Gastrointestinally-distributed UDP-glucuronosyltransferase1A10 which metabolizes estrogens and nonsteroidal anti-inflammatory drugs depends upon phosphorylation

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Among gastrointestinal (GI)-distributed isozymes encoded at the UGT1 locus, UGT1A10 metabolized a number of important chemicals. Similar to broad conversion of phytoestrogens [Basu et al., *J. Biol. Chem.* 279, 1429-1441 (2004)], UGT1A10 metabolized estrogens and their derivatives, whereas UGT1A1, 1A3, 1A7, and 1A8 differentially exhibited reduced activity toward the same. 1A10, compared with 1A7, 1A8, and 1A3, generally exhibited high activity toward acidic nonsteroidal antiinflammatory drugs and natural benzaldehyde derivatives, while 1A3 metabolized most efficiently aromatic transcinnamic acids known to be generated from flavonoid-glycosides by microflora in the lower GI tract. Finally, UGT1A10, 1A7, 1A8, and 1A3 converted plant-based salicylic acids; methyl salicylic acid was transformed at high levels and acetyl salicylic- (aspirin) and salicylic acid at moderate to low levels. Atypically, UGT1A10 transformed estrogens between pH 6 and 8, but acidic structures preferentially at pH 6.4. Furthermore, evidence indicates UGT1A10 expressed in COS-1 cells depends upon phosphorylation; 1A10 versus its single, double, and triple mutants at 3 predicted protein kinase-C (PKC) phosphorylation-sites incorporated [33P]-orthophosphate showing a progressive decrease with no detectable label or activity for the triple T73A/T202A/S432G-1A10 mutant. Single and double mutants revealed either null/full activity or null/additive activity, respectively. Additionally, UGT1A10-expressing cultures glucuronidated [14C]-17β-estradiol, whereas cultures containing null mutants at PKC sites showed no estrogen conversion. Importantly, UGT1A10 in cells supported 10-fold higher glucuronidation of 17β-estradiol than UGT1A1. In summary, our results suggest gastrointestinal-distributed UGT1A10 is important for detoxifying estrogens/phytoestrogens and aromatic acids with complementary activity by UGT1A7, 1A8, 1A3, and/or 1A1 evidently dependent upon phosphorylation.
Many aromatic chemicals are taken into the body unwittingly as components of plant-based diets, as well as wittingly as therapeutic agents. Although there are several different categories of chemicals in dietary plants, the flavonoids are by far the largest group and include quasi-active phytoestrogens, which often mimic endogenous estrogens due to structural similarities (1). Additionally, there are non-flavonoid-type estrogenic chemicals in plants, e.g. coumestrol and zearalenone, which are reported to have toxic effects in the body (2). While it is known that phytoestrogens can have beneficial tumor-suppressing activity (3) and other effects (4), it is not generally known that the chemicals can also be destructive to animal reproductive systems, as shown by the collapse and emaciation of the reproductive and urinary systems of livestock that grazed on clovers and crops with high phytoestrogen content (2). Additionally the phytoestrogens, genistein, daidzein, and equol, as well as estrogenic flavonoid precursors (5), chalcones, are shown to have potent effects on cardiac contractility (6). While modest accumulations of phytoestrogens may be inconsequential or beneficial, absorption of high levels of phytoestrogens (isoflavones) can lead to adverse and serious disruption of systems and individual enzymes that could possibly initiate disease(s).

Many ingested chemicals also have the potential to interact with each other or with conventional therapeutics. The plant antistress agent, salicylic acid, is ingested unwittingly (7) and has the potential to interact with non-steroidal anti-inflammatory drugs (NSAID). While both chemicals depress the proinflammatory cyclooxygenase enzyme, the combination of these two antiplatelet chemicals enhances the risks of bleeding (7). To the extent that our plant-based diet is a source of large quantities of these exogenous chemicals with many structural variations, such conditions, no doubt, have forced animal systems to limit absorption or to adapt to both natural and synthetic chemicals. Notably, a study detected less than 1% uptake in the blood of an ingested flavonoid
compared to 98% metabolism and binding to protein in the body when 40-fold less of the same chemical was administered intravenously (8). The results suggest the chemical was not absorbed when administered via the gastrointestinal (GI) tract or was metabolized and excreted.

