexamethasone in 17-day foetuses *in utero*. 5. Natural development of hepatic transferase activity between days 18 and 20 of gestation is retarded after foetal hypophysectomy by decapitation *in utero*. 6. Overall glucuronidation of *o*-aminophenol, as observed in foetal rat liver, is also precociously stimulated by dexamethasone. 7. From this and from evidence previously presented we suggest that glucocorticoids, which are known to increase in rat foetuses between days 17 and 20 of gestation, trigger the normal development *in utero* of hepatic transferase activity towards *o*-aminophenol which occurs at that time. We also suggest that these hormones are responsible for the rise in activity of the enzyme in foetal lung, kidney and upper alimentary tract which occurs during the same gestational period.

Glucuronidation of potentially toxic molecules, which is the major conjugatory detoxicating pathway in adult mammals, is virtually absent from early foetal tissues and develops to adult values perinatally (see Dutton & Burchell, 1977). Because lack of this process renders the foetus and early newborn susceptible to the adverse effects of toxic lipophiles, the search for endogenous factors responsible for triggering the perinatal appearance of glucuronidation has been extensive. We have shown (Wishart *et al.*, 1977*a*) that the activity towards *o*-aminophenol of UDP-glucuronosyltransferase (EC 2.4.1.17), one of the rate-limiting enzymes in the glucuronidation process (Fyffe & Dutton, 1975; see Dutton & Burchell, 1977), develops from negligible to adult values in foetal rat liver between days 17 and 20 of gestation. We also demonstrated (Wishart & Dutton, 1976; Wishart *et al.*, 1977*a*) that glucocorticoid hormones precociously induce transferase activity in cultured explants of 15-day rat foetal liver. Since there is increased glucocorticoid output in rat foetuses between the gestational ages of 17 and 20 days (Kamoun, 1970), we proposed (Wishart *et al.*, 1977*a*) that these hormones may indeed be responsible for triggering the surge of transferase activity that occurs *in vivo* at that time.

As the behaviour of foetal liver cultured as explants may differ from that *in utero* (see Wishart *et al.*, 1977*a*), we have examined the effects of glucocorticoids on transferase activity in foetal tissues *in utero*. We find that injection of glucocorticoids into pregnant rats will precociously stimulate transferase activity towards *o*-aminophenol in several foetal tissues, and also stimulate in foetal liver the process of overall glucuronidation as subsequently measured in small pieces of intact tissue. Preliminary accounts of parts of this work have been published (Wishart & Dutton, 1977*a*,*b*). The role of glucocorticoids in the regulation of UDP-glucuronosyltransferase activity to substrates other than *o*-aminophenol is discussed elsewhere (Wishart & Dutton, 1977*a*; Wishart *et al.*, 1977*b*).

Materials and Methods

All steroids and UDP-glucuronic acid were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Wistar rats were used in all experiments. They were time-mated. Conception occurred at night; during the following 24h (i.e. their first day of life) foetuses were considered to be 0 days of age; day 1 was therefore reckoned as the period 24–48h after conception, and so on. Steroids were injected intraperitoneally, dissolved or sus-

pended in arachis oil. Maternal adrenalectomy and foetal decapitation *in utero* (Jacquot & Kretchmer, 1964) were carried out in the laboratory of Dr. R. Jacquot, Reims, France, under the guidance of Dr. J.-M. Félix.

'Optimally activated' UDP-glucuronosyltransferase (*o*-aminophenol as substrate) was assayed in homogenates as described previously (Wishart *et al.*, 1977*a*). The glucuronidation capacity of portions of liver (each approx. 1 mg wet wt.) snipped from the lobular edges was measured as for liver slices by the method of Dutton & Storey (1962), except that the assay mixture was sonicated (Leakey *et al.*, 1976) before the addition of trichloroacetic acid. Protein was measured (in samples taken before trichloroacetic acid addition) by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Results are expressed as means ± S.E.M., unless otherwise indicated. 'Control' foetuses are those from mothers injected with arachis oil only. 'Normal' foetuses are those from uninjected mothers.

Results

Precocious development of rat foetal liver UDP-glucuronosyltransferase activity towards o-aminophenol in utero after maternal injection of dexamethasone

To attempt to stimulate foetal transferase activity with glucocorticoids *in utero* it was not necessary to inject the foetuses directly, because these hormones freely pass the placental barrier (Zarrow *et al.*, 1970). We therefore injected the mothers.

