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CONJUGATION REACTIONS IN HEPATOCYTES ISOLATED FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract—The activities of three drug conjugation reactions, glutathione, glucuronic acid and sulphate conjugation and the synthesis of glutathione, have been measured in hepatocytes isolated from streptozotocin-induced male diabetic rats.

The intracellular content of reduced glutathione (GSH) was decreased in diabetic rat hepatocytes compared with controls. Following depletion of the intracellular GSH stores with diethylmaleate, the resynthesis of GSH in the presence of 0.5 mM L-methionine, occurred faster in diabetic rat hepatocytes than in those from control rats indicating that the cystathione pathway may be more efficient in the diabetic animals. In contrast, there was no significant difference in the resynthesis of GSH between control and diabetic rat hepatocytes in the presence of L-cysteine.

The GSH conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) was deficient in diabetic rat hepatocytes, although only the effect on the former reaction was statistically significant ($P < 0.05$). The V_{max} for CDNB conjugation was significantly lower ($P < 0.05$) in cytosolic fractions prepared from diabetic rat liver than in control rat liver fractions. This was accompanied by an increase in the affinity of the enzyme for CDNB. In contrast, the V_{max} and K_m for the conjugation of DCNB in cytosolic fractions were unaffected by the induced-diabetes. Glucuronic acid conjugation of both 1-naphthol and phenolphthalein was markedly deficient in diabetic rat hepatocytes. The intracellular concentrations of the cofactor for glucuronidation, UDP–glucuronic acid, were decreased in diabetic rat liver and this was thought to contribute to the defect in glucuronidation. The sulphation of 1-naphthol was not significantly altered by the induced diabetes.

Deficiencies in glutathione and glucuronic acid conjugation in streptozotocin-induced diabetic rats may result in an increased susceptibility to xenobiotic induced cytotoxicity.

Alterations in drug oxidation and conjugation are known to occur in experimentally-induced diabetes, however, most of the work has been carried out using hepatic microsomal fractions [1–11]. The results of these studies are often difficult to interpret, particularly where membrane bound enzymes, such as UDP-glucuronyltransferase, are involved in the metabolic pathways. This paper demonstrates that the activities of two conjugation enzymes, UDP-glucuronyltransferase (EC 2.4.1.17) and glutathione-*S*-transferase (EC 2.5.1.18) are altered in hepatocytes isolated from streptozotocin-induced diabetic rats, whilst the activity of a third enzyme, phenolsulphotransferase (EC 2.8.1), is unaltered.

We have previously reported that the formation of glucuronic acid conjugates from 1-naphthol and 4-nitrophenol was defective in untreated "native" microsomes from streptozotocin-induced diabetic rats [9, 10]. Streptozotocin-induced diabetes did not alter the glucuronidation of either phenolphthalein or paracetamol by "native" microsomes [10]. This indicates that the defect in UDP-glucuronyltransferase activity may be isozyme specific. The defect in 4-nitrophenol glucuronidation was abolished by maximal detergent (Triton X-100) activation of the microsomes. In contrast, allosteric activation by UDP *N*-acetyl glucosamine and/or magnesium ions did not significantly alter the defect [9]. Increased latency of the transferase enzyme in streptozotocin-induced diabetes was thought to be responsible for the defect in hepatic microsomal 4-nitrophenol glucuronidation. Rouer *et al.* [11] found similar results in experimentally and spontaneously (db/db and ob/ob mice) diabetic mice; 4-nitrophenol glucuronyltransferase activity was lower in both experimentally-induced and spontaneously occurring diabetes and the defect was abolished by digitonin activation of the microsomes.

In marked contrast to these reports of deficient glucuronyltransferase activity in hepatic microsomes from diabetic animals, the conjugation of 4-nitrophenol in hepatocytes prepared from streptozotocin-induced diabetic rats has been shown to be increased compared with controls [12, 13]. The reason for this apparent discrepancy is unclear, but the relevance of the results obtained in the microsomal subfractions of liver to metabolism in the whole cell has been questioned. UDP-glucuronyltransferase is a membrane-bound enzyme and its activity may be differentially modified during preparation of microsomes from diabetic rat liver.

The activity of glucocorticoid sulfotransferases is known to be elevated in streptozotocin-treated rats [14]. However, there is little information available on the sulphation of xenobiotics in experimentally-induced diabetes. In general, the sulphation of xenobiotics seems less sensitive to the effects of enzyme inducers and fasting than glucuronidation [15, 16].

The blood concentration of reduced glutathione (GSH)* has been shown to be decreased in untreated ketotic diabetic patients [17, 18] and liver GSH content is decreased in steptozotocin treated female rats [19]. There are contradictory reports in the literature concerning the effect of experimentally-induced diabetes on glutathione-*S*-transferase activity [11, 20], and little or no data are available on the ability of diabetic rat liver to synthesis GSH when the intracellular supply has been depleted.

In the present study the activities of UDP-glucuronyltransferases, phenolsulphotransferase and glutathione-*S*-transferase were measured in hepatocytes isolated from streptozotocin induced male diabetic rats. Glucuronyltransferases exist as a family of isozymes with different substrate specificities, induction and developmental characteristics [21, 22]. The activity of the 3-methylcholanthrene inducible form of the enzyme (GT_1) was measured using 1-naphthol as substrate and that of the phenobarbitone inducible isozyme (GT_2) using phenolphthalein [10]. In addition, the hepatic UDP-glucuronic acid content was measured in both control and diabetic liver. Phenolsulphotransferase activity was measured using 1-naphthol. Intracellular GSH content and the rate of GSH synthesis following depletion by diethylmaleate (DEM) were measured in addition to GSH conjugation. 1-Chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) were used as substrates for at least four isozymes of rat liver glutathione-*S*-transferase [23].

MATERIALS AND METHODS

Materials

Phenolphthalein glucuronide, 1-naphthol sulphate, 1-naphthol glucuronide, tetrabutylammonium hydrogen sulphate, reduced glutathione, streptozotocin, 1-chloro-2,4-dinitrobenzene, DL-buthionine-[*S*,*R*]-sulfoximine, UDP-glucuronic acid and Trypan Blue were obtained from Sigma. Collagenase was from Boehringer Mannheim, 3,4-dichloronitrobezene from Aldrich and William's E medium from Flow Laboratories.

Methods

(i) *Treatment of animals*

Male Sprague–Dawley rats (180–220 g) were used. Rats were starved for 24 hr prior to receiving 60 mg/kg streptozotocin, intravenously, in acetate buffer, pH 4.5, on day 1. On day 6 the rats were divided into two groups. Hepatocytes were prepared from the first group and the second group received daily treatment with 16 IU insulin (Port Insulatard, Wellcome), subcutaneously, for 6 days before hepatocytes were prepared on day 12.

(ii) *Assessment of the induced diabetes*

The induced diabetes was assessed as described previously [9, 10]. Blood and urinary glucose concentrations were determined by a specific enzymatic method using O-dianisidine as the chromogen according to Sigma Technical Bulletin No. 310. On day 6 after treatment, streptozotocin-treated rats had blood glucose concentrations over 250 mg/100 ml compared with 80–100 mg/100 ml in control rats. Between 4 and 6 days after treatment diabetic rats excreted approximately 16 g glucose/24 hr in the urine. All signs of streptozotocin-induced diabetes were readily reversed within 48 hr of initiating insulin treatment.

(iii) *Preparation and incubation of hepatocytes*

Hepatocytes were prepared by collagenase perfusion as described previously [24] and the viability of the preparations was assessed by Trypan Blue exclusion. Control rat preparations were $90 \pm 1\%$ ($N = 12$) viable and diabetic rat preparations $87 \pm 2\%$ ($N = 14$). Incubations were carried out at 10^6 viable cells/ml in Krebs–Henseleit buffer, pH 7.4, containing 10 mM Hepes, in 50 ml round bottomed flasks at 37° under 95% O_2/5% CO_2 [25].

(iv) *Preparation of hepatic cytosol*

Rat livers were washed in ice-cold 0.01 M Tris buffer, pH 7.4, containing 1.15% (w/v) KCl and homogenised in 4 vol. of ice-cold 0.1 M Tris buffer, pH 7.4, containing 1.15% (w/v) KCl and 15% (v/v) glycerol. The homogenate was centrifuged at 15,000 *g* for 20 min at 0–4°, and the supernatant centrifuged again at 105,000 *g* for 50 min at 0–4° to separate the cytosolic (supernatant) and microsomal fractions. The cytosol was stored in 1 ml aliquots at −80° until required.

(v) *Analytical methods*

(i) *Measurement of UDP-glucuronic acid.* UDP-glucuronic acid was measured essentially using the procedure of Bock and White [26]. Approximately 2 g of liver was excised and homogenised in 10 ml 0.3 M glycine–0.2 M trichloroacetic acid buffer, pH 2.2, and 0.05 μCi UDP-^{14}C-glucuronic acid was added. The homogenate was centrifuged at 100,000 *g* for 10 min, and 5 ml of the supernatant removed and adjusted to pH 7.4 with 5 M NaOH. Acetone (6 vol.) at −20° was added and after standing for 30 min at −20° the precipitated UDP-glucuronic acid was recovered by centrifugation (3000 *g* for 5 min). The precipitate was washed with ether, recentrifuged and excess ether removed *in vacuo*. The precipitate was dissolved in 0.5 ml 0.1 M Tris–HCl, pH 7.4. Recovery of UDP-glucuronic acid (based on UDP-^{14}C-glucuronic acid) extracted from 8 rat livers was $77 \pm 12\%$ (mean ± SD). The concentration of UDP-glucuronic acid in the preparations was determined by an enzymatic procedure using 4-nitrophenol as the substrate for the GT reaction in maximally detergent activated guinea pig microsomes prepared as described for rats [27]. Each incubation contained 5 mM $MgCl_2$, 1 mM 4-nitrophenol and 2 mg of microsomal protein in a final volume of 1 ml of 0.1 M Tris–HCl buffer, pH 7.4.

(ii) *Measurement of GSH content and synthesis.* GSH concentrations were measured by the method of Saville [28]. This method measures cellular

* Abbreviations used: GSH, reduced glutathione; GT, UDP-glucuronyltransferase; DEM, diethylmaleate; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloronitrobenzene; Hepes, *N*-2-hydroxyethyl-piperazine-*N*^{1}-2-ethane sulfonic acid; BSO, DL-buthionine-*SR*-sulfoximine.

reduced thiol content of which 90–95% is GSH. To measure resynthesis of GSH, the cellular GSH content was depleted by incubation with 0.02% (v/v) DEM for 30 min at 37°. After this period the cells were allowed to sediment and resynthesis was measured after resuspension of the cells (10^6 cells/ml) in William's E medium supplemented with either 0.5 mM L-methionine or 0.5 mM L-cysteine. In some experiments 5 mM DL-buthionine-[*S,R*]-sulfoximine (BSO) was included as an inhibitor of GSH synthesis [29]. One-millitre samples were removed at timed intervals up to 2 hr for the determination of GSH.

(iii) *Measurement of conjugation reactions.* Glucuronic acid and sulphate conjugation of 100 μM 1-naphthol and 100 μM phenolphthalein was measured in hepatocytes (10^6/ml) as described previously [30] and the conjugates separated by reversed-phase ion-pair high pressure liquid chromatography [31] using tetrabutylammonium hydrogen sulphate as the ion-pairing agent. The GSH conjugation of 50 μM CDNB and 50 μM DCNB was measured in hepatocytes (10^6/ml). These reactions were also carried out in hepatic cytosol in the presence of 5 mM GSH at a range of concentrations between 1 mM and 1 μM for CDNB and 1 mM and 10 μM for DCNB. Reactions were carried out under conditions of linearity with respect to both time and protein content and the conjugation rate was quantified by direct spectrophotometry as described by Habig and Jakoby [23]. Protein concentrations were determined by the method of Lowry *et al.* [32] using bovine serum albumin as the standard. The Michaelis–Menten parameters, K_m and V_{max}, were calculated by non-linear regression analysis using BMDP/PAR derivative free non-linear regression analysis [33].

RESULTS

Table 1 shows that the GSH content of hepatocytes from streptozotocin-induced diabetic rats was significantly ($P < 0.02$) lower than in control rat hepatocytes. Incubation of the hepatocytes with 0.02% (v/v) DEM for 30 min decreased cellular GSH levels to approximately 10% of the initial fresh cell content (after DEM treatment control rat cells contained 3.3 nmol GSH/10^6 cells and diabetic rat cells contained 3.8 nmol GSH/10^6 cells). The viability of the cell suspension was not altered by this treatment. Following DEM depletion GSH resynthesis occurred readily in the presence of 0.5 mM L-methionine or 0.5 mM L-cysteine. The time course of GSH resynthesis in the presence of L-methionine and of L-cysteine are shown in Fig. 1A and 1B respectively, for both control and diabetic animals. Resynthesis was linear for at least 45 min, and in the presence of L-methionine resynthesis proceeded more rapidly in diabetic rat cells than in those from control rats, particularly over the first 30 min of incubation. GSH resynthesis in the presence of L-cysteine was similar in both groups of animals. BSO (5 mM) inhibited the synthesis of GSH in the presence of both L-methionine and L-cysteine. The rate of GSH synthesis was calculated over the linear part of the resynthesis curve for each experiment (Table 1). In the presence of 0.5 mM L-methionine the rate of GSH synthesis was significantly faster ($P < 0.01$) in diabetic rat hepatocytes than in controls.

As observed previously [23] CDNB conjugation with GSH is faster than that with DCNB in rat liver. In hepatocytes from diabetic animals the formation of GSH conjugates with both substrates (50 μM) was deficient (Table 2), although the effect was significant only with CDNB. Figures 2A and B show the formation of GSH conjugates with CDNB and DCNB in cytosol prepared from diabetic and control rat livers. The transferase activity towards CDNB was significantly lower in diabetic animals at 1 μM, 5 μM, 250 μM, 500 μM and 750 μM concentrations. The activity towards DCNB was similar in both groups of animals. Using these data the V_{max} and K_m of the reactions were calculated. The V_{max} for CDNB was significantly lower in diabetic rat cytosol, whereas that for DCNB conjugation was unchanged (Table 3). The affinity of the enzyme for CDNB was also altered by the induced diabetes. Figures 3A and B show that the formation of the glucuronic acid conjugates of both 1-naphthol and phenolphthalein was linear with time for at least 15 min in hepatocytes. 1-Naphthol glucuronidation was 2-fold faster than that of phenolphthalein. The glucuronic acid conjugation of both substrates was markedly decreased in diabetic rat hepatocytes. Table 3 shows that the rate of glucuronidation (calculated from the data shown on Figs 3A and B) was 2–3-fold higher in control rats that in diabetic rats ($P < 0.01$).

The UDP-glucuronic acid content of diabetic rat

Table 1. GSH content and rate of GSH synthesis in hepatocytes from control and diabetic rats

	GSH content (nmol/10^6 cells)	Rate of GSH synthesis (nmol/min/10^6 cells)	
		L-met	L-cys
Control	39.6 ± 3.2 (7)	0.19 ± 0.02 (8)	0.21 ± 0.03 (5)
Diabetic	29.2 ± 1.9* (12)	0.37 ± 0.04** (9)	0.20 ± 0.04 (5)

Data are presented as mean ± SEM, with the number of experiments in parentheses.
Statistical significance was determined using a non-paired Student's *t*-test.
*$P < 0.02$, **$P < 0.01$.
Significance values refer to differences between control and diabetic animals.
L-met, L-methionine; L-cys, L-cysteine.

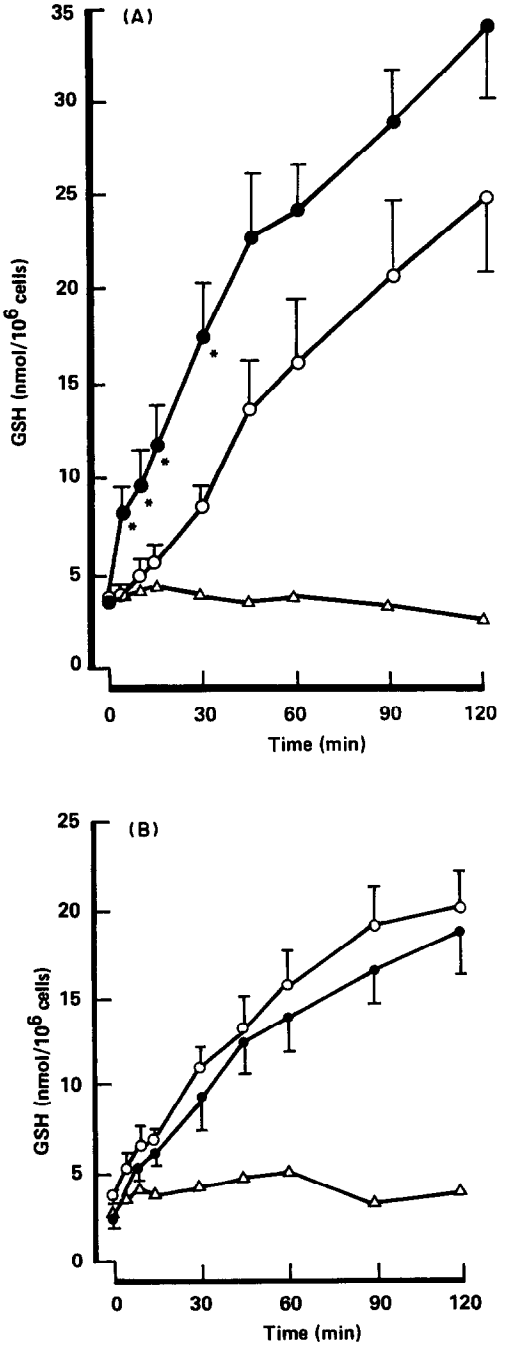

Fig. 1. GSH resynthesis in the presence of L-methionine (A) and L-cysteine (B) after diethylmaleate treatment of control (○, N = 5) and diabetic (●, N = 5) rat hepatocytes. In some control rat incubations buthionine sulfoximine was present in addition to either L-methionine or L-cysteine (△, N = 2). Error bars represent the SEM. *P < 0.05, by unpaired Student's *t*-test. Significance values refer to differences between control and diabetic rats.

livers was markedly reduced compared with that of control rat liver (216 ± 17 (N = 4) compared with 314 ± 18 (N = 4) nmol/g liver in control livers, P < 0.01 by non-paired Student's *t*-test).

Table 2. GSH conjugation with 50 μM 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) in hepatocytes

	CDNB (nmol/min/10^6 cells)	DCNB (nmol/min/10^6 cells)
Control	3.4 ± 0.4 (6)	2.6 ± 0.6 (4)
Diabetic	2.1 ± 0.2* (6)	1.3 ± 0.2 (5)

Data are presented as the mean ± SEM, with the number of experiments in parentheses.

Statistical significance was determined by non-paired Student's *t*-test.

* P < 0.05.

Significance values refer to differences between control and diabetic rats.

Phenolphthalein was not significantly sulphated by rat hepatocytes as found by previous authors [34], so sulphation was measured using only 1-naphthol. In contrast, to the marked effect of streptozotocin-induced diabetes on glucuronic acid conjugation, there is no significant alteration in the sulphation of 1-naphthol in diabetic rats (Fig. 4 and Table 4).

Treatment of the diabetic rats for 6 days with insulin abolished all the alterations in both glucuronic acid and GSH conjugation pathways (data not shown).

DISCUSSION

The alterations in conjugation reactions observed in this study will have important consequences for the detoxification of xenobiotics by diabetic rats. It has been shown previously that in conditions where glucuronidation is deficient, the cytotoxicity, mutagenicity and carcinogenicity of many compounds is exacerbated [35, 36]. Thus, defective glucuronic acid conjugation could lead to increased susceptibility to xenobiotic induced toxicities in the diabetic rats. A decrease in hepatic GSH content has been previously demonstrated in streptozotocin-induced diabetic rats and this decrease led to a greater susceptibility to paracetamol-induced hepatic GSH depletion [19]. This is likely to result in increased susceptibility to paracetamol induced hepatocellular damage. The decrease in potential for GSH protection against cell toxicity may also be partly responsible for the increased susceptibility to the toxicity of chloroform and other chlorinated hydrocarbons observed in experimentally-induced diabetic rats [37, 38].

Following depletion by DEM, control rat hepatocytes can resynthesise GSH equally well when supplied with either L-methionine or L-cysteine as precursor amino acids. The hepatocytes therefore contain an efficient cystathionine pathway for the conversion of L-methionine to L-cysteine as found by previous authors [39]. BSO effectively inhibits GSH synthesis in the presence of both L-methionine and L-cysteine. Diabetic rat hepatocytes synthesise GSH using L-cysteine at approximately the same rate as those from control rats. In contrast they are capable of producing GSH from L-methionine twice as fast as control rat hepatocytes indicating that

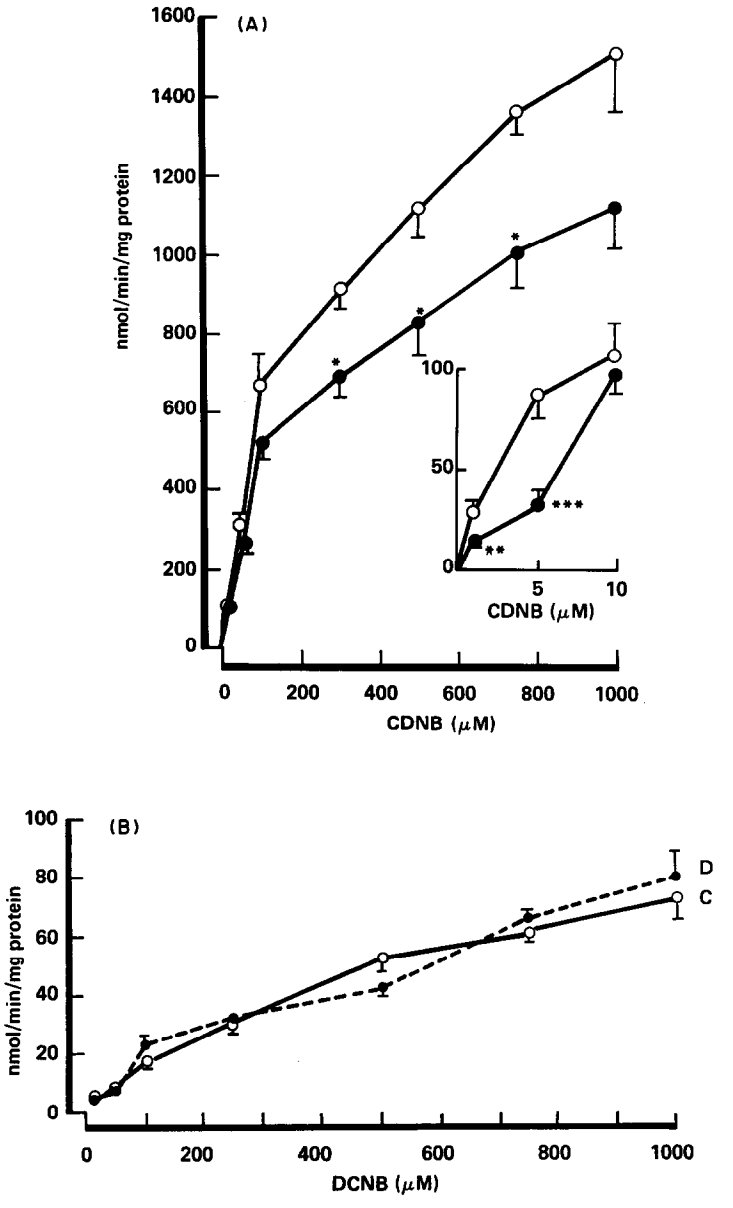

Fig. 2. Formation of GSH conjugates from 1-chloro-2,4-dinitrobenzene (A) and 3,4-dichloronitrobenzene (B) by hepatic cytosol from control (○, N = 5) and diabetic (●, N = 7) rats. Error bars represent the SEM. *P < 0.05; **P < 0.02; ***P < 0.01, by non-paired Student's *t*-test. Significance values refer to differences between control and diabetic animals.

Table 3. Michaelis–Menten kinetics for the GSH conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) in hepatic cytosol

	CDNB		DCNB	
	K_m	V_{max}	K_m	V_{max}
Control	229.5 ± 16.0 (5)	1815.3 ± 210.6 (5)	469.7 ± 65.0 (5)	84.9 ± 18.0 (5)
Diabetic	180.6* ± 10.5 (7)	1209.4* ± 115.6 (7)	499.2 ± 145.2 (7)	102.1 ± 17.2 (7)

K_m is expressed as μM and V_{max} as nmol/min/mg cytosolic protein.
Data are presented as mean ± SEM, with the number of experiments in parentheses.
Statistical significance was determined by non-paired Student's *t*-test.
* P < 0.05.
Significance values refer to differences between control and diabetic animals.

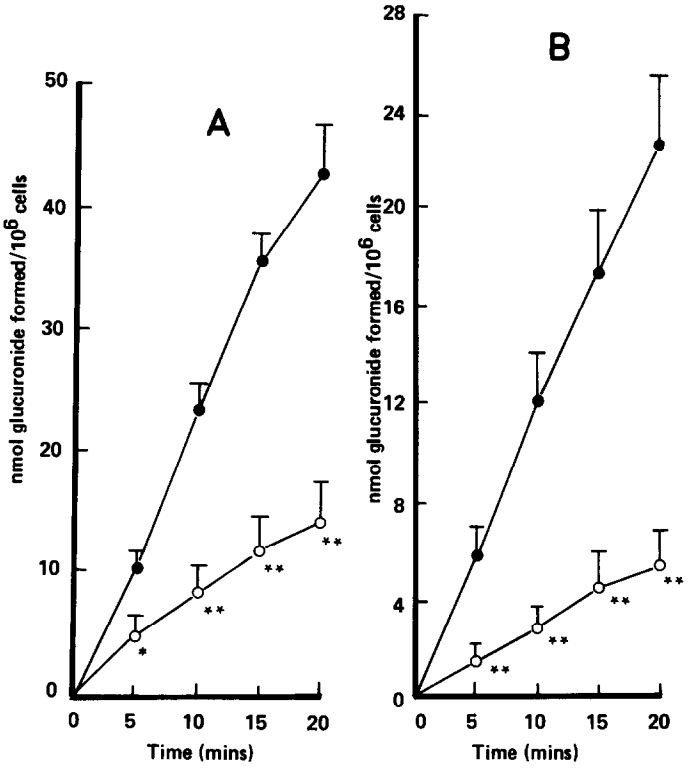

Fig. 3. Glucuronic acid conjugation of 1-naphthol (A) and phenolphthalein (B) in hepatocytes from control (●, N = 9) and diabetic (○, N = 6) rats. Error bars represent the SEM. *P < 0.02, **P < 0.01, by unpaired Student's *t*-test. Significance values refer to differences between control and diabetic rats.

the cystathionine pathway may be more efficient in diabetic rat cells than in those from control rats. This may be due to induction of the enzymes involved, and we are at present investigating this possibility.

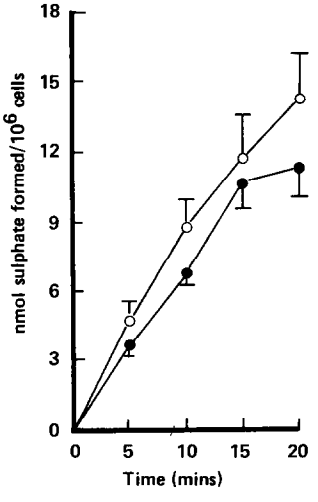

Fig. 4. Sulphation of 1-naphthol in hepatocytes from control (●, N = 9) and diabetic (○, N = 6) rats. Error bars represent the SEM.

Hassing *et al.* have also shown that recovery of hepatic GSH levels following depletion by paracetamol is faster in steptozotocin-induced diabetic rats in controls [19].

Diabetic rat hepatocytes contain less GSH than controls despite their increased capacity for synthesis. This may be due to either increased intrahepatic catabolism of GSH or to increased GSH efflux from the liver. The major enzyme catabolising GSH is γ-glutamyl transferase and hepatic levels of this enzyme are not significantly altered by the induced diabetes (unpublished observation). During fasting the hepatic GSH content has been shown to be decreased [40–42]. This decrease was also accompanied by an increase in the ability of the liver to synthesise GSH [41] and an increase in GSH turnover [42]. Lauterburg and coworkers have shown that the alterations in hepatic GSH status during fasting are due to an increased efflux of GSH from the liver [42]. This may also occur during streptozotocin-induced diabetes and is currently under investigation.

The GSH conjugation of CDNB was decreased both in hepatocytes and in hepatic cytosol prepared from diabetic animals. This defect was apparently due to a decrease in the V_{max} of the transferase enzyme activity which was accompanied by an increase in the affinity of the enzyme for CDNB. In contrast, DCNB conjugation was unaltered in the cytosol, whereas a decrease in activity, although not

Table 4. Glucuronidation and sulphation of 1-naphthol (N) and phenolphthalein (P) by hepatocytes from control and diabetic rats

	N-glucuronidation	*N*-sulphation (nmol/min/10^6 cells)	*P*-glucuronidation
Control	2.91 ± 0.17 (9)	0.80 ± 0.05 (9)	1.20 ± 0.14 (9)
Diabetic	1.15 ± 0.30* (6)	0.99 ± 0.14 (6)	0.36 ± 0.11* (6)

Data are presented as mean ± SEM, with the number of experiments in parentheses.
Statistical significance was determined by non-paired Student's *t*-test.
* $P < 0.01$.
Significance values refer to differences between control and diabetic rats.

statistically significant, was observed in diabetic rat hepatocytes. Excess GSH (5 mM) was added to the incubations with cytosol while the conjugation reactions in hepatocytes relied upon the intracellular supply of GSH. The decrease in GSH conjugation in hepatocytes may be partly due to the observed decrease in intracellular GSH. However, this is unlikely as it has previously been demonstrated that GSH conjugation is unaffected even when the intracellular content falls below 50% of the normal hepatic levels [43, 44]. There may be an alteration in the structure of hepatic cytosolic GSH-*S*-transferase in diabetes analogous to changes in the structure of the cytochrome P-450 enzyme proteins which have previously been demonstrated in streptozotocin-induced diabetes [45, 46]. Younes *et al.* also found that GSH-*S*-transferase activity towards CDNB decreased in streptozotocin induced diabetic rats, whereas the GSH conjugation of an epoxide substrate was unaffected [20]. In contrast, in streptozotocin-induced diabetic mice the conjugation of CDNB was increased [11]. The effect of streptozotocin-induced diabetes on the activity of GSH-*S*-transferase is, therefore, both isozyme and species dependent.

This study has shown that glucuronic acid conjugation of both 1-naphthol and phenolphthalein is markedly deficient in hepatocytes isolated from diabetic rats. In contrast we have previously found that in "native" microsomes from diabetic rat liver only the GT_1 type of substrates, 1-naphthol and 4-nitrophenol, showed a decrease in glucuronic acid conjugation; the glucuronidation of phenolphthalein and paracetamol (GT_2 substrates) being unaltered [10]. The observed decreases in the activity of GT_1 substrates in diabetic rat liver microsomes were shown to be due to a decrease in the V_{max} of the reaction: the k_m of the enzyme for 4-nitrophenol being unaltered [47]. Increased membrane constraint was proposed as the mechanism responsible for the transferase defect in microsomal experiments [9, 10]. In isolated hepatocytes it appears that an additional factor may be contributing to the altered glucuronic acid conjugation, causing a defect in phenolphthalein glucuronidation in addition to that of 1-naphthol.

An important difference between the experiments carried out using the hepatic microsomal system and the hepatocytes for studying glucuronic acid conjugation lies in the supply of the cofactor for the reaction, UDP-glucuronic acid. For microsomal experiments, UDP-glucuronic acid was supplied to the reaction in excess, whereas the glucuronidation of substrates in hepatocytes relies entirely upon the endogenous concentration of UDP-glucuronic acid. The intracellular UDP-glucuronic acid concentration is known to control the extent of glucuronidation of substrates in hepatocytes to a large extent [15, 16, 48]. This study has shown that the UDP-glucuronic acid content of diabetic rat liver is significantly lower than that in control rat liver and this is in agreement with two previous reports [6, 49] of decreased hepatic UDP-glucuronic acid content in alloxan-induced diabetes. This decrease in UDP-glucuronic acid availability may be caused by lower NAD levels in the livers of streptozotocin-induced diabetic animals [50] and/or a decrease in the activity of hepatic UDP-glucose dehydrogenase [6, 49]. There has also been a contradictory report of increased liver UDP-glucuronic acid concentrations in streptozotocin-induced diabetic rats [51]. However, these authors appear to have found very low levels of UDP-glucuronic acid in control rat livers. The concentration of UDP-glucuronic acid in hepatocytes is likely to be limiting the extent of glucuronidation of both 1-naphthol and phenolphthalein in diabetic rat hepatocytes. This limiting factor may be operating in addition to alterations in the GT enzyme concentration and/or structure and its membrane constraint.

In contrast to the results obtained in this study using hepatocytes and also to those obtained in perfused livers from streptozotocin-induced diabetic rats [47], Eacho and and coworkers observed an increase in the conjugation of 4-nitrophenol in hepatocytes isolated from male streptozotocin-induced diabetic rats. They attributed this to an increased availability of UDP-glucuronic acid in diabetic rat liver, despite the fact that sulphation was predominant in their system at the concentrations of 4-nitrophenol used, up to 100 μM. They substantiated this conclusion with evidence that the V_{max} of 4-nitrophenol glucuronidation was not altered in microsomes from diabetic rat liver. However, they investigated enzyme kinetics only in microsomes which had been maximally activated by Triton X-100, and, therefore, no difference in activity would have been expected between the diabetic and control livers [9]. The reason for the discrepancy between the results of the present study and those of Eacho and coworkers may be because Eacho *et al.* starved their control rats

overnight prior to experiments in order to achieve glycogen depletion in the livers analogous to that present in the diabetic rat livers. Starvation is known to decrease hepatic glucuronide formation [15, 16] and this would account for the apparent increase in GT activity they observed in hepatocytes from the non-starved diabetic rats.

The sulphation of 1-naphthol was increased slightly in diabetic rat hepatocytes after 20 min incubation (Fig. 4). This is thought to be as a result of the decrease in the glucuronidation of 1-naphthol and not due to a specific effect of the induced diabetes on sulphation. Sulphation is, generally, less sensitive to the effects of nutrition and enzyme inducers than glucuronidation [15, 16].

Insulin treatment of the diabetic rats abolished both the signs of the induced diabetes, for example the hyperglycaemia, glycosuria and polyuria, and all the alterations in metabolism observed with GSH and glucuronic acid conjugation. This suggests that the defects in conjugation were due to the induced diabetes and not to an effect of the streptozotocin *per se*. The diabetes induced defects in conjugation remained unaltered 12 days after the administration of streptozotocin, in the absence of insulin treatment.

Deficient monooxygenase activity occurs in spontaneously diabetic Wistar rat liver analogous to that observed in streptozotocin-induced diabetic rat livers [52]. Hepatic microsomes from spontaneously diabetic mice also show defects in glucuronyltransferase activity towards 4-nitrophenol [11]. These are important findings and the implications of the results obtained in diabetic animals must be assessed for their relevance to diabetic patients. These patients often receive a plethora of different drugs concomitantly with either oral hypoglycaemic or insulin therapy [53]. Any deficiency in the drug detoxifying mechanisms could lead to a greater incidence of adverse side effects of drugs in diabetic patients.

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