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DISTRIBUTION OF GLUCURONIDATION CAPACITY (1-NAPHTHOL AND MORPHINE) ALONG THE RAT INTESTINE

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Abstract—The distribution of glucuronidation capacity along the rat intestine was investigated using mucosal cells, isolated from the small intestine, the caecum, and the colon *plus* rectum. The glucuronidation capacity for 1-naphthol decreases from 787 ± 75 (duodenum) to 128 ± 13 (colon plus rectum) pmoles/min.mg cell protein. The ratio between 1-naphthol and morphine glucuronidation was constant throughout the intestine (7.15 ± 0.37). The distribution of maximal activity of UDP-glucuronosyltransferase in intestinal cell homogenates follows the same pattern. The maximal activity of UDPglucose dehydrogenase in homogenates corresponds closely to the glucuronidation rate in mucosal cells. The activity of β-glucuronidase in intestinal cell homogenates is constant along the duodenum and jejunum but increases throughout the terminal ileum, caecum, colon and rectum. Subcellular fractionation studies using marker enzymes indicate that UDPglucose dehydrogenase and β-glucuronidase are cytosolic enzymes in intestinal mucosal cells. Although UDP-glucuronosyltransferase activity is found in both the mitochondrial and the microsomal fractions, no indications for a mitochondrial localization of this enzyme can be found. Activity in the mitochondrial fraction appears to be due to endoplasmic reticulum, associated with the mitochondrial fraction.

It has been often observed that the specific activities of biotransformational [1–3] and other enzymes [4] varies along the length of the intestine, the highest activity being found in the duodenum. A gradual decrease is observed further down the intestinal tract. The longitudinal distribution of UDPGT* appears to follow this general pattern [2, 5]. Unfortunately, most of these studies were done with intestinal homogenates, supplied with high UDP-glucuronic acid concentrations. The *in vivo* significance of these observations remains, therefore, to be established because maximal capacity of UDPGT seems not to be rate-limiting for intestinal glucuronidation [6, 7]. In earlier studies with tissue slices a fivefold difference in glucuronidation capacity (for *o*-aminophenol) was found between duodenum and terminal ileum [5]. However, in more recent studies with perfused segments [8] or *in situ* perfused loops [9] of the rat intestine no significant differences in the total glucuronidation capacity of proximal, distal and intermediate segments of the small intestine could be found. We, therefore, decided to reinvestigate the longitudinal distribution of glucuronidation capacity using mucosal cells, isolated from the small and large intestine. Because it is becoming increasingly clear that activity towards both the GT-1 and the GT-2 group of substrates [10] is present in the intestinal wall [11–13], 1-naphthol and morphine were used to measure glucuronidation capacity.

We also determined the specific activities and the subcellular localization of the major enzymes involved in glucuronide formation (UDPGDH, UDPGT, βG). The measurement of βG and UDPGDH is particularly interesting because these enzymes may be rate-limiting for glucuronide breakdown, and synthesis [7], respectively. The longitudinal distribution of these enzymes along the intestine was investigated because earlier literature indicates a non-uniform distribution over the gastrointestinal tract [5, 14].

In the rat liver UDPGT can be found in most cellular membranes [15–17] but the contribution of the nuclear envelope and the plasma membrane to the total glucuronidation capacity is negligible [16]. The contribution of mitochondrial glucuronidation may be considerable [15], but the presence of UDPGT in rat liver mitochondria has been challenged recently [18]. βG in the rat liver has a multiple localization [19] but is found in the particle-free supernatant of intestinal homogenates [20]. The subcellular localization of UDPGDH in the intestine has not been investigated in detail.

