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GLUCURONIDATION IN THE RAT INTESTINAL WALL

COMPARISON OF ISOLATED MUCOSAL CELLS, LATENT MICROSOMES AND ACTIVATED MICROSOMES

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Abstract—Glucuronidation and sulphation of 1-naphthol and 7-hydroxycoumarin was studied in isolated rat intestinal epithelial cells and in microsomes prepared from these cells. In the isolated cells formation of 1-naphthol sulphate could not be detected. Sulphate conjugates of 7-hydroxycoumarin constitute a minor portion of total conjugates formed. Maximum glucuronidation rates for 1-naphthol and 7-hydroxycoumarin do not differ significantly from each other (approximately 12.5 nmoles/min·g intestine). The intestinal microsomal UDP-glucuronosyltransferase, prepared from isolated cells, could be activated *in vitro* by Triton X-100 and $MgCl_2$. Activation increased both K_m^{app} and V_{max} for 1-naphthol; K_m^{app} for UDP-glucuronic acid was decreased by activation with $MgCl_2$ but increased again by further addition of Triton X-100. In fully activated microsomes K_m^{app} for 1 naphthol was 69.7 ± 13.9 μM and V_{max} was 70.0 ± 3.9 nmoles/min·mg microsomal protein; K_m^{app} for UDP-glucuronic acid was 0.67 ± 0.06 mM. The glucuronidation rate (expressed as nmoles/min·g intestine) in microsomes is substantially higher than in isolated cells. It appears that glucuronidation in intact cells is limited by factors other than the extracellular substrate concn. Both cellular uptake of the substrate and availability of UDP-glucuronic acid can play a significant role. It is concluded that isolated mucosal cells are more suitable for predicting intestinal first-pass metabolism of phenolic xenobiotics than intestinal microsomes, because cellular substrate uptake and cosubstrate availability appear to be important determinants of the maximum glucuronidation rate.

Drug metabolism activity in the intestinal wall is considerably less than in the liver [1, 2]. However, in comparison with phase I drug metabolism, phase II metabolism is relatively important [2, 3] and can affect bioavailability of drugs to a large extent. Up to 60% of an oral or intraduodenal dose of phenolic drugs like salicylamide [4], ethinyl estradiol [5] or morphine [6] can be metabolized in the intestinal wall during absorption.

Conjugation with both sulphate and glucuronic acid can take place in the intestinal wall [7]. The relative proportions of sulphate and glucuronide production appear to be species-specific [4, 8], but can also be dependent on the substrate used [9]. Functional heterogeneity of UDP-glucuronosyltransferase (UDPGT)* towards two groups of substrates has been identified in the rat [10] and mouse [11] intestinal wall. Furthermore, the distribution between sulphate and glucuronide formation may depend on the extracellular substrate concn, or can be influenced by artefacts arising from isolation and/or incubation conditions [12, 13]. We recently found that the glucuronidation rate in rat intestinal epithelial cells, isolated by a low-amplitude high-frequency method, is about two-fold higher than in cells isolated with an enzymic procedure [14].

In order to estimate the *in vivo* significance of intestinal conjugative metabolism, several models including microsomes [15–17], isolated epithelial cells [9, 14, 18, 19], everted sacs [8, 20–22], vascularly perfused isolated intestinal segments [23], and *in situ* perfused intestinal loops [8, 16, 20, 24, 25] have been used. In addition to drugs [15, 16, 19, 20, 22], model xenobiotic substrates like 1-N [16, 17, 19, 21, 24, 25] and 7-HC [8, 9, 14, 18] have been used extensively. For making adequate extrapolations to the *in vivo* situation it is helpful to know the exact quantitative relationship between different *in vitro* model systems. Direct comparisons between perfused livers, isolated hepatocytes and subcellular systems [26, 27] suggest that perfused livers and isolated hepatocytes more closely mimic the *in vivo* situation than do subcellular fractions. Concerning the intestinal wall no detailed comparison of the conjugative capacity of cellular and subcellular systems is available (but see Refs 17 and 18).

Until now all published studies of the intestinal microsomal UDPGT have used fully activated microsomes [15–17, 28]. Activation is considered to be the result of the isolation procedure [16, 29]. It seems unlikely, however, that the intestinal UDPGT in its normal cellular environment is fully activated. Evidence obtained from hepatic microsomes indicates that the UDPGT in its cellular environment

* Abbreviations: 7-HC, 7-hydroxycoumarin; LDH, lactate dehydrogenase (EC 1.1.1.27); 1-N, 1-naphthol; PBS, phosphate-buffered saline; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucuronosyltransferase (EC 2.4.1.17).

is neither fully activated nor fully latent; partial activation by endogenous substances may be important [26, 30]. Since we have recently developed an intestinal microsomal model, in which the UDPGT exhibits an appreciable and reproducible degree of latency [31], it is now possible to make a comparison of the glucuronidation rate in latent and activated intestinal microsomes. We decided to make a comparison of these subcellular systems with the cellular system.

The results reported here indicate that the use of microsomes may lead to a systematical overestimation of the glucuronidation capacity of the intestinal wall, especially at higher substrate concns. The cellular system seems to correspond more closely to the *in vivo* situation. It is suggested that the glucuronidation of 1-N and 7-HC in intact epithelial cells is limited by other factors than the extracellular substrate concn. Both cellular uptake of the substrate and availability of UDPGA may play a significant role.

MATERIALS AND METHODS

Chemicals. UDPGA (disodium salt), 1-N (grade III), 1-naphthyl-β-D-glucuronide (sodium salt), β-glucuronidase (type B3, bovine liver) and arylsulphatase (type V, limpet) were obtained from Sigma. 7-HC (umbelliferone) was purchased from Fluka. Crystalline bovine serum albumin was obtained from Poviet (Oss, The Netherlands). All other chemicals were of analytical-grade purity and used as supplied.

Animals. Adult male Wistar rats (Cpb:WU) (200–250 g) were obtained from T.N.O. (Zeist, The Netherlands) and allowed free access to water and a commercially available diet (RMH-B) (Hope Farms, Woerden, The Netherlands).

Isolation of intestinal epithelial cells. The animals were killed by cervical dislocation between 9.00 and 10.00 a.m. and the small intestine, with the omission of the duodenum, was immediately flushed free of intestinal contents with ice-cold PBS (90 mM $NaCl_2$, 9 mM KH_2PO_4 and 34 mM $Na_2HPO_4 \cdot 2H_2O$, pH 7.4). All further work was done at 0–4° using polyethylene and polypropylene materials. Epithelial cells were isolated by a high-frequency low-amplitude vibration method [32, 33] as described previously [14, 31]. Cells were harvested by 5 min centrifugation at 850 g_{max} in a Beckman TJ-6 centrifuge (TH-4 rotor). Isolated cells were washed twice by resuspending in PBS followed by centrifugation at 850 g_{max}. Washed cells were suspended (cells of 0.5 g intestine/ml) in phosphate buffer (9 mM KH_2PO_4, 34 mM $Na_2HPO_4 \cdot 2H_2O$, 0.1 mM EDTA-Na_2, pH 7.4) for preparing microsomes (see later) or in Krebs–Ringer (94 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11.6 mM glucose), saturated with carbogen gas [5% CO_2 and 95% O_2 (v/v), pH 7.4 after saturation], for cell incubations.

Preparation of intestinal microsomes. Washed cells suspended in phosphate buffer were homogenized using an Ultra-Turrax (Janke & Kunkel KG, Staufen im Breisgau) (11,000 rpm, four bursts of 10 sec) [31, 34]. Intestinal microsomes were prepared by differential centrifugation in a MSE-Prepspin 50 ultracentrifuge (4°, titanium fixed-angle rotor, 20 min at 15,000 g_{max}, 60 min at 95,000 g_{max}), resuspended in phosphate buffer (microsomes of 1 g intestine/ml), quick-frozen and stored at −80° until use.

Incubation of cells. Incubations were done within 2 hr of cell isolation in polypropylene incubation vessels in a shaking water bath (60 cycles/min) at 37°. Each incubation contained cells obtained from 0.25 g intestine in a final vol. of 4 ml Krebs–Ringer, saturated with carbogen gas (0.6–1.2 mg cell protein/ml). After preincubation for 10 min, the reaction was started by addition of the substrate (final concn 10–200 μM) in Polysorbate-80 [final concn 0.0025% (v/v)]. The reaction was stopped after 15 min by the addition of 0.5 ml 15% (w/v) trichloroacetic acid. Reactions were linear with time for at least 30 min.

Conjugates of 7-HC were quantitated as follows. Unmetabolized substrate was removed by two extractions with 6 ml chloroform (efficiency 95–98%), and two 1-ml aliquots of the aqueous layer were treated with β-glucuronidase and arysulphatase as described elsewhere [14]. Liberated 7-HC was extracted and quantitated according to Greenlee and Poland [35] using a Perkin–Elmer LS-3 spectrofluorimeter ($\lambda_{exc.}$ = 368 nm, $\lambda_{em.}$ = 456 nm). Net amounts of glucuronides and sulphates were calculated by correcting for non-extracted 7-HC.

Since no sulphate conjugates of 1-N could be detected (see Results) 1-N glucuronide could be quantitated directly by measuring the fluorescence of the conjugates (1-naphthyl sulphate and 1-naphthyl-β-D-glucuronide having the same maximum excitation and emission wavelengths). A modification of the method of Bock and White [26] was used. After centrifugation of the reaction mixture 50 μl of the clear supernatant was added to 4.5 ml of a 0.01 N NaOH/1 M NaCl solution. The fluorescence of 1-N glucuronide was read at $\lambda_{exc.}$ = 290 nm and $\lambda_{em.}$ = 330 nm and compared with the fluorescence of a series of 1-naphthyl-β-D-glucuronide standards. The presence of 1-N in the reaction mixture did not interfere with the determination.

Incubation of microsomes. Incubations were done in a shaking water bath (90 cycles/min) at 37°. Each incubation contained microsomes obtained from 0.05 g intestine in a final vol. of 1 ml phosphate buffer (0.10–0.15 mg microsomal protein/ml). Cofactors, activators and microsomes were preincubated for 10 min, after which the reaction was started by adding the substrate (20–200 μM final concn) in Polysorbate-80 [final concn 0.002% (v/v)]. The reaction was stopped after 10 min with 125 μl 15% (w/v) trichloroacetic acid. Reactions were linear with time for at least 30 min. Incubation blanks (no UDPGA added) showed no reaction. Addition of 1 mM saccharo-1,4-lactone (to inhibit β-glucuronidase) or 10 mM EDTA (to inhibit UDP-pyrophosphatase) did not increase the glucuronidation rate.

Glucuronidation of 7-HC was quantitated by measuring the disappearance of the substrate [31, 35]. In preliminary experiments it was established that all disappeared 7-HC could be recovered by treatment with β-glucuronidase. Glucuronidation of 1-N was measured by quantitating the formed 1-N glucuronide as described earlier.

Table 1. Characteristics of isolated rat intestinal epithelial cells and microsomes

Cell yield	$(40 \pm 4) \times 10^6$	cells/g intestine
Cell protein	18.1 ± 2.2	mg/g intestine
Cell protein	0.45 ± 0.08	mg/10^6 cells
Microsomal protein	2.39 ± 0.11	mg/g intestine
Microsomal protein	0.060 ± 0.008	mg/10^6 cells
Microsomal protein	13.2 ± 2.4	mg/mg cell protein × 100%

Values represent the means ± S.E.M. of at least six different preparations of cells and microsomes.

Other techniques. The number of cells isolated was estimated by suspending an aliquot of cells (0.1 ml) in 0.9 ml of a solution containing 0.16% (w/v) trypan blue and 3 mM sodium tetraphenylborate [32] and counting the number of cells in a Bürker chamber. Only well-separated cells were counted. Viability was assessed by trypan blue exclusion and LDH leakage [14]. Protein was determined according to Lowry *et al.* [36] using crystalline bovine serum albumin as a standard.

Statistical methods. All incubations were done in duplicate with six or three batches of cells or microsomes, respectively. Apparent kinetic parameters for 7-HC, 1-N and UDPGA in microsomal incubations were determined for each batch of microsomes separately using a non-linear least-squares fitting programme [37] implemented on an Apple II microcomputer. The proportional S.D. and bisquare weighting options were used. Coefficients of variation of the estimated parameters never exceeded 10% (with 1-N as substrate). Means and S.E.M. of parameters were calculated according to standard statistical methods and comparisons between parameters were made by Student's *t*-test for paired or unpaired samples [38].

RESULTS

The cell isolation procedure employed yields a reproducible number of cells (Table 1). The cells are shed from the intestine villi as sheets and remain in mutual contact at their luminal surface.* Cell counting can only be done after separation of the cells with sodium tetraphenylborate. Even then, some large aggregates are still apparent. Therefore the number of cells counted tends to be an underestimate. As a base of reference for quantitating the cellular metabolism we prefer to use the amount of cellular protein. Cell viability, as assessed by trypan blue exclusion and LDH leakage, ranged between 80 and 90%, as reported before [14, 33].

The formation of glucuronic acid and sulphate conjugates of 7-HC and 1-N is illustrated in Fig. 1. No free 1-N could be detected after prolonged treatment (90 hr at 37°) of the aqueous phase (after two chloroform extractions) with 10 U/ml arylsulphatase. The amount of 1-N, liberated by treatment with β-glucuronidase, corresponded to the amount of conjugates determined by the direct fluorimetric assay (see Materials and Methods). We must conclude, therefore, that no measurable amounts of 1-N sulphate have been formed. Conjugation of 7-HC with sulphate appears to be linear with substrate concn in the concn range tested (Fig. 1). At 100 μM substrate concn sulphate conjugates accounted for about 9% of total conjugates formed.

Conjugation with glucuronic acid in the cellular system is saturated at low substrate concns [50 μM for 7-HC and 20 μM for 1-N (Fig. 1)]. The maximal glucuronidation rates for both substrates do not differ significantly from each other ($P > 0.30$, Student's *t*-test for unpaired samples). At 100 μM substrate concn the glucuronidation rates for 7-HC and 1-N are 13.1 ± 2.2 and 11.9 ± 2.2 nmoles/min·g intestine, respectively (0.326 ± 0.089 and 0.296 ± 0.097 nmoles/min·10^6 cells).

The intestinal microsomal UDPGT activity towards 7-HC and 1-N can be activated two- to three-fold by relatively high $MgCl_2$ concns. A further two-fold activation by Triton X-100 is possible (Fig. 2). The effect of activation on the kinetic parameters of the enzyme was further investigated using 1-N as a substrate. Activation increases the V_{max} and K_m^{app} values for 1-N (Fig. 3) in a statistically significant way (Table 2). The K_m^{app} for UDPGA is lowered by adding $MgCl_2$ but increased by further addition of Triton X-100 (Table 2).

A direct comparison between the glucuronidation rate of 1-N in latent microsomes, activated microsomes and intact epithelial cells is presented in Fig. 4. Activities were calculated on a common basis (nmoles/min·g intestine) using the data presented in Table 1. It can be seen that the glucuronidation rate in microsomes (whether activated or not) is

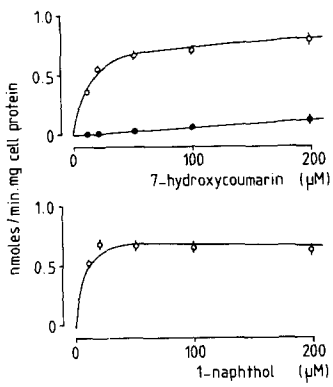

Fig. 1. Glucuronidation (○) and sulphation (●) of 7-hydroxycoumarin (upper panel) and 1-naphthol (lower panel) in isolated rat intestinal epithelial cells. Cells were incubated at 37° in Krebs–Ringer (pH 7.4). Formed conjugates were quantitated by treatment with β-glucuronidase and arylsulphatase, respectively. No 1-naphthol sulphate could be detected. Data points represent the means ± S.E.M. of duplicate determinations with six batches of cells, each prepared from two rats.

* A. Sj. Koster *et al.*, to be published.

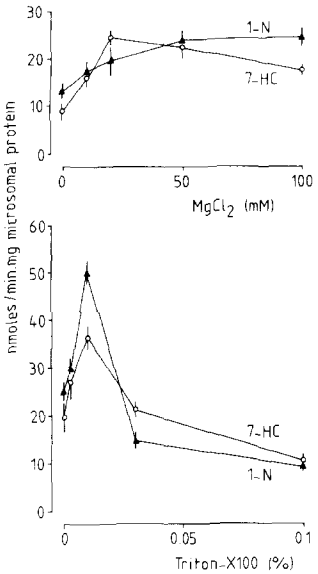

Fig. 2. Influence of $MgCl_2$ (upper panel) and Triton X-100 (lower panel) on the glucuronidation of 7-hydroxycoumarin (○) and 1-naphthol (▲) in rat intestinal microsomes. Microsomes were incubated at 37° in phosphate buffer (pH 7.4) in the presence of 3 mM UDPGA and 200 μM 7-hydroxycoumarin or 1-naphthol, respectively. The Triton X-100 experiment was carried out in the presence of optimal concns of $MgCl_2$ (20 mM for 7-hydroxycoumarin, 50 mM for 1-naphthol). Data points represent the means ± S.E.M. of duplicate determinations with three batches of microsomes, each prepared from four rats.

several-fold higher than in isolated epithelial cells. The same conclusion holds for 7-HC.

DISCUSSION

Glucuronidation and sulphation in mucosal cells

Isolated intestinal epithelial cells have been used previously for the study of phase II drug metabolism [9, 18, 19]. Cells have been isolated by enzymic treatment of intact intestinal segments [9, 18] or mucosal scrapings [19]. We adopted a high-frequency, low-amplitude vibration method [32, 33], because this procedure results in a higher cell yield and a higher viability of isolated cells [14, 39]. Moreover, the cells isolated by this method are superior to enzymically isolated cells as far as glucuronidation is concerned [14]. Metabolic and structural integrity of the vibration-isolated cells has been demonstrated [33, 40].

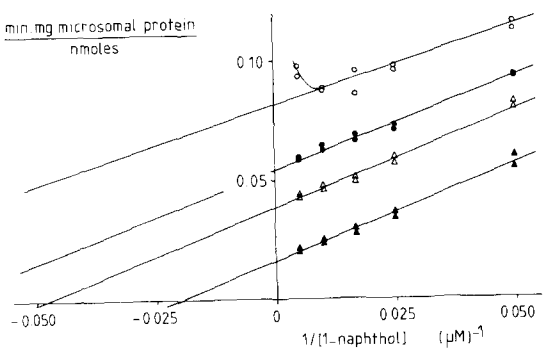

Fig. 3. Glucuronidation rate of 1-naphthol in rat intestinal microsomes. Incubations were done at 37° in phosphate buffer (pH 7.4) with latent microsomes (○) and with microsomes activated by 5 mM $MgCl_2$ (●), 50 mM $MgCl_2$ (△) or a combination of 50 mM $MgCl_2$ and 0.01% Triton X-100 (▲). UDPGA was present at a final concn of 3 mM. Data points represent individual measurements with one batch of microsomes. Essentially similar results were obtained with two other batches of microsomes.

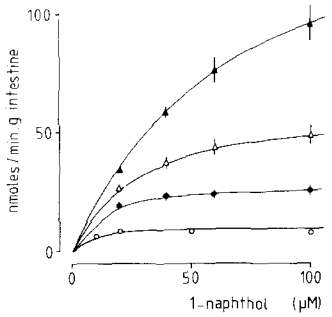

Fig. 4. Comparison of the glucuronidation of 1-naphthol in isolated rat intestinal epithelial cells (○) and microsomes (●△▲). Cells were incubated at 37° in Krebs–Ringer (pH 7.4). Microsomes were incubated at 37° in phosphate buffer (pH 7.4) with 3 mM UDPGA in the presence of 50 mM $MgCl_2$ (△), a combination of 50 mM $MgCl_2$ and 0.01% Triton X-100 (▲), or without further addition (latent microsomes) (●). Data points represent the means ± S.E.M. of duplicate determinations with six batches of cells or three batches of microsomes, prepared from two and four rats, respectively.

No sulphate conjugates could be detected when isolated cells were incubated with 1-N. This observation is in accordance with other studies, using the rat intestine [19, 21, 24, 25], where only trace amounts of 1-N sulphate could be detected. Upon incubation with 7-HC, small but measurable amounts of 7-HC sulphate were found, as was previously reported [18]. This low proportion of sulphate conjugation appears to be species-specific [4, 8, 9] and may be caused by the presence in the adult rat intestine of an inhibitor of the sulphotransferase [41]. The linear dependence of sulphation rate on extracellular substrate concn and the presence of 1.2 mM $MgSO_4$ in the incubation medium argue against limitation by endogenous cofactors or by availability of inorganic sulphate [12, 42]. The maximum amount of glucuronides formed (for 1-N as well as 7-HC) in the isolated cells is comparable to earlier reported values obtained with isolated rat intestinal epithelial cells [18, 19].

In vitro *activation of intestinal microsomes*

In contrast with the hepatic microsomal UDPGT, the intestinal microsomal UDPGT is generally considered to be inactivatable *in vitro* or, conversely, to be fully activated during the isolation procedure [16, 29, 30]. The results obtained in this study clearly show that the intestinal microsomal UDPGT, prepared by the presently described procedure, can be activated *in vitro*, as was reported before [31]. It cannot be excluded, however, that some activation does occur during preparation. Ultra-Turrax homogenization is known to activate the guinea pig hepatic microsomal UDPGT [43]. The latent microsomes used in this study may, therefore, not be fully

Table 2. Influence of $MgCl_2$ and Triton X-100 on the apparent kinetic parameters for the glucuronidation of 1-naphthol and 7-hydroxycoumarin in rat intestinal microsomes

Addition to the assay	Substrate (in the presence of 3 mM UDPGA)		UDPGA (in the presence of 200 μM substrate)	
	K_m^{app} (μM)	V_{max} (nmoles/min · mg microsomal protein)	K_m^{app} (mM)	V_{max} (nmoles/min · mg microsomal protein)
		1-Naphthol as substrate		
None	9.4 ± 1.2	12.8 ± 2.0	0.589 ± 0.037	16.6 ± 2.7
50 mM $MgCl_2$	25.5 ± 2.4*	27.6 ± 3.8*	0.331 ± 0.027*	27.6 ± 3.3*
50 mM $MgCl_2$ + 0.01% Triton X-100	69.7 ± 13.9*	70.0 ± 3.9*†	0.699 ± 0.056†	73.0 ± 5.3*†
		7-Hydroxycoumarin as substrate		
20 mM $MgCl_2$	155 ± 33	34.4 ± 4.8	0.927 ± 0.257	36.7 ± 2.6

* Significantly different ($P < 0.05$) from incubations without added $MgCl_2$ and Triton X-100 (Student's *t*-test for paired samples).
† Significantly different ($P < 0.05$) from incubation without added Triton X-100 (Student's *t*-test for paired samples). Microsomes were incubated at 37° in phosphate buffer (pH 7.4) as detailed in Materials and Methods. K_m^{app} and V_{max} values represent the means ± S.E.M. of independent non-linear least squares estimates of K_m^{app} and V_{max} values, obtained from three batches of microsomes. Each batch of microsomes was prepared from four rats.

latent. Nevertheless, the microsomes prepared by the presently described procedure exhibit an appreciable latency since five- to six-fold activation is possible by using a combination of $MgCl_2$ and Triton X-100. Activation of the rat liver enzyme by Triton X-100 is a well-known phenomenon [30, 44, 45], while activation by $MgCl_2$ seems to be rather variable and may be dependent on the subcellular system and the substrate studied [31, 44, 45]. Activation of the intestinal microsomal UDPGT by $MgCl_2$ and Triton X-100 affects the kinetic parameters for the glucuronidation of 1-N in a similar way. Both V_{max} and K_m^{app} are increased. In rat liver microsomes only V_{max}, and not K_m, was increased by Triton X-100 activation [44]. Parallel displacement of the curves in the Lineweaver–Burk plots suggests that in the absence of activators uncompetitive inhibition occurs [46]. The maximal glucuronidation rate for 1-N in fully activated microsomes is substantially higher than previously reported values; K_m values are comparable [10, 17, 28].

Glucuronidation in the cellular and subcellular systems

The glucuronidation rate in intestinal microsomes is substantially higher than in isolated cells. This discrepancy becomes even greater when a microsomal recovery of approximately 50% is taken into account [recovery measured with arylesterase as a microsomal marker enzyme (data not shown)]. It seems, therefore, probable that at extracellular substrate concns above 50 μM glucuronidation in intact cells is limited by factors other than the extracellular substrate concn *per se*.

1-N and 4-methylumbelliferone belong to the 3-methylcholanthrene-inducible UDPGT-1 group of substrates. Activity towards this group of substates has been identified in the rat and mouse intestinal wall [10, 11]. In the rat liver these substrates are glucuronidated by one UDPGT form [47]. We assume that 7-HC belongs to the UDPGT-1 group of substrates as well. The observation, that 1-N and 7-HC in intact mucosal cells are glucuronidated at equal maximal rates, supports therefore the hypothesis that in intact rat intestinal epithelial cells glucuronidation is limited by endogenous factors. We suggest that UDPGA availability is crucial, as was observed in isolated hepatocytes [48] and perfused livers [13, 26]. UDPGA levels in isolated mucosal cells are not known. It seems, however, unlikely that the UDPGA level in mucosal cells will prove to be higher than in hepatocytes, where concns of approximately 0.3 mM have been measured [13, 26, 48]. When it is assumed that the latent microsomes closely mimic the endoplasmic reticulum in the mucosal cells and that the intracellular UDPGA level is the only rate-limiting factor, intracellular UDPGA levels of approximately 0.3 mM would be required to obtain maximum glucuronidation rates of 12.5 nmoles/min · g intestine (the cellular rate). However, uncertainties in these assumptions militate against quantitatively reliable conclusions concerning the intracellular UDPGA level. Nevertheless, we established empirically that cellular glucuronidation rates correspond to the glucuronidation rates measured in latent microsomes in the presence of non-maximal

UDPGA levels. Essentially the same conclusion was reached by Bock and White [26], who studied the glucuronidation of 1-N in perfused livers and hepatic microsomes.

At extracellular substrate concns below 50 μM cellular uptake of the substrate may become rate-limiting. This is suggested by the observation that in isolated cells 1-N glucuronidation is saturated at 20 μM, while saturation of the less lipophilic substrate 7-HC [49] occurs at about 50 μM extracellular concn. This implies, however, that once it has penetrated into the cell the substrate is very efficiently glucuronidated. The low K_m^{app} for 1-N in latent microsomes suggests that this is indeed possible.

The maximum glucuronidation rate of 1-N and 7-HC in isolated cells is comparable to the rate observed in everted sacs [21], *in situ* perfused intestinal loops [25] and *in vitro* perfused intestinal segments.* A further comparison of isolated cells with intact intestinal segments and *in vivo* experiments is necessary to see if extrapolation of data obtained with isolated cells is possible. The isolated mucosal cells appear to be a relatively simple model system, which can be used to predict intestinal conjugation of phenolic xenobiotics. A more reliable prediction can be made by using cells than by using activated or even latent microsomes, because cellular substrate uptake and cosubstrate availability appear to be important determinants of the maximum conjugation rate in the intact cellular system.

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