Because of its persistence *in vivo* (see Bush *et al.*, 1968; Wong *et al.*, 1976), dexamethasone was preferentially used in the present studies. It was injected (300 μg) into pregnant rats successively on days 14, 15 and 16 of gestation. As shown in Fig. 1, transferase activities in the livers of foetuses from these mothers rose to reach values at day 17 within the range of those in livers of adult male rats (30–40 nmol of *o*-aminophenyl glucuronide formed/h per mg of protein). Fig. 1 shows that this precocious rise was already evident within 24 h of the first injection, and also that it preceded the initiation of the surge in normal foetuses by 2–3 days. Indeed, on day 17 the transferase activity in control foetuses had not yet begun its perinatal rise. Once begun, transferase activity developed at similar rates in livers of foetuses either exposed to dexamethasone or left to develop the enzyme naturally (Fig. 1).

A direct relationship appeared between the amount of dexamethasone injected and the transferase activity stimulated (Fig. 2).

No effect of dexamethasone injection was observed on the values of the transferase in the maternal liver, when examined on day 17 of gestation.

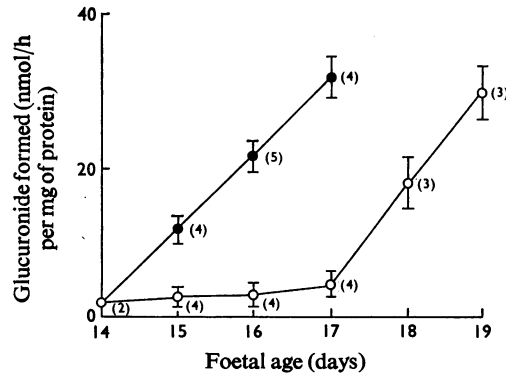

Fig. 1. *Precocious stimulation of UDP-glucuronosyltransferase development in liver of foetal rats exposed in utero to dexamethasone*

Pregnant rats were injected intraperitoneally with 300 μg of dexamethasone dissolved in 0.1 ml of arachis oil on each of days 14, 15 and 16 of gestation. Transferase activity was assayed in the livers of their foetuses (●) on the days shown. Activity was also measured (on days 15, 16 and 17) in the livers of foetuses from mothers similarly injected with arachis oil only, and (on days 14, 18 and 19) in those of foetuses from uninjected mothers; transferase activity has been previously shown (Wishart *et al.*, 1977*a*) not to differ significantly between these last two 'control' groups. Both are therefore depicted by the symbol (○). Each result shows the mean ± S.E.M. of the indicated number of pooled foetal liver samples. This number of samples was obtained from at least two mothers.

Effect of natural steroids on foetal liver UDP-glucuronosyltransferase activity in utero

When using chick embryo we encountered great difficulty in stimulating the hepatic transferase *in ovo* by single injection of the natural glucocorticoids; continuous infusion of these compounds was, however, clearly effective (Leakey *et al.*, 1976). Presumably the natural glucocorticoids are rapidly inactivated *in vivo*. As continuous infusion of rat foetuses *in utero* with these compounds appeared difficult, we injected steroids into the mother in large doses (25 mg) twice daily from day 14 for 3 days. On day 17 transferase activity in the foetal liver was clearly raised over that in control foetal liver when cortisol was administered (Table 1), although, as expected, values were below those obtained after dexamethasone injection. A possibly stimulatory effect was noted with corticosterone or progesterone (Table 1).

Effect of foetal decapitation on development of UDP-glucuronosyltransferase

A surge of glucocorticoids occurs naturally in foetal rat plasma over days 18–20 of gestation

(Kamoun, 1970). It is believed to be stimulated by enhanced secretion of corticotropin by the foetal hypophysis (Milković *et al.*, 1972). Ablation of the foetal hypophysis should therefore inhibit the natural prenatal rise of transferase activity towards *o*-aminophenol if foetal corticotropin and glucocorticoids are involved in that phenomenon.

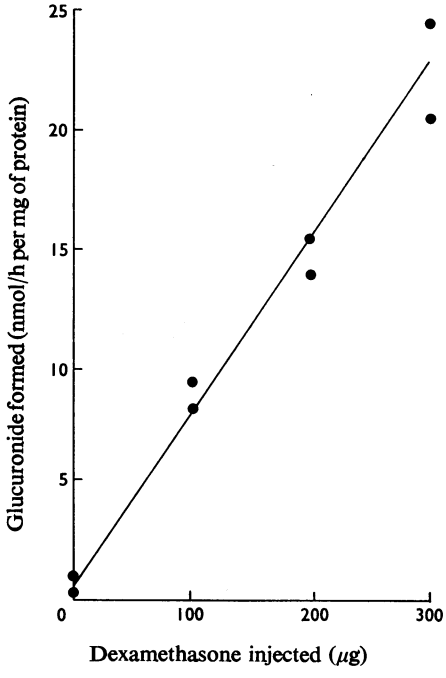

Fig. 2. *Relationship of maternally administered dexamethasone to UDP-glucuronosyltransferase activity in foetal liver in utero*