Also, a variety of simple phenols are generated in the GI system from plant constituents (9, 10). Flavonoid-glycosides reach the lower GI tract where the microflora both hydrolyze the glycosides and further metabolize free flavonoids to form a range of aromatic chemicals (9). The metabolites include various aromatic acids and aldehydes as break-down products. Hence, absorption of ingested chemicals and those generated in the GI determine chemical exposure. As these lipophiles are both simple and polyaromatic containing hydroxyl and/or carboxyl group(s), they are potential substrates of UDP-glucuronosyltransferase (UGT) isozymes, which convert chemicals to glucuronides to enhance water solubility and excretion from cells. To that end, the cluster--- UGT1A1, 1A7, 1A8, 1A9, and 1A10--- of the human UGT1-encoded isozymes (11) was recently shown to be differentially distributed throughout the GI, but strategically located in the mucosal layer of tissues (12). The observation that the Gunn rat model (UGT1<sup>−/−</sup>) is 100-fold more sensitive to acetaminophen than the wild-type Wistar rat (13) indicates glucuronidation is protective against chemical toxins. Because UGT1A10 --among the isozyme-cluster-- was effective in metabolizing all categories of phytoestrogens (12), it suggested the isozyme would also metabolize structurally similar endogenous estrogens. Furthermore, the high abundance of the isozyme in the lower-GI led to speculation it may play a role in converting aromatic acids formed in that region by microflora, as well as convert ingested therapeutic acidic drugs.

In this study, we show UGT1A10 glucuronidated 3 different categories of chemicals including estrogens, plant-based cinnamic- and aromatic-acid structures, nonsteroidal antiinflammatory drugs, and salicylic-acid derivatives. While UGT1A10 was the primary metabolizer, studies showed
differential overlapping activity with UGT1A1, 1A3, 1A7, and 1A8. The isozyme exhibited different pH optima for catalysis, and it showed a dependency on phosphorylation that is linked to protein kinase C (PKC). Additionally, UGT1A10-transfected COS-1-cell cultures glucuronidated $[^{14}C]-17\beta$-estradiol, unlike those transfected with its null mutants at PKC phosphorylation sites.

**EXPERIMENTAL PROCEDURES**

*Chemicals-* All substrates were obtained from Sigma Chemical Co. (St. Louis, Mo), Fluka (Milwaukee, WI), Aldrich (Milwaukee, WI), or Wako Pure Chemical Industries (Richmond, VA).

*Preparation of Microsomes-* Human tissue adjacent to that used for in-situ analysis (12) was snap-frozen and stored at -80°C until microsomes were prepared (14). Microsomes were resuspended in phosphate buffered saline and stored at -80°C until UGT was analyzed.

*Source of UGT1A1, 1A7, 1A8, and 1A10 expression units-* The pSVL-based UGT1A1 expression unit was previously described (15). UGT1A7, 1A8, and 1A10 were constructed as described (12). Similarly, UGT1A3 and 1A5 were constructed using the UGT1A4-cDNA (15) as template instead of UGT1A9-cDNA and an appropriate genomic subclone as described (12).

*Transfection of UGT1-cDNA-expression Units into COS-1 cells-* All cDNA expression units were transfected into COS-1 cells using DEAE-Dextran as the carrier (15).

*Western Blot Analysis of UGT-cDNA-dependent Expression-* To establish the relative amounts of each UGT protein, an antibody (16) prepared by Veritas (Rockville, MD) was raised in rabbit against the common 245-amino acid carboxyl-peptide present in each UGT1-encoded isozyme and used for Western blot analysis (16). pSVL-based cDNA-dependent expression of UGT1A1, 1A3, 1A7, 1A8, or 1A10 protein in COS-1 cells was harvested 72 hr after transfection; 100 μg cellular protein was prepared and electrophoresed in a 7.5 or 15 % polyacrylamide-SDS gel and electrotransblotted onto nitrocellulose membranes, which were processed as described (16). Proteins
were visualized by immunoreacting with rabbit anti-UGT1 and goat anti-rabbit second antibody-HRP-conjugate according to the ECL protocol (Amersham Life Science, Arlington Heights, IL), which exposed x-ray films for development and quantitation.