MATERIALS AND METHODS

Chemicals. UDP-glucuronic acid, UDP-glucose, 1-naphthyl-β,D-glucuronide (all sodium salts) and 1-naphthol (grade III) were obtained from Sigma. NADH, NAD^+ and sodium pyruvate were purchased from Boehringer-Mannheim; INT, indoxylacetate and sodium succinate from Fluka. Morphine.HCl, *p*-nitrophenylphosphate and crystalline bovine serum albumin were obtained from

* Abbreviations used; AE, arylesterase (EC 3.1.1.2); AP, alkaline phosphatase (EC 3.1.3.1); INT, 2(*p*-iodophenyl)3-(*p*-nitrophenyl)5-phenyltetrazolium chloride; LDH, lactate dehydrogenase (EC 1.1.1.27); SDH, succinate dehydrogenase (EC 1.3.99.1); UDPGDH, UDP-glucose dehydrogenase (EC 1.1.1.22); UDPGT, UDP-glucuronosyltransferase (EC 2.4.1.17); βG, β-glucuronidase (EC 3.2.1.21).

Brocacef (Maarssen, The Netherlands), Merck and Poviet (Oss, The Netherlands), respectively. Morphine 3-glucuronide was kindly donated by Dr. R. Hawks (National Institute on Drug Abuse, Rockville, U.S.A.). Morphine.HCl was of Dutch pharmacopeial grade. 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 (Muracon-1, Trouw, Putten, The Netherlands).

Isolation of intestinal epithelial cells. Mucosal cells from the small intestine were isolated by vibration in EDTA-containing (5 mM) phosphate-buffered saline as described before [6, 21]. With this method a mixed population of villus and crypt cells is obtained [22]. For the study of longitudinal distribution the small intestine was divided in eight segments, each approximately 10 cm long (segment 1–8). Mucosal cells from the caecum (segment 9) and the colon *plus* rectum (segment 10) were isolated by shaking the everted segments, filled to distention with saline, EDTA-containing (5 mM) phosphate buffered saline for 40 min. Intestinal segments were washed extensively before EDTA-treatment to remove intestinal contents as far as possible. Preparation of cells was done at 0–4°.

Subcellular fractionation. For subcellular fractionation cells isolated from the intestinal segments 1, 2, 3, 9 and 10 were used; cells from segments 7 and 8 were combined. Washed cells were resuspended in 0.25 M sucrose buffered at pH 7.4 with 5 mM Tris–HCl and disrupted using a Polytron-homogenizer (type P10/35, Kinematica, Luzern, Switzerland): four bursts of 7 sec [23]. Cell débris, brush borders and nuclei were spun down by centrifugation at 1000 g_{max} (5 min) in a Beckman TJ-6 centrifuge (TH-4 rotor). The resulting ("nuclear") pellet was washed with 0.25 M sucrose (pH 7.4) and the combined supernatants were centrifuged at 12,000 g_{max} (20 min) in a MSE-Prepspin 50 ultracentrifuge (titanium fixed-angle rotor). The postmitochondrial supernatant was centrifuged at 105,000 g_{max} (60 min). The microsomal pellet was washed by resuspending in 0.15 M KCl and microsomes were pelleted by recentrifugation at 105,000 g_{max} (60 min). The nuclear, mitochondrial and microsomal pellets were resuspended in 0.25 M sucrose (pH 7.4), quick-frozen and stored at −80° until use. The particle-free supernatant (sup-1) and washing (sup-2) were stored identically. All procedures were carried out at 0–4°.