Injections of 0, 100, 200 and 300 μg of dexamethasone in 0.1 ml of arachis oil were given intraperitoneally to pregnant Wistar rats on days 14 and 15 of gestation. Transferase activity was assayed in foetal livers on day 16 of gestation. Each result represents the activity from a pool of at least six foetal livers, two pools being provided by one mother for each dose.

Foetal hypophysectomy by decapitation *in utero* was carried out in Reims (see the Materials and Methods section) on three foetuses in each of three mothers on day 18 of gestation. These mothers had been adrenalectomized 4 days previously. Transferase activities were assayed on day 20 in livers of decapitated and untreated foetuses from the same litter. Activities were 14.2±5.6 nmol of *o*-aminophenyl glucuronide formed/h per mg of protein in the three pools of liver from decapitated foetuses; this value is well below the value of 44.0±6.1 of the above units obtained with the six pools (two from each mother) of untreated littermates. As the Reims and Dundee Wistar colonies possessed comparable foetal liver transferase activities and rate of transferase development (e.g. 20-day Dundee foetuses had a value of 53.4±7.2), these results suggest that no development of transferase occurred beyond the day of ablation [i.e. day 18, when Dundee foetuses (see Fig. 1) possessed activities of 17.7±4.8 of these units]. These results would support the role of the foetal hypophysis in initiation of transferase development.

Effect of dexamethasone on overall glucuronidation in foetal liver tissue

Both UDP-glucuronosyltransferase and UDP-glucuronic acid are needed for glucuronidation. Glucuronidation in intact tissues or cells must involve synthesis of the nucleotide, and although both the transferase and UDP-glucose dehydrogenase may be considered rate-limiting in natural development of glucuronidation (Fyffe & Dutton, 1975), the complex cellular regulation of transferase activity (see Zakim & Vessey, 1976) makes this a simplification. It was therefore necessary to know if dexamethasone stimulated not only the transferase assayed in broken cells (with added UDP-glucuronic acid), but also the glucuronidation observable in intact tissue.

Small pieces were cut from livers of 17-day foetal rats whose mothers had been injected with 300 μg of dexamethasone on each of the 3 preceding days,

Table 1. *Effect of natural steroids on UDP-glucuronosyltransferase in utero*

Pregnant Wistar rats were injected twice daily at 09:00h and 17:00h on each of days 14, 15 and 16 of gestation with 25mg of steroid suspended in 0.25ml of arachis oil. Control animals received arachis oil only. Transferase activity was assayed in the foetal livers on day 17, as described in the text. Each result represents enzyme activity in a pool of livers comprising half of one litter.

Steroid injected	17-day foetal liver UDP-glucuronosyltransferase activity (nmol of *o*-aminophenyl glucuronide formed/h per mg of protein)
Cortisol (11β,17,21-trihydroxypregn-4-ene-3,20-dione)	14.4, 11.4
Corticosterone (11β,21-dihydroxypregn-4-ene-3,20-dione)	4.9, 7.2
Progesterone (pregn-4-ene-3,20-dione)	8.4, 7.5
None (oil only)	5.4, 2.3, 6.1, 4.1

Table 2. *Effect of dexamethasone on extrahepatic UDP-glucuronosyltransferase activity in utero*

Animals were injected with dexamethasone as described under Fig. 1. 'Controls' were in this case all normal uninjected animals. Each result from 17-day foetuses represents transferase activity from a pool of organs from two litters, i.e. more than 20 foetuses. Results from 20-day foetuses are from a pool of organs from one litter. Upper-alimentary-tract samples contained the stomach, oesophagus and duodenum.

Source of tissue	UDP-glucuronosyltransferase activity (nmol of *o*-aminophenyl glucuronide formed/h per mg of protein)		
	Lung	Upper alimentary tract	Kidney
17-day normal foetus	0.8, 0	4.5, 1.8	0.7, 0.5
17-day foetus exposed to dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione)	3.8, 2.2	17.2, 20.5	3.4, 2.1
20-day normal foetus	2.2, 1.8	7.5, 15.4	3.4, 1.2

and assayed for overall glucuronidation of *o*-aminophenol (see the Materials and Methods section). Control samples, from arachis oil-injected mothers, were treated similarly. The glucuronidating capacity of pooled liver pieces from two control litters was 0.086 and 0.080 nmol of *o*-aminophenyl glucuronide formed/h per mg of protein, whereas that of pooled liver pieces from two dexamethasone-treated litters were 0.382 and 0.669 of these units. Glucuronidation had therefore increased 4–8-fold in livers of foetuses exposed to the glucocorticoid.

Stimulation of extrahepatic UDP-glucuronosyltransferase activity

Extrahepatic organs represent important sites for detoxication by glucuronidation (Aitio, 1973; see Dutton & Burchell, 1977). As in liver, the transferase activity towards *o*-aminophenol in foetal rat lung, upper alimentary tract and kidney develops several-fold between days 17 and 20 of gestation (Table 2). These activities were all activatable with digitonin; the optimally activated values are reported here.

Administration of dexamethasone to the mother precociously stimulated, in 17-day foetuses, transferase activities in lung, kidney and the alimentary tract up to or above those found normally in 20-day foetuses (Table 2).

Discussion

The above results clearly demonstrate that administration of dexamethasone to the mother will precociously stimulate liver UDP-glucuronosyltransferase activity in foetal Wistar rats from negligible to adult male values, and that its development at several extrahepatic sites is also accelerated. Dexamethasone and other glucocorticoids cross the rat placenta (Zarrow *et al.*, 1970) and thus the intact foetus must have been exposed to these compounds. The transferase may therefore have been stimulated by the same mechanism that operates at the isolated tissue exposed to the glucocorticoids in culture (Wishart *et al.*, 1977*a*). This mechanism was shown to require amino acid incorporation (Wishart *et al.*, 1977*a*) and may be provisionally considered as involving induction of transferase enzyme.

The relevance of this induction to the natural prenatal surge of transferase activity towards *o*-aminophenol must be briefly discussed. In the rat, foetal adrenocorticoid activity increases between days 17 and 20 of gestation (Dupouy *et al.*, 1975), being stimulated by foetal hypophysial corticotropin (Milković *et al.*, 1972), and increasing foetal plasma glucocorticoids above those in the maternal plasma (Kamoun, 1970). Over this period we have shown that transferase activity towards *o*-aminophenol in our rat colony increases from negligible to adult values (Wishart *et al.*, 1977*a*). In the present paper we have described how ablation of the foetal hypophysis arrests this transferase development.

The conclusion appears inescapable that, for *o*-aminophenol at least, and in the rat, the prenatal surge of UDP-glucuronosyltransferase activity is brought about initially by foetal corticotropin stimulating production of foetal glucocorticoids which act at the liver cell to induce the enzyme. The mechanism thus resembles that proposed for initiation of the surge of transferase in chick embryo (Leakey *et al.*, 1976), and closely parallels the well-documented initiation of glycogen synthase in foetal rat liver (Jacquot *et al.*, 1973).

Dexamethasone did not affect the maternal enzyme. Neither did it stimulate the enzyme in neonatal rats (G. J. Wishart, unpublished work); and adrenalectomy of adult males did not, at least within 10 days, lower the activity in their livers (M. T. Campbell, A. M. Donald & G. J. Wishart, unpublished work). These findings are not inconsistent with the proposed role of glucocorticoids perinatally. Adrenalectomy did not lower glycogen synthase activity in adult rat liver (Parvez *et al.*, 1976), and Greengard (1971) points out that factors triggering perinatal onset of an enzyme need not be required for its subsequent maintenance.

The low effectiveness of administered natural

glucocorticoids *in utero* as opposed to tissue culture is explicable by their rapid metabolism. For example, when radioactive glucocorticoids were injected maternally, the ratio foetal tissue/maternal plasma radioactivity for corticosterone was one-fifth that for dexamethasone; moreover, the ratio of unmetabolized to metabolized steroid per g of foetal tissue was, for corticosterone, some 10% of that for dexamethasone (Wong *et al.*, 1976). Progesterone had no effect on transferase activity in cultured foetal liver (Wishart *et al.*, 1977*a*); its minor stimulation on injection, if real, could be attributed to its metabolism to glucocorticoids.

Further support for the role of glucocorticoids in the natural development of transferase activity towards this substrate comes from the precocious stimulation of transferases extrahepatically and of glucuronidation in pieces of foetal liver. In these latter preparations, stimulation by glucocorticoids of UDP-glucose dehydrogenase (EC 1.1.1.22; C. Petrou & G. J. Wishart, unpublished work) may play a part.

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