**Assay for Glucuronidation of Chemicals by UGT1A1, 1A3, 1A7, 1A8, and 1A10** - The modified glucuronidating assay system has been described (17, 18). The common donor substrate, UDP-[\(^{14}\)C]glucuronic acid (1.41 mM, 1.4 µCi/µmol), was used in all in-vitro reactions with an unlabeled acceptor/aglycone substrate. Similar to reactions for Km determinations, pH profiles reflect product generated in 2 hr at 37°C with 300 µg protein from pSVL(UGT)-transfected COS-1 cells. Linear glucuronidation reactions for substrate screens were conducted at both pH 6.4 and 7.6 over 4 hr and defined as glucuronide accumulated/4 hr as described (19). All chemicals were solubilized in fresh DMSO.

**Mutagenesis of Predicted PKC Phosphorylation Sites in UGT1A10** - Site-directed mutagenesis at amino acid 73, 202 or 432 in UGT1A10 was carried out as described (18). Oligos for converting the common PKC site, S432G, in UGT1A10 were: sense (214C) (5-ctgcttggtcacccgatgaccc-3) and antisense (463C) (5-ggcgcatgatgttctccttgtaaccttg-3) for fragment 1 and sense (435C) (5-caaagttacaagggacaacatgcgcc-3) and antisense (BamH1Stop) (5-cccggatccaccacctttcaatggtgtt-3) for fragment 2. For T73A mutation of UGT1A10, primers were: sense Xho1A10S (5-ccctcgagggagctgctggctcgggct-3) and antisense 73A10AS (5-cgaggttgagtaagtcttcactgcggca-3) for fragment 1; for its overlapping unit, the sense was the complement of 73A10AS, and the antisense was PXAS6 (18). Finally, for T202A mutation for UGT1A10, primers were: sense Xho1A10S and antisense 202A10AS (5-ccatactctctctgtgagctgttcgtcgc-3), and its overlapping fragment was synthesized using the complement of 202A10AS as sense and PXAS6 as antisense.
$^{33}$P-Orthophosphate Labeling of UGT1A10 in COS-1 Cells - Sixty hr after transfection with a pSVL-based construct containing either UGT1A10, single mutant (T73A-, T202A-, or S432G-1A10), double mutant (T73A/ T202A-, T73A/S432G-, or T202A/S432G-1A10), or triple mutant (T73A/T202A/S432G-1A10) was compared to COS-1 control cells. Cells were conditioned in phosphate-free medium for 12 hr and also serum-free conditions for the last 3 hr before exposure to $^{33}$P orthophosphate (5.0 mCi/mL) for 8 hr as described (20,21). With all manipulations carried out at 0-4°C, attached cells were washed 5 times with phosphate-buffered saline, solubilized in 1% Triton X-100, 0.5 % sodium deoxycholate, 0.1 % sodium dodecylsulfate, 1.0 mM EDTA in 25mM Tris-borate-137mM NaCl (TBS) pH 7.4 (buffer A), passed through a 26-gauge needle to reduce viscosity, and spun in microfuge tubes to remove insoluble cellular debris. Supernatants were recovered, protein estimations were carried out, and equal amounts of protein reacted with anti-UGT for 1hr before addition of Protein-A-Sepharose equilibrated in buffer A to allow immunocomplexes to form for 12 hr with sample-rotation (21). UGT-immunocomplexes bound to Protein-A-Sepharose were washed 4 times with buffer A for 1 hr, washed overnight with buffer A/containing 1M KCl, and finally once each with TBS and water. After samples were boiled in SDS sample-buffer to detach protein, each sample was spun through a microSpin column (Amersham, Piscataway, NJ) to remove beads. Duplicate sets of radiolabeled UGT1A10 and mutants were electrophoresed in a SDS-7.5 % polyacrylamide gel; one gel was processed for exposure to x-ray film, and the duplicate gel was analyzed by Western blot as described above.

$^{14}$C Estradiol Glucuronidation by COS-1 Cells Transfected with UGT1A10 or its Mutants at PKC Phosphorylation Sites or UGT1A1- Forty-eight hr after transfection with
pSVL-based UGT1A1, UGT1A10, or T73A-, T202A- or S432G-1A10 mutants, COS-1 cells were conditioned in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 4% charcoal stripped (cs) fetal bovine serum (FBS) for 24 hr before adding 20 or 40 μM [14C]17β-estradiol (2.5 μCi/40 μM) in fresh DMEM/csFBS. Also, a concentration range of 17β-estradiol was studied. After 3 hr, 500 μL of medium in triplicates were diluted 1:2 with cold ethanol and spun to remove protein. Recovered supernatant was spun dry and resuspended in phosphate buffered saline (PBS). One-half of each sample was treated with buffer or 50 units of β-glucuronidase in 50 μM phosphate buffer, pH 6.8, for 2 hr at 37°C. Samples were vacuum-dried, resuspended in 70% ethanol, and applied to TLC plates for elution as described (18) for quantitation (19).