Cell incubation and determination of specific activities. The glucuronidation of 1-naphthol and morphine in mucosal cells was measured as described before [6, 13] using saturating concentrations of 1-naphthol (100 μM) or morphine (1 mM). Marker enzyme activities in all subcellular fractions after appropriate dilution were measured at 37° as described: AP at pH 9.4 [24], SDH at pH 7.4 [25], AE at pH 6.8 using indoxyl-acetate as a substrate [26] and LDH at pH 7.4 (by NADH-conversion using sodium pyruvate). Glucuronidation of 1-naphthol was measured under maximally activated conditions (50 mM $MgCl_2$, 0.01% Triton X-100, ref. 6). UDPGDH was measured at 37° in 40 mM Tris-buffer pH 8.7 by quantitation of NADH-production from NAD^+ (3 mM) and UDPglucose (1 mM). Activity of βG was determined by measuring (37°, 100 mM acetate buffer pH 5.0) the formation of 1-naphthol from 1-naphthyl-β-D-glucuronide (1 mM). The reaction (1 ml total volume) was terminated after 10 min by adding 125 μl 15% (w/v) trichloroacetic acid. After centrifugation 0.1 ml of the reaction mixture was added to 4.5 ml of 0.01 N NaOH/1 M NaCl solution and the fluorescence of 1-naphtol was measured using a Perkin-Elmer LS-3 spectrofluorimeter (λ_{exc} = 330 nm, λ_{em} = 460 nm). Activities of AP, AE, LDH and UDPGDH were followed by measuring absorbance changes *vs.* time on an Aminco DW-2a UV-Vis spectrofotometer in the split-beam mode. Protein was determined [27] using crystalline bovine serum albumin as a standard. Cellular glucuronidation capacity and the activity of UDPGT and βG were quantified by including a series of 1-naphthyl-β,D-glucuronide, morphine 3-glucuronide or 1-naphthol standards in each experiment. All activities were expressed as nmoles/min.mg protein. The specific activity of UDPGDH (measured as NADH-production) was divided by two in order to represent UDP-glucuronic acid production [28]. Specific activities in the total homogenate were calculated from protein content and specific activities of the subcellular fractions.

Experimental design and statistical analysis. Cell incubations were done in duplicate with three batches of cells isolated from two rats each. Subcellular fractions were prepared from three (segments 1–8) or two (segments 9, 10) batches of isolated cells, each prepared from two (segments 1–8) or four (segments 9, 10) rats. All experiments were analysed by one-way analysis of variance, in which the within-segment mean square was used as error variance. Significance of longitudinal gradients was investigated by comparing the between-segment variances and error-variances in an F-test [29]. No statistically significant difference in subcellular distribution of enzyme activities was observed between different intestinal segments and data obtained from all segments were pooled for the representation of the subcellular fractional results. Analyses of variance were implemented on an Apple IIe microcomputer. Subcellular distribution of enzyme activities is illustrated using De Duve-plots, including the nuclear fraction, mitochondrial fraction, microsomal fraction, particle-free supernatant and microsomal washing.

RESULTS

The cellular glucuronidation capacity for 1-naphthol and morphine is illustrated in Fig. 1. An approximately twofold difference in glucuronidation capacity between the duodenum (segment 1) and the terminal ileum (segment 8) is observed. When the amount of cellular protein is taken into account a three- to fourfold difference between small intestinal segments can be calculated (Table 1). The glucuronidation capacity of the large bowel is considerably lower, both on a cellular basis (Fig. 1) and on an intestinal segment basis (Table 1). The ratio between glucuronidation capacity for 1-naphthol and

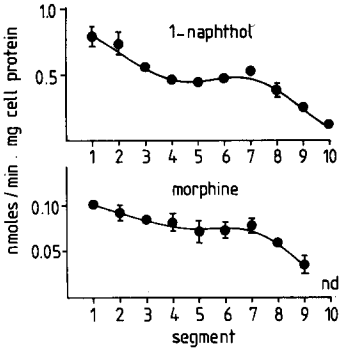

Fig. 1. Distribution of glucuronidation capacity along the rat intestine. Glucuronidation of 1-naphthol (100 μM) and morphine (1 mM) in isolated mucosal cells was measured as described in Materials and Methods. Data points represent the means ± S.E.M. of duplicate determination with three batches of cells, each prepared from two rats. When no error bar is given, the S.E.M. was smaller than the symbol used. The longitudinal distribution of glucuronidation capacity was statistically significant ($P < 0.001$; one-way analysis of variance).

morphine was constant along the entire intestine (ratio = 7.15 ± 0.37). The longitudinal distribution of UDPGT and UDPGDH in cell homogenates (Fig. 2) follows the same pattern as the cellular glucuronidation capacity.

The subcellular localization of marker enzyme activities indicates that a reasonable separation between brush-border, mitochondrial, and cytosol fractions is obtained (Fig. 3). At least 55% of the enzyme activities, characteristic for these organelles are found in the 1000 *g* pellet, the 12,000 *g* pellet and the particle-free supernatant, respectively (Table 2). AE appears not to be a good microsomal marker, because most of the activity of this enzyme is found in the particle free supernatant. However, the absence of UDPGT (Table 2) and cytochrome P-450 (data not shown) in the particle-free supernatant, indicates that liberation of enzyme activity from the endoplasmic reticulum is restricted to AE under our conditions. UDPGDH and βG activity are almost exclusively found in the particle-free supernatant, while UDPGT is found in both the mitochondrial and microsomal fraction (Fig. 3, Table 2).

DISCUSSION

Longitudinal distribution of glucuronidation capacity

The longitudinal distribution of glucuronidation capacity for 1-naphthol and morphine in isolated mucosal cells, described in this study, corresponds to the earlier described distribution of *o*-aminophenol glucuronidation in whole intestinal wall slices [5]. Also, the distribution of UDPGT, UDPGDH and βG confirms earlier studies [2, 5, 14]. We could, however, not confirm the high activities of UDPGT in the colon [5] and of UDPGDH in the caecum [14]. In the present study a gradual decrease of UDPGT and UDPGDH activities and a gradual increase in βG activity was observed along the entire small and large intestine. The gradual decrease in glucuronidation capacity from duodenum to the large bowel is the result of both a decrease in cellular glucuronidation rate and a decrease in mucosal cell protein, isolated *per* intestinal segment. The latter difference results from a difference in villus length and surface area between proximal and distal intestinal segments [30, 31].

The gradual decrease of glucuronidation capacity along the rat intestine, found in isolated cells or whole intestinal wall slices [5], is not observed in perfused intestinal segments [8] or in *in situ* perfused intestinal loops [9]. This suggests that other factors than glucuronidation capacity alone are affecting the glucuronation rate in intact intestinal segments. In everted gut sacs of the guinea-pig a gradual decrease of glucuronidation-capacity (7-hydroxycoumarin) from duodenum to terminal ileum was observed [32]. However, only mucosal conjugates were measured by these authors and it can, therefore, not be decided if total glucuronide production in everted gut sacs of the guinea-pig intestine decreases from duodenum to ileum.

The constant ratio of 1-naphthol and morphine glucuronidation along the intestine indicates that the UDPGT-forms, involved in the glucuronidation of group-1 and group-2 substrates [10, 12, 13], are present in a constant ratio along the intestine. The large difference between maximal activity of UDPGT in cell homogenates and glucuronidation capacity in intact mucosal cells confirms that UDPGT-activity *per se* is not rate-limiting for intestinal conjugation [6, 7, 12].

Table 1. Distribution of glucuronidation capacity along the rat intestine

Intestinal segment	Number of observations	Cell protein isolated (mg/segment)	Glucuronidation capacity* (nmoles/min segment)	
			1-naphthol	morphine
1. Duodenum	7	37.9 ± 4.4	29.8 ± 4.5	3.90 ± 0.50
2. Proximal jejunum	7	38.9 ± 7.5	28.7 ± 6.4	3.58 ± 0.77
3.	7	34.2 ± 6.8	19.4 ± 4.0	2.90 ± 0.60
4.	4	44.8 ± 6.4	21.1 ± 3.2	3.64 ± 0.71
5.	4	31.0 ± 2.9	13.9 ± 1.6	2.21 ± 0.46
6.	4	24.3 ± 5.8	11.5 ± 2.8	1.79 ± 0.48
7.	7	18.6 ± 2.2	9.7 ± 1.4	1.45 ± 0.22
8. Terminal ileum	7	20.6 ± 1.7	8.0 ± 1.2	1.22 ± 0.13
9. Caecum	6	11.0 ± 1.7	2.8 ± 0.5	0.39 ± 0.13
10. Colon + rectum	6	3.7 ± 0.7	0.5 ± 0.1	<0.2

* Calculated from cellular glucuronidation rate (Fig. 1) and protein content *per* segment.

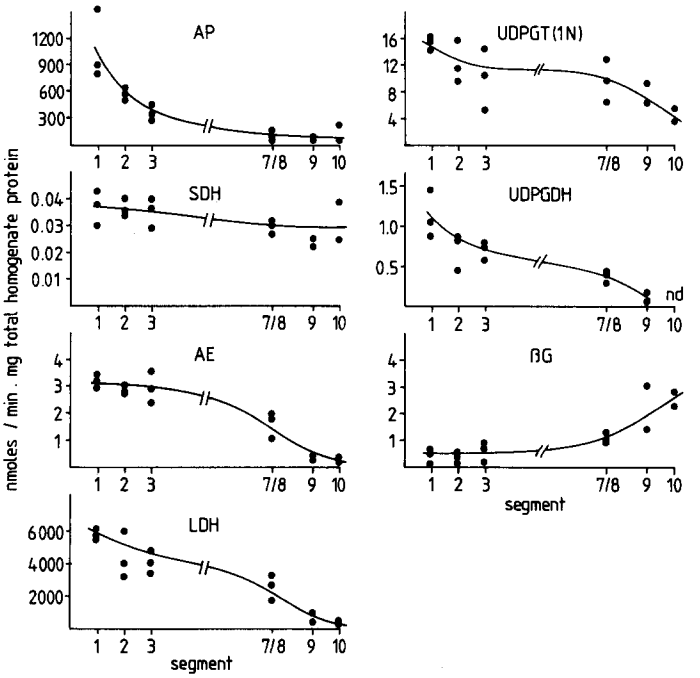

Fig. 2. Distribution of enzyme activities along the rat intestine. All enzyme activities were measured in different subcellular fractions as described in Materials and Methods and the activity of the total homogenate was calculated from protein content and specific activities of all fractions. Data points represent individual measurements obtained from different batches of cell homogenates. A statistically significant longitudinal distribution ($P < 0.05$) was observed for all enzyme activities except SDH (one-way analysis of variance).

Subcellular localizatiion of UDPGT, UDPGDH and βG

The results of our cellular fractionation-experiments are comparable to earlier published studies [23, 24, 33]. Our method seems to be more effective in disrupting mucosal cells because only 5–10% of the microsomal and cytosolic enzyme activities are found in the 1000 *g* pellet. In earlier studies higher values (20% or more) are found [23, 24, 33]. The more vigorous homogenization used in the present study results in the partial disruption of brush-borders and mitochondria. This is demonstrated by the occurrence of AP in the mitochondrial fraction and by the presence of AP and SDH in the microsomal

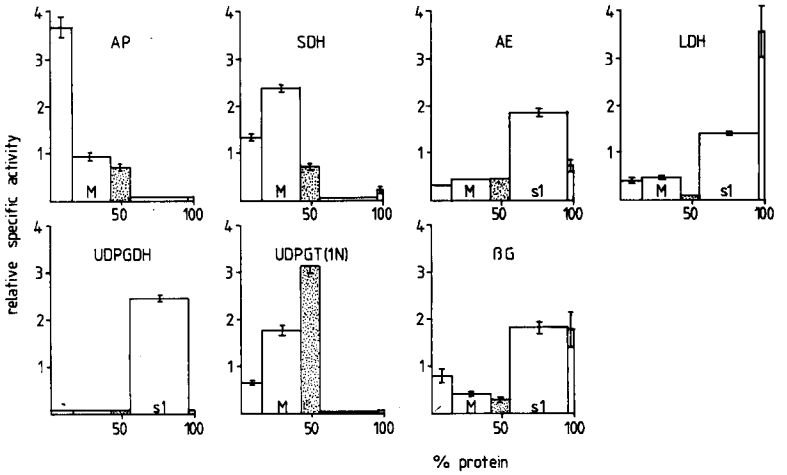

Fig. 3. Subcellular localization of enzyme activities in intestinal cell homogenates. Specific activities ± S.E.M. in subcellular fractions are expressed relative to the activity in the total homogenate (see Fig. 2). No statistically significant differences in subcellular localization of enzyme activities was observed between different intestinal segments and data from different segments were, therefore, pooled. The amount of protein in each subcellular fraction (from left to right: nuclear fraction, mitochondrial fraction M, microsomal fraction, particle-free supernatant S1 and microsomal washing) is given on the abscissa.

Table 2. Subcellular localization of UDP-glucuronosyltransferase, UDP-glucose dehydrogenase and β-glucuronidase in intestinal cell homogenates

Enzyme (assumed location)	Percentage ± S.E.M. of total activity in subcellular fraction				
	1000 *g* pellet	12,000 *g* pellet	105,000 *g* pellet	Particle free supernatant	Microsomal washing
Protein	15.1 ± 1.2	27.2 ± 0.9	13.1 ± 0.7	41.4 ± 1.2	3.5 ± 0.8
AP (brush-border)	56.8 ± 3.4	28.8 ± 3.5	9.4 ± 0.7	4.6 ± 0.3	0.4 ± 0.1
SDH (mitochondria)	21.0 ± 2.2	65.9 ± 2.1	9.2 ± 0.7	3.4 ± 0.3	0.7 ± 0.2
AE (microsomes)	5.3 ± 0.6	11.1 ± 0.5	6.3 ± 0.7	75.4 ± 1.3	1.8 ± 0.2
LDH (cytosol)	5.5 ± 0.8	12.8 ± 1.4	1.0 ± 0.1	68.9 ± 3.0	11.7 ± 1.8
UDPGT	10.4 ± 1.4	48.0 ± 3.5	41.5 ± 3.3	<1	<1
UDPGDH	<1	<1	<1	>95	<1
βG	11.0 ± 1.7	11.4 ± 1.3	3.1 ± 0.8	68.5 ± 2.4	6.0 ± 0.9

fraction. The liberation of AE from the endoplasmic reticulum by our procedure is more extensive than in earlier studies, in which a Potter-Elvehjem type of homogenizer was used [24, 33]. The choice of a cell fractionation method will depend on the purpose of the study. High recovery and purity of the fractions obtained are conflicting requirements [23]. Our procedure results in a recovery of 42% of the enzyme activity of UDPGT in the microsomal fraction and of 95 and 69% of the enzyme activities of UDPGDH and βG in the particle free supernatant fraction. Specific enzyme activities are increased two- to threefold with respect to the total intestine homogenate.

A cytosolic localization of UDPGDH is clearly indicated by the present results. More than 95% of the activity of this enzyme is found in the particle-free supernatant. In the rat liver βG has a multiple localization [19] but in the rat intestine more than 60% of the βG activity (with *p*-nitrophenol-β,D-glucuronide as a substrate) is found in the particle-free supernatant [20]. This is confirmed by our present results. Since no germ-free animals were used, it cannot be excluded that some of the βG activity is of bacterial origin. We consider this possibility unlikely because the intestine was washed extensively before cell isolation and because βG activity was measured at pH 5.0. Definitive conclusions about the subcellular localization of UDPGT are difficult to make due to the lack of a suitable marker enzyme for endoplasmic reticulum. AE (see above) and glucose-6-phosphatase [33] are easily solubilized during homogenization of intestinal cells. The amount of AE activity in the mitochondrial fraction is 1.76 times higher than in the microsomal fraction, while the amount of UDPGT activity in the mitochondrial fraction is only 1.16 times the UDPGT activity in the microsomal fraction. When it is assumed that AE is not present in mitochondria and that AE from endoplasmic reticulum in the mitochondrial fraction is as easily solubilized as from endoplasmic reticulum in the microsomal fraction, no indication for a mitochondrial localization of UDPGT can be derived from the present results. It appears, therefore, that UDPGT activity in the mitochondrial fraction is due to associated endoplasmic reticulum [18, 34]. Our data are not detailed enough to draw any conclusions about the occurrence of UDPGT in the nuclear envelope and/or other cellular membranes of rat intestinal mucosal cells.

REFERENCES

1. G. Clifton and N. Kaplowitz, *Cancer Res.* **37**, 788 (1977).
2. C. R. Nair, D. P. Chauhan, P. H. Gupta and S. K. Mehta, *Digestion* **24**, 190 (1982).
3. H. Hoensch, C. H. Woo, S. B. Raffin and R. Schmid, *Gastroenterology* **70**, 1063 (1976).
4. G. Crouzoulon, *Comp. Biochem. Physiol.* **62A**, 789 (1979).
5. O. Hänninen, A. Aitio and K. Hartiala, *Scand. J. Gatroenterol* **3**, 461 (1968).
6. A.Sj. Koster and J. Noordhoek, *Biochem. Pharmac.* **32**, 895 (1983).
7. A.Sj. Koster, C. P. J. Meewisse and J. Noordhoek, *Archs Toxicol.* **55**, 123 (1984).
8. A.Sj. Koster and J. Noordhoek, *J. Pharmac. exp. Ther.* **226**, 533 (1983).
9. J. Lasker and D. E. Rickert, *Xenobiotica* **8**, 665 (1978).
10. K. W. Bock, B. Burchell, G. J. Dutton, O. Hänninen, G. J. Mulder, I. S. Owens, G. Siest and T. R. Tephly, *Biochem. Pharmac.* **32**, 953 (1983).
11. K. W. Bock, U. C. von Clausbruch, R. Kaufmann, W. Lilienblum, F. Oesch, H. Pfeil and K. L. Platt, *Biochem. Pharmac.* **29**, 495 (1980).
12. A.Sj. Koster, in *Advances in Glucuronide Conjugation* (Eds. S. Matern, K. W. Bock and W. Gerok), p. 177. MTP-Press, Lancaster, U.K. (1984).
13. A.Sj. Koster, A. C. Frankhuijzen-Sierevogel and J. Noordhoek, *Drug Metab. Dispos.* **13**, 232 (1985).
14. O. Hänninen, K. Alanen and K. Hartiala, *Scand. J. Gastroenterol.* **1**, 152 (1966).
15. P. Stasiecki, F. Oesch, G. Bruder, E.-D. Jarasch and W. W. Franke, *Eur. J. Cell. Biol.* **21**, 79 (1980).
16. J. Zaleski, S. K. Bansal and T. Gessner, *Can. J. Biochem.* **60**, 972 (1982).
17. S. C. Hauser, J. C. Ziurys and J. L. Gollan, *J. biol. Chem.* **259**, 4527 (1984).
18. J. Zaleski and T. Gessner, *Res. Commun. Chem. Pathol. Pharmac.* **37**, 279 (1982).
19. J. W. Owens, K. L. Gammon and P. D. Stahl, *Archs Biochem. Biophys.* **166**, 258 (1975).
20. W. H. Curley, G. W. Lucier and C. M. Schiller, *Comp. Biochem. Physiol.* **68B**, 1 (1981).
21. P. J. A. Borm, A.Sj. Koster, A. C. Frankhuijzen-Sierevogel and J. Noordhoek, *Cell. Biochem. Function* **1**, 161 (1983).

22. A.Sj. Koster, P. J. A. Borm, M. R. Dohmen and J. Noordhoek, *Cell Biochem. Function* **2**, 95 (1984).
23. W. C. Hülsmann, J. W. O. van den Berg, and H. R. De Jonge, *Meth. Enzymol.* **32**, 665 (1973).
24. G. M. Jones and R. J. Mayer, *Biochim. biophys. Acta* **304**, 634 (1973).
25. R. J. Pennington, *Biochem. J.* **80**, 649 (1961).
26. E. H. Shephard and G. Hübscher, *Biochem. J.* **113**, 429 (1969).
27. A. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
28. G. J. Dutton, *Glucuronidation of Drugs and Other Compounds*. CRC Press, Boca Raton, FL (1980).
29. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th Edn. Iowa State University Press, Ames, IA (1967).
30. R. Ecknauer, B. Sircar and L. R. Johnson, *Gastroenterology* **81**, 781 (1981).
31. J. S. Trier and J. L. Madara, in *Physiology of the Gastrointestinal Tract* (Ed. L. R. Johnson), p. 925. Raven Press, New York (1981).
32. J. R. Dawson and J. W. Bridges, *Biochem. Pharmac.* **28**, 3291 (1979).
33. R. J. Shirkey, J. Chakraborty and J. W. Bridges, *Analyt. Biochem.* **93**, 73 (1979).
34. C. B. Pickett, D. Montisano, D. Eisner and J. Cascarano, *Expl. Cell Res.* **128**, 343 (1980).