Immunochemical comparison of UDP-glucuronyltransferase from Gunn- and Wistar-rat livers

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1. Antiserum was raised against purified Wistar-rat liver UDP-glucuronyltransferase.
2. UDP-glucuronyltransferase activities towards 4-nitrophenol, bilirubin, 1-naphthol and morphine were co-immunoprecipitated from solubilized Wistar-rat liver preparations.
3. UDP-glucuronyltransferase activities towards 1-naphthol, 2-aminophenol and 4-nitrophenol were precipitated from solubilized Gunn-rat liver preparations by this antiserum.
4. UDP-glucuronyltransferase activities towards 1-naphthol, 4-nitrophenol and bilirubin, from Wistar-rat liver, were slightly inhibited by antiserum, whereas 1-naphthol UDP-glucuronyltransferase activity from Gunn-rat livers was greatly inhibited.
5. Measurable Wistar-rat liver glucuronyltransferase activities in washed immunoprecipitates indicate that the enzyme(s) were not merely inhibited by antiserum.
6. Immunoglobulin G purified from this antiserum immunoprecipitated transferase activities towards 4-nitrophenol, bilirubin and 1-naphthol.
7. The washed immunoprecipitates from both rat strains, containing UDP-glucuronyltransferase activity, appear to be similar when analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.
8. Radial-immunodiffusion studies suggest that a smaller amount of UDP-glucuronyltransferase protein is present in Gunn-rat liver than in Wistar-rat liver.
9. The significance of these results in relation to the genetic deficiency in the Gunn rat is discussed.

The molecular basis of the genetic deficiency of human hepatic UDP-glucuronyltransferase is not well understood. However, the Gunn strain of rat, which has an hereditary hyperbilirubinaemia (Gunn, 1938), may be a useful animal model to help the study of the human deficiency diseases, the Crigler-Najjar and Gilbert's syndromes. The biochemical lesion in the Gunn rat is recognized by a complete inability to glucuronidate bilirubin *in vivo* (Lathé & Walker, 1957) and a much diminished ability to glucuronidate 2-aminophenol (Arias, 1961). The isolation of UDP-glucuronyltransferase(s) and the study of the inherited deficiency existing in Gunn rats may provide an insight into these human diseases.

Evidence from the study of development (Wishart *et al.*, 1977; Wishart, 1978; Lucier & McDaniel, 1977) and inducibility by glucocorticoids (Wishart *et al.*, 1977; Wishart, 1978) and xenobiotics (Bock *et al.*, 1973, 1977) of UDP-glucuronyltransferase with up to 12 aglycone substrates suggests that at

Abbreviations used: SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

least two functionally heterogeneous forms of the enzyme may exist. UDP-glucuronyltransferase activities towards 1-naphthol or 4-nitrophenol appear to have been separated from the activity towards morphine (Del Villar *et al.*, 1975, 1977; Bock *et al.*, 1977) and bilirubin (Burchell, 1980). If more than one UDP-glucuronyltransferase does exist, then the genetic deficiency in the Gunn rat may be due to the absence of one or more of the different catalytic forms.

In the present paper, antiserum raised in rabbits against UDP-glucuronyltransferase purified to apparent homogeneity (Burchell, 1978) has been used to find out (a) whether multiple forms of UDP-glucuronyltransferase can be identified and (b) whether amounts of UDP-glucuronyltransferase protein can be measured by using this specific immunochemical reagent, thereby facilitating the investigation of the genetic deficiency of this enzyme in Gunn rat liver.

A preliminary account of a small part of this work has been reported elsewhere (Burchell & Weatherill, 1978).

Experimental

Materials

Lubrol 12A9 (a condensate of dodecanol with approx. 9.5 mol of ethylene oxide/mol) was obtained from ICI Organics Division, Manchester, U.K. UDP-glucuronic acid (triammonium salt), bilirubin, bovine serum albumin and Coomassie Brilliant Blue protein stain were all purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. The radioactive materials 1-[1-¹⁴C]naphthol and [*N*-methyl-¹⁴C]morphine hydrochloride were from The Radiochemical Centre, Amersham, Bucks., U.K. 2-Aminophenol was from BDH Chemicals, Poole, Dorset, U.K., and resublimed twice before use. All other chemicals, where available, were analytical-reagent grade.

The protein standards used for SDS/polyacrylamide-gel electrophoresis, i.e. ovalbumin, pyruvate kinase and carbonic anhydrase, were purchased from Boehringer, Mannheim, West Germany.

Protein A-Sepharose CL-4B was from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE-cellulose (DE 52) was obtained from Whatman Biochemicals, Maidstone, Kent, U.K. Agarose powder was purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A.

Wistar and Gunn rats were from colonies maintained in the Institute animal unit.

Enzyme assays

UDP-glucuronyltransferase activity towards various substrates was assayed by the following methods: bilirubin (Heirwegh *et al.*, 1972), 1-naphthol (Otani *et al.*, 1976), morphine (Del Villar *et al.*, 1974), 2-aminophenol and 4-nitrophenol (Winsnes, 1969). Epoxide hydratase was assayed by the method of Oesch (1974). Protein concentrations were measured by the method of Bradford (1976) with bovine serum albumin as standard.

Gel electrophoresis

Polyacrylamide-gel electrophoresis was performed in 7.5% acrylamide gels in the presence of 0.1% SDS at 20°C, pH 7.2, at a constant 60 V for 2 h. Samples for electrophoresis were prepared and polypeptide bands were stained with Coomassie Brilliant Blue as described by Weber & Osborn (1969). The molecular weights of the polypeptide bands were estimated by comparing their mobilities with the mobilities of standard proteins of known molecular weights.

Production of antiserum

Specific antiserum to UDP-glucuronyltransferase was raised in White Lop-eared rabbits by the method described by Burchell (1978). Pre-immune serum was obtained from rabbits before injection

with UDP-glucuronyltransferase, and did not contain immunoglobins capable of cross-reacting with UDP-glucuronyltransferase when analysed by Ouchterlony double diffusion (Ouchterlony, 1949).

Purification of IgG

IgG was purified from crude sera, in approx. 80% yield, by two methods.

(a) Ion-exchange chromatography on DEAE-cellulose (Fahey & Terry, 1978). IgG was bound to a DEAE-cellulose column (18 cm × 2 cm) equilibrated with 5 mM-Tris/phosphate buffer, pH 8.0, and eluted with a 100 ml linear gradient of 5–50 mM-Tris/phosphate buffer, pH 8.0. The IgG was further purified by (NH₄)₂SO₄ fractionation (0–45% saturation). The precipitated product was dissolved in 0.1 M-potassium phosphate, pH 7.0, and dialysed against 50 vol. of the same buffer for 16 h.

(b) Affinity chromatography on Protein A-Sepharose CL-4B (Hjelm, 1974). IgG was bound to Protein A-Sepharose equilibrated with 0.1 M-potassium phosphate, pH 7.0, and eluted with 0.1 M-glycine/HCl buffer, pH 3.0. Fractions were immediately adjusted to pH 7.0 by the presence of an equal volume of 0.5 M-potassium phosphate, pH 7.0, in the collection tubes.

Immunoprecipitation of UDP-glucuronyltransferase

Solubilized UDP-glucuronyltransferase preparations were prepared from male Wistar- or Gunn-rat livers as previously described (Burchell, 1977). Then 1 vol. of these preparations was mixed with 5 vol. of either pre-immune serum, antiserum or purified IgG in the presence of 0.2 mM-benzamide. These mixtures were incubated for 24 h at 4°C. They were then centrifuged at 10000 *g* for 5 min and the supernatants were removed for assay. The immunoprecipitates were washed by gentle resuspension in 1.0 ml of 0.25 M-sucrose and the pellet was recovered by further centrifugation at 10000 *g* for 5 min. This washing procedure was repeated five times. The washed immunoprecipitates were resuspended in a volume of 0.05% Lubrol/25 mM-potassium phosphate/2 mM-EDTA/5 mM-2-mercaptoethanol buffer, pH 7.4, equal to half of the volume of the original incubation mixture.

Radial immunodiffusion (Mancini *et al.*, 1965).

Radial immunodiffusion plates containing purified IgG from antiserum (0.5–1.0 mg/ml) were prepared with 0.9% (w/v) agarose in 0.7 M-glycine/0.06 M-NaCl/0.4 M-glycerol/0.015 M-NaN₃/0.35% (w/v) Lubrol 12A9 buffer, pH 7.4.

Lubrol-solubilized preparations of hepatic microsomal UDP-glucuronyltransferase were prepared from adult Gunn and Wistar rats (Weatherill & Burchell, 1978) of approximately the same age and

weight. The protein content of each preparation was adjusted to 12mg/ml by dilution with solubilizing buffer. Samples (8μl) of each of the Gunn- and Wistar-rat soluble fractions (containing 24–96μg of protein) were placed in the wells. Immunodiffusion was performed at room temperature in a humid chamber until no further increase in ring diameter was observed (5 days). The diameter of the immunoprecipitin line was measured by projection by using a photographic enlarger.

Results

Comparison of hepatic microsomal UDP-glucuronyltransferase activities of Gunn and Wistar rats

Gunn- and Wistar-rat liver microsomal UDP-glucuronyltransferase activities were assayed by using four aglycone substrates in the presence of Lubrol and/or pentan-3-one (Lalani & Burchell, 1979) to compare activities in the two strains and assess the enzymological nature of the transferase deficiency in our Gunn-rat colony. UDP-glucuronyltransferase activity towards bilirubin was not detectable in Gunn-rat liver microsomal fractions even in the presence of Lubrol, pentan-3-one or both activators (Table 1).

The UDP-glucuronyltransferase activities of freshly prepared microsomal fractions from Gunn-rat liver ('native' microsomal fraction) towards 2-aminophenol and 1-naphthol were 18.0 and 32.0% respectively of those observed in the comparable Wistar-rat liver preparation. Activity towards morphine was not significantly different in these 'native' Gunn- and Wistar-rat microsomal fractions.

In the presence of 0.05% Lubrol, apparent

transferase activities of Gunn-rat preparations increased toward all substrates except bilirubin (Table 1). Only one outstanding inter-strain difference was noted after treatment with Lubrol; transferase activity towards 1-naphthol was increased twice as much in Wistar- as in Gunn-rat preparations.

The presence of 10mM-pentan-3-one in the enzyme assay only abolished the apparently defective function of UDP-glucuronyltransferase towards 2-aminophenol; it did not significantly alter UDP-glucuronyltransferase activities towards the other substrates tested, even when assayed in the presence of detergent.

Therefore microsomal fractions of Gunn-rat livers from the colony maintained in our animal unit exhibit the enzymological defects characteristic of this strain of rat, as previously reported (see Dutton & Burchell, 1977, for references).

Immunoprecipitation of rat liver UDP-glucuronyltransferase by using crude antiserum

Purified Wistar-rat liver UDP-glucuronyltransferase, which catalyses the glucuronidation of 2-aminophenol, 1-naphthol, 4-nitrophenol, 2-aminobenzoate and morphine, but not bilirubin (Burchell, 1978), was used to raise an antiserum in rabbits (see the Experimental section). This antiserum was used to compare UDP-glucuronyltransferase from Gunn- and Wistar-rat livers by Ouchterlony double-diffusion analysis. Fig. 1 shows the appearance of a sharp continuous immunoprecipitin line with no spur formation after the interaction of liver (NH₄)₂SO₄ preparations from both rat strains with antiserum, suggesting that the transferase proteins from both strains are immunologically similar.

Table 1. UDP-glucuronyltransferase activities in Gunn- and Wistar-rat liver microsomal fractions

Microsomal pellets were resuspended in 0.25M-sucrose and UDP-glucuronyltransferase activities assayed as described in the text. Specific-activity units are nmol of glucuronide formed/min per mg of protein, and are means ± S.D. for the numbers of experiments shown in parentheses. N.D., not detectable. The final concentrations of pentan-3-one and Lubrol used were 10 mM and 0.05% respectively.

UDP-glucuronyltransferase activity										
Gunn						Wistar				
Substrate	Additions	...	None	Pentan-3-one	Lubrol	Lubrol + pentan-3-one	None	Pentan-3-one	Lubrol	Lubrol + pentan-3-one
2-Aminophenol	(6)		0.022 ±0.005	0.202 ±0.053	0.089 ±0.041	0.850 ±0.140	0.122 ±0.040	0.199 ±0.040	0.414 ±0.100	0.913 ±0.063
Bilirubin	(5)		N.D.	N.D.	N.D.	N.D.	0.033 ±0.019	0.038	0.367 ±0.082	0.387 ±0.047
Morphine	(8)		0.48 ±0.30	0.62 ±0.13	3.48 ±0.32	3.70 ±0.85	0.56 ±0.35	0.56 ±0.35	5.25 ±0.13	4.59 ±1.19
1-Naphthol	(10)		0.56 ±0.25	0.71 ±0.26	4.82 ±2.46	5.63 ±2.41	1.75 ±0.76	1.78 ±0.64	30.7 ±16.3	35.8 ±15.7

The same preparation of antiserum was also used to analyse UDP-glucuronyltransferase by immunoprecipitation from solution. Gunn- or Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with antiserum for 24 h at 4°C and the resulting antigen-immunoglobulin complexes were precipitated by centrifugation (see the Experimental section). The immunoprecipitation of UDP-glucuronyltransferase activities from Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions is shown in Fig. 2. Incubation in the absence of antiserum (i.e. 100% preimmune serum) did not precipitate any UDP-glucuronyl-

transferase or epoxide hydratase activities. Increasing amounts of antiserum gradually immunoprecipitated up to 95% of the UDP-glucuronyltransferase activity. Epoxide hydratase, the most likely contaminant of the Wistar-rat liver UDP-glucuronyltransferase antigen used to produce antiserum, was not precipitated, indicating the purity of

Fig. 1. Ouchterlony double-diffusion analysis of UDP-glucuronyltransferase from Gunn- and Wistar-rat liver. The experiments were performed in 1% agar plates and allowed to develop for 48 h at 15°C before photography. Antiserum raised against UDP-glucuronyltransferase purified from Wistar-rat liver was placed in the centre wells and allowed to react with $(\text{NH}_4)_2\text{SO}_4$ fractions from Gunn-rat liver (well 1), Wistar-rat liver (well 2) and phenobarbital-treated Wistar-rat liver (well 3). Wells 4, 5 and 6 were empty.

Fig. 2. Immunoprecipitation of UDP-glucuronyltransferase activity from $(\text{NH}_4)_2\text{SO}_4$ fractions of Wistar-rat livers

The results are expressed as percentages of the activity of samples treated similarly with serum obtained from rabbits before immunization against UDP-glucuronyltransferase as antigen. UDP-glucuronyltransferase was measured with as substrate 4-nitrophenol ($\square$), morphine (▨) or bilirubin (▩). Epoxide hydratase activity was measured with styrene oxide as substrate ($\bullet$). The mean values of four determinations are shown and the ranges of these values are indicated by bars.

Table 2. Inhibition of Wistar-rat liver UDP-glucuronyltransferase by antiserum

Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with antiserum at 4°C for 24 h and were then assayed before and after centrifugation at 10000 g for 5 min for UDP-glucuronyltransferase activity. The values shown are the averages of three results, with the ranges shown in parentheses.

		UDP-glucuronyltransferase activity			
		In supernatant before centrifugation (%)		In supernatant after centrifugation (%)	
Antiserum (%) in incubation mixture	Substrate ...	4-Nitrophenol	Bilirubin	4-Nitrophenol	Bilirubin
0		100	100	100	100
40		72.1 (56.1–92.0)	83.7 (74.6–91.2)	45.3 (20.0–83.8)	31.6 (24.5–36.4)
100		55.7 (37.8–70.9)	67.9 (60.2–74.1)	16.6 (0.0–33.3)	0.0

the antigen and the selectivity of the immunoprecipitation procedure.

Inhibition of UDP-glucuronyltransferase activity by antiserum

Incubation mixtures were assayed before and after centrifugation to determine whether antiserum might be merely inhibiting UDP-glucuronyltransferase. Table 2 shows that UDP-glucuronyltransferase activities in Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were inhibited by approx. 30–40% by antiserum. After centrifugation of the incubation mixtures, 85–100% of the transferase activities were removed from the supernatant by antiserum.

UDP-glucuronyltransferase activity was measured in precipitated enzyme-immunoglobulin complexes. Table 3 shows that approx. 40% of the bilirubin UDP-glucuronyltransferase activity was recovered in the immunoprecipitate, and confirms that antiserum only partially inhibits UDP-glucuronyltransferase activity. The specific activity of the isolated bilirubin UDP-glucuronyltransferase was 1.2 nmol/min per mg of protein, representing a 6-fold purification over the $(\text{NH}_4)_2\text{SO}_4$ fraction. Thus the antiserum only partially inhibited UDP-glucuronyltransferase activity towards the two substrates tested and also immunoprecipitated activity on centrifugation.

Immunoprecipitation of 1-naphthol glucuronyltransferase activity from Gunn- and Wistar-rat liver preparations

Immunoprecipitation of UDP-glucuronyltransferase activity from Gunn- and Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions was compared with 1-naphthol as substrate, because this transferase activity is easily detected in Gunn-rat liver preparations by a sensitive radioactive assay. The protein concentrations of the $(\text{NH}_4)_2\text{SO}_4$ fractions from the two strains were adjusted to the same value before incubation with antiserum. The results of the determination of 1-naphthol UDP-glucuronyltrans-

ferase activity remaining in the supernatant after immunoprecipitation are shown in Fig. 3. An apparent difference in the percentage of UDP-glucuronyltransferase activity immunoprecipitated was observed during this titration experiment. 1-Naphthol UDP-glucuronyltransferase was maximally immunoprecipitated from Gunn-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions at a much lower antiserum concentration than was required to immunoprecipitate activity from the Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fraction (Fig. 3). This result suggests that lower amounts of 1-naphthol UDP-glucuronyltransferase protein may exist in Gunn-rat liver. However,

Fig. 3. Immunoprecipitation of 1-naphthol UDP-glucuronyltransferase from Gunn- and Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions

(●) Gunn- or (○) Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with antiserum at 4°C for 24 h and were then assayed after centrifugation at 10000g for 5 min for UDP-glucuronyltransferase activity. One unit of activity represents 1 nmol of glucuronide formed/min.

Table 3. Bilirubin UDP-glucuronyltransferase activity of resuspended immunoprecipitates

Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with antiserum at 4°C for 24 h and then centrifuged at 10000g for 5 min. The washed immunoprecipitates and supernatants (see the text) were assayed for bilirubin UDP-glucuronyltransferase activity. One unit of activity represents 1 nmol of glucuronide formed/min. The data are expressed as means $\pm$ S.D., with the ranges of results obtained shown in parentheses; *n* is the number of experiments performed.

Antiserum (%) in incubation mixture	(n)	Bilirubin UDP-glucuronyltransferase activity		Recovery (%)
		In supernatant	In resuspended immunoprecipitate	
0	17	1.56 $\pm$ 0.77 (0.34–2.87)	0.0	0.0
100	17	0.0	0.48 $\pm$ 0.26 (0.11–0.99)	37.5 $\pm$ 25.1 (12.8–91.1)

inhibition of 1-naphthol UDP-glucuronyltransferase by antiserum may account for the difference. Indeed, Table 4 shows that 10% antiserum in the incubation mixture inhibits 62.5% of the 1-naphthol glucuronyltransferase activity of Gunn-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions, whereas the activity of the equivalent Wistar-rat liver preparation was only inhibited by 20%. Thus the difference in the titration curves in Fig. 3 can be explained by a much greater inhibition of Gunn-rat liver 1-naphthol UDP-glucuronyltransferase by antiserum.

UDP-glucuronyltransferase activities towards 2-aminophenol and 4-nitrophenol were also immunoprecipitated from solubilized Gunn-rat liver preparations with this antiserum.

Immunoprecipitation of rat liver UDP-glucuronyltransferase by using purified IgG

In order to facilitate identification and quantification of the UDP-glucuronyltransferase(s) present in Gunn- and Wistar-rat livers, the IgG fraction was purified from crude antiserum or pre-immune serum as described in the Experimental section.

Initially it was important to determine whether purified IgG immunoprecipitated UDP-glucuronyltransferase activities. Table 5 shows that UDP-glucuronyltransferase activities towards 4-nitrophenol, 1-naphthol and bilirubin were co-immuno-

precipitated from Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions by the IgG fraction of antiserum. Similarly 1-naphthol UDP-glucuronyltransferase activity was also immunoprecipitated from Gunn-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions. Further, UDP-glucuronyltransferase activity towards 4-nitrophenol and 1-naphthol can be measured in the resuspended immunoprecipitates obtained from Wistar-rat liver preparations. Thus UDP-glucuronyltransferase was indeed precipitated by the IgG fraction isolated from crude antiserum. As UDP-glucuronyltransferase activities towards a number of substrates are immunoprecipitated by crude antiserum, presumably all these activities are immunoprecipitated by the IgG fraction of antiserum.

Sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic analysis of immunoprecipitates

The immunoprecipitates from Gunn and Wistar rat liver were compared by SDS/polyacrylamide-gel-electrophoretic analysis (Fig. 4). In both cases three polypeptide bands of mol.wts. 59 000, 56 000 and 29 000 were observed and were identified as heavy chain of IgG, purified UDP-glucuronyltransferase and light chain of IgG respectively. No other polypeptides that might be identified as different-molecular-weight forms of UDP-glucuronyltransferase were observed in Wistar-rat liver immuno-

Table 4. *Inhibition of 1-naphthol UDP-glucuronyltransferase activity from Gunn and Wistar rat liver by antiserum*
Gunn- or Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with antiserum at 4°C for 24 h and were then assayed before and after centrifugation at 10 000 g for 5 min for 1-naphthol UDP-glucuronyltransferase activity. Values shown are the averages of three results, with the ranges shown in parentheses.

Antiserum (%) in incubation mixture	Strain ...	UDP-glucuronyltransferase activity			
		In supernatant before centrifugation (%)		In supernatant after centrifugation (%)	
		Gunn	Wistar	Gunn	Wistar
0		100.0	100.0	100.0	100.0
10		37.5 (32.0–42.6)	80.0 (67.8–86.1)	20.2 (6.8–40.0)	48.2 (36.5–42.1)

Table 5. *Immunoprecipitation of UDP-glucuronyltransferase with purified immunoglobulin G*
Gunn- or Wistar-rat liver $(\text{NH}_4)_2\text{SO}_4$ fractions were incubated with purified IgG at 4°C for 24 h and were assayed, after centrifugation at 10 000 g for 5 min, for UDP-glucuronyltransferase activity. The results are expressed as a percentage of the activity in controls incubated with IgG purified from pre-immune serum. The values shown are an average of four results, with the range shown in parentheses.

IgG in incubation mixture (%)	Substrate Strain ...	UDP-glucuronyltransferase activity in supernatant after centrifugation (%)			
		4-Nitrophenol Wistar	Bilirubin Wistar	1-Naphthol	
				Gunn	Wistar
0		100.0	100.0	100.0	100.0
100		19.1 (4.8–33.3)	13.2 (0.0–39.7)	3.78 (0.0–6.9)	9.66 (2.9–16.2)

Fig. 4. *SDS/polyacrylamide-gel electrophoresis of immunoprecipitates obtained from Gunn- and Wistar-rat liver*

Disc electrophoresis was performed with cylindrical 7.5% acrylamide gels (7.0 cm × 0.6 cm) in the presence of 0.1% SDS. Gel A, resuspended immunoprecipitate from Gunn-rat liver (NH_4)₂SO₄ fraction; gel B, resuspended immunoprecipitate from Wistar-rat liver (NH_4)₂SO₄ fraction. Gels were stained with 0.25% Coomassie Blue for 60 min and destained with acetic acid/methanol/water (7:5:43, by vol.). The direction of migration is from the top to the bottom; df, dye front; a, b and c indicate molecular weights of 59 000, 56 000 and 29 000 respectively.

Fig. 5. *Immunochemical quantification of microsomal UDP-glucuronyltransferase from Wistar- and Gunn-rat liver*

Radial immunodiffusion of Lubrol-solubilized liver microsomal fraction from Wistar (O) and Gunn (●) rats was performed as described in the Experimental section. 'Δ Area' is the area of the immunoprecipitin ring minus the area of the sample well.

weight may be hidden by the heavy chain of IgG. Qualitatively, the same complement of UDP-glucuronyltransferase protein(s) appears to have been immunoprecipitated from both rat strains.

Radial-immunodiffusion analysis

Radial immunodiffusion (see the Experimental section) was used as a method to quantify the amount of UDP-glucuronyltransferase protein in Gunn- and Wistar-rat microsomal fractions. Fig. 5 shows one of these experiments where the largest difference between the amounts of UDP-glucuronyltransferase in Gunn- and Wistar-rat liver was observed. A linear relationship exists between the area of the immunoprecipitin ring and the amount of Lubrol-solubilized protein from either Gunn- or Wistar-rat liver applied into each well in both cases, indicating that this method can be used to measure amounts of UDP-glucuronyltransferase protein. Four Gunn-rat and four Wistar-rat livers were assayed in four separate experiments, with different batches of purified IgG. The amount of UDP-glucuronyltransferase protein present in the deficient Gunn strain was observed to be 64% (43–95%) of the amount of UDP-glucuronyltransferase in Wistar-rat livers, and therefore it is possible that one form of UDP-glucuronyltransferase is absent from the Gunn-rat liver.

precipitates, although both bilirubin and 4-nitrophenol UDP-glucuronyltransferase activities were co-precipitated. However, a second UDP-glucuronyltransferase of slightly higher molecular

Discussion

Antiserum and the IgG fraction of antiserum slightly inhibited the solubilized Wistar-rat liver UDP-glucuronyltransferase, whereas the solubilized Gunn-rat liver 1-naphthol UDP-glucuronyltransferase is greatly inhibited by low concentrations of antiserum. Presumably the binding of IgG to the enzyme from Gunn-rat liver causes a greater steric hindrance of and prevents substrate access to the enzyme active site. The interaction of IgG and 1-naphthol UDP-glucuronyltransferase suggests that a conformational difference may exist between the Gunn- and Wistar-rat enzymes, which is also reflected by the lower activity of the solubilized Gunn enzyme.

This specific antiserum or the IgG fraction of this antiserum also forms precipitable complexes with solubilized Wistar-rat liver UDP-glucuronyltransferase such that enzyme activities towards 1-naphthol, 4-nitrophenol, morphine and bilirubin are detectable in resuspended immunoprecipitates. Indeed, immunoaffinity chromatography may be a good method for isolation and purification of bilirubin UDP-glucuronyltransferase.

It is important to note that the antigen that does not catalyse the glucuronidation of bilirubin is capable of eliciting the synthesis of antibodies that can precipitate this activity. If there is more than one UDP-glucuronyltransferase protein in the apparently homogeneous preparation of antigen (Burchell, 1978), inactive bilirubin UDP-glucuronyltransferase may be present and more than one antibody may have been produced in response to this antigen. However, different forms of this enzyme are likely to have some similar antigenic properties. One transferase form used as antigen may induce synthesis of antibodies specific for a dozen or more antigenic determinants on a protein of this size (Milstein *et al.*, 1979), and thus an IgG fraction may immunoprecipitate all UDP-glucuronyltransferase activities.

Different molecular-weight forms of UDP-glucuronyltransferase were not detected by SDS/polyacrylamide-gel electrophoresis of the immunoprecipitates, and only one immunoprecipitin line was observed during Ouchterlony double-diffusion analysis. However, different forms of UDP-glucuronyltransferase that have very similar molecular weights would not be detected by these immunochemical experiments. Multiple forms of UDP-glucuronyltransferase may exist, but may only be separable on a charge basis such as DEAE-cellulose chromatography (Del Villar *et al.*, 1977; Tukey *et al.*, 1978). More recently, Bock *et al.* (1979) have reported that morphine UDP-glucuronyltransferase may have a slightly higher mol.wt. (56000) than 4-nitrophenol UDP-glucuronyltransferase (54000), although the definitive

experiment of gel electrophoresis of mixed preparations was not done. To date we have not been able to identify any different forms of UDP-glucuronyltransferase on a molecular-weight basis.

A functionally defective form of UDP-glucuronyltransferase with potential activity towards 2-aminophenol, 4-nitrophenol and 1-naphthol has been purified from Gunn-rat liver (Weatherill & Burchell, 1978), and in Gunn-rat liver amounts of this transferase and of those with activity towards morphine and testosterone may exist similar to those in Wistar-rat liver (Lalani & Burchell, 1979; B. Burchell & K. W. Bock, unpublished work; Abrams & Elliott, 1974; Jacobson *et al.*, 1975). Only bilirubin UDP-glucuronyltransferase protein may be absent from the Gunn-rat liver, consistent with the complete absence of this enzyme activity as illustrated by Table 1, and a small decrease in total UDP-glucuronyltransferase protein as measured by radial immunodiffusion. If so, the genetic deficiency of UDP-glucuronyltransferase appears to be extremely complicated by the existence of a functional defective form, the possible absence of a second form and an induction deficiency (Vainio & Hietanen, 1974). Only a large genetic deletion would explain this enzyme deficiency. The most simple hypothesis, proposing the existence of one form of UDP-glucuronyltransferase with a single altered amino acid in the active site restricting access of certain substrates, does not currently explain all of the accumulated data.

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