RESULTS

Glucuronidation of Epigallocatechin 3-O-gallate, 17β-Estradiol, and Flurbiprofen by Microsomes isolated from Human GI-tissues- To gain insight into the function of the UGT1-encoded isozymes, we previously compared their pattern of tissue-distribution and cellular location by Northern blot analysis and in-situ hybridization of messenger RNAs (12). We found UGT1A10 is primarily distributed in the mucosal layer of the GI tract below the stomach (12) with substantial amounts in other tissues. We also used microsomes prepared from adjoining tissue to that used for in-situ hybridization in conjunction with the highly specific UGT1A10-substrate, epigallocatechin 3-O-gallate (ECG) (see reference 12), to assess its relative level of activity in GI tissues.

[Determination of substrate specificity was based on a screen of 40 chemicals using recombinant UGT1A1, 1A7, 1A8, 1A9, and 1A10 (12) at both pH 6.4 and 7.6. Under similar conditions and for a given substrate, isozyme specificity was determined as the ratio of an isozyme-activity to the sum of all isozyme-activities.] In this study with GI microsomes, we compared ECG with two other
UGT1A10-substrates, flurbiprofen (pH 6.4) and 17β-estradiol (pH 7.0), which were 83- and 89 %-
specific, respectively. UGT 1A10 appeared to be present in
duodenum:ileum:colon:stomach:esophagus at relative levels of 9:8:3:2:1 (Fig. 1). While the
isozyme was most abundant in small and large intestines, which was followed by stomach, and it
showed greater turnover of 17β-estradiol than ECG and flurbiprofen, it appeared detectable in
esophageal microsomes by substrate analysis. Although UGT1A8 was detectable only in the
stomach by Northern blot analysis (12), glucuronidation studies with phloretin at pH 7.0 and in-situ
hybridization with specific probes demonstrated moderately high to high levels in esophagus,
duodenum, ileum and colon (12). Except for stomach, UGT1A8 is concentrated in a highly erratic
pattern in limited regions of all GI tissues. UGT1A7, on the other hand, was highest in esophagus
and at low to intermediate levels in nearly every tissue with the highest level in thyroid and adrenal
glands (12). While Northern blot detected UGT1A3 only in liver (unpublished data), it was shown
that the isozyme, like UGT1A8 (12), can be found in GI tissues following RT-PCR amplification of
mRNA (23). In a recent study with bilirubin as UGT1A1-specific substrate (12), we found 1A1 was
distributed in duodenum, ileum and stomach at relative levels of approximately 10:4:1 with activities
expressed as pmol/hr/mg prot.

*Western Blot Analysis of UGT1A1-, 1A3-, 1A7-, 1A8-, and 1A10-expressed in COS-1 cells*- To
normalize the relative levels of UGT proteins, we carried out Western blot analysis (Table 1A) with
the UGT1A common-end antibody (16), and the relative levels of UGT1A1, 1A3, 1A7, 1A8, and
1A10 synthesized in COS-1 cells were determined by scanning blots with a UMAX system and
quantifying with Adobe Photoshop software. After normalizing relative protein levels, specific
activities were calculated and are shown.
pH Optimization and Km Determination for Glucuronidation of 17β-Estradiol and Flurbiprofen

by UGT1A10- Since our earlier studies with UGT1-encoded isozymes demonstrated optimal conditions for glucuronidation depended, to a large extent, upon the acceptor substrate (12), we optimized conditions for the type of chemical. UGT1A10 metabolized 17β-estradiol with a pH optimum between 6 and 8 and flurbiprofen with a narrower pH range between 6 and 6.4 (Fig. 2A and 2C); typical pH-7.6 assay conditions did not show glucuronidation of the latter aglycone. The pH profile for glucuronidation of ECG (not shown) was essentially the same as for flurbiprofen.

Since increasing concentrations of phloretin and eugenol up to 200 μM stimulated UGT1A10 activity and higher levels showed progressive inhibition that culminated at 100% by 800 μM (12), we examined effects of 17β-estradiol or flurbiprofen concentration on activity. Concentrations of 17β-estradiol and flurbiprofen from 200 to 400 μM stimulated activity, which was sustained up to 800 μM. The Km value for both 17β-estradiol and flurbiprofen was 64 μM (Fig. 2B and D). Complete inhibition of UGT1A10 by 800 μM phloretin or eugenol (12), but not by high concentrations of 17β-estradiol and flurbiprofen, indicates inhibition is substrate-dependent.

Because published studies (24,25) indicate UGT1A also glucuronidates 17β-estradiol, we compared its metabolism by 1A10 to that by 1A1. Increasing the concentration of 17β-estradiol from 2 to 20 μM caused a 2 fold increase in UGT1A10 activity, as seen in Fig. 2A, and further increases in concentrations up to 100 μM (Fig. 3) showed almost a linear increase in activity. To the contrary, 20 μM 17β-estradiol showed no detectable conversion by UGT1A1, but 40 μM elicited a substantial 1A1 activity that was barely enhanced further by increases up to 100 μM (Fig. 3). Unlike its limited concentration effects on UGT1A1 conversion, 17β-estradiol supported a high rate of...
metabolism by UGT1A10 between 2 and 200 μM (Figs. 2B and 3). At 40 μM, UGT1A10 showed >10-fold greater activity than 1A1 (Fig. 3).

*Glucuronidation of Estrogens, Plant-derived Aromatic Acids, and Nonsteroid Anti-inflammatory Drugs by UGT1A10, 1A1, 1A3, 1A7, and 1A8* - In an earlier study (12), we observed that UGT1A10 metabolized two different types of phytoestrogens nearly equally at both pH 6.4 and 7.6. Furthermore, we found that UGT1A4, 1A5, 1A6, 1A9, UGT2B7 and 2B15 did not show a significant activity toward the primary estrogens (data not shown) (26), although recombinant 2B7 was previously reported to have detectable activity when microsomes were analyzed (24). Since phytoestrogens and mammalian estrogens often exhibit similar actions and show competitive interactions via the estrogen receptor (1,6), we compared the metabolism of endogenous estrogens by UGT1A10 to that by 1A8, 1A7, 1A3, and 1A1 using equal specific protein (Table 1A). Eight out of 9 estrogens, including their derivatives, were excellent substrates for UGT1A10 at both pH 6.4 and 7.6 (Table 1B). UGT1A10, 1A8, 1A7, and 1A3 showed a 5 to >10-fold preference for the predictably more hydrophobic 2-CH₃O(methoxy)-estradiol than 2-OH(hydroxyl)-estradiol; similarly, this enzyme set exhibited from 6- to 17-fold greater preference for 4-OH-estrone than 2-OH-estrone. UGT1A10 showed exceptional and equally high activity towards 3 estriol isomers, which contained 3 different versions of 16- and 17-hydroxyls that project in versus out of the plane of the core steroid structure (see Table IB); the estriols turnover were only some 23 to 30% less than that for estrone and 2-CH₃O-estradiol, which gave the highest activities for steroids. UGT1A8 showed more than 4-fold preference for the 16,17-estriol than 17-estriol. Interestingly, the two least effective estrogenic substrates-- 2-OH-estradiol and 2-OH-estrone -- for UGT1A10 were converted at the highest level by UGT1A1, which was 2.5- to 4.5-fold higher than by 1A10. Hence, the two
Isozymes showed complementary activity toward the 2-OH estrogens, and, overall, UGT1A10 was superior to 1A1, 1A8, 1A7, and 1A3 in metabolizing estrogens.

With few exceptions, all isozymes metabolized the estrogens and their derivatives nearly equally under both pH conditions, suggesting a broad pH optimum as seen in Fig. 2A for 17β-estradiol. UGT1A9 inefficiently metabolized certain estrogen derivatives at either pH 6.4 or 7.6 (Legend of Table I).

In a previous report (12), UGT1A10 was shown to glucuronidate 32/40 chemicals with a 1.5 to 10-fold preference for pH-6.4 optimum. Substrates included anthraquinones and related chemicals, flavonoids/isoﬂavones (phytoestrogens) and related chemicals, polycyclic aromatic hydrocarbons- and simple-phenols, and lactone-containing phytoestrogens. In contrast, UGT1A10 converted 7/8 estrogens (this study) and 6/7 phytoestrogens (12) nearly equally at pH 6.4 and 7.6; only estrogenic 2-OH-estradiol and phytoestrogenic daidzein showed a pH-6.4 preference. Among the 40 chemicals (12), UGT1A10 metabolized isoflavones or phytoestrogens the most efﬁciently; UGT1A7 had comparably high turnover for the lactone-containing phytoestrogens (coumestrol and zearalenone) and the isoflavan, equol. Among estrogenic compounds, UGT1A3 effectively conjugated only 2-CH3O-estradiol.

Because UGT1A10 exhibited a high activity toward many dietary agents, including phytoestrogens (12), and it is distributed primarily in GI mucosa from duodenum through colon, we carried out substrate analysis on carboxyl-containing chemicals known to form in the lower GI tract by microflora from ﬂavonoids found in high levels in our plant-based diet (9) or to be ingested as dietary constituents or as therapeutic agents such as NSAIDs. Studies with such substrates, which included salicylates, transcinnamic acids, β-phenylpyruvic through diphenyl/biphenyl acids, as well as 14 therapeutic NSAIDs (Tables II and III), show UGT1A10 exhibited the major activity, whereas
UGT1A7, 1A8, and 1A3 exhibited significant activity. For plant-based salicylates, methysalicylate was the most effective substrate in this study and by UGT1A10; 1A7 and 1A8 were approximately one-half as effective; and 1A3 exhibited 3% the level of 1A10 activity. Acetylsalicylic acid (aspirin) was best converted by UGT1A10, but there was little conversion of salicylic acid by any isozyme. Among metabolites (aldehydes and acids) (Table II) originating from plants, UGT1A10 conversion of aldehyde-containing vanillins was second to methylsalicylate showing 50% the level of conversion; 1A8 and 1A7 also showed high activity toward the vanillins yielding 30 to 50% that of 1A10 at both pH values. Oxidation of the aldehyde group in 4-OH, 3-CH₃O-benzoic acid (vanillic acid) dramatically reduced turnover by 1A8, 1A10, and 1A8 (Table II) showing only 6 to 37% the activity for the best aldehyde analog.

Also UGT1A10 expressed the highest conversion of transcinnamic acids, including caffeic and ferulic acids at pH 6.4. For the pyruvic, propionic-, and isovaleric-phenyl acids (Table II), UGT1A3 showed approximately 2-fold greater activity than other isozymes. Diphenyl- and biphenyl acetic acids, with free rotation of phenyl groups versus fixed groups, were metabolized nearly equally by the isozymes examined.

Results with therapeutic polyaromatic acids, NSAIDs (Tables II and III), show UGT1A10 converted mefenamic acid, diflunisal, flurbiprofen, fenoprofen, ibuprofen, and indomethacin at levels comparable to the vanillins and certain estrogens, but with pH 6.4 preference. Overall, UGT1A3 metabolized the NSAID at a moderate level with pH 6.4 preference, except for indoprofen.

It is notable that the nonacidic, but aromatic acetaminophen is not a substrate for either of these isoforms or a poor substrate for UGT1A3 and UGT1A10.

There are other significant observations concerning glucuronidation of these chemicals. While UGT1A1 metabolized certain estrogens, it did not metabolize any of the aromatic acids in this study.
This is notably since this isozyme forms ester-linked glucuronides via the propionic acid groups of the bilirubin IXα molecule (15).

$[^{33}P]$-Orthophosphate Labeling of UGT1A10 with Effects of Mutations at Predicted PKC Phosphorylation Sites and on In-vitro Glucuronidation- Because exposure of colon cells to the dietary constituent, curcumin, at 50 µM rapidly inhibited UGT activity for test substrates, eugenol, phloretin, capsaicin, ECG, and bilirubin (12,21), we carried out experiments to assess the substrate range of inhibition. Earlier results showed calphostin-C, a highly specific PKC inhibitor, irreversibly down regulated glucuronidation suggesting UGT proteins undergo phosphorylation. Further, we carried out computer searches for consensus sequences for phosphorylation and uncovered 4 to 5 predicted PKC phosphorylation sites in each protein (21). Consistent with these findings and under conditions of equal protein (Fig. 4, top panel), the radiogram (Fig. 4, bottom panel) shows UGT1A10 incorporated $[^{33}P]$phosphate, which when compared with predicted PKC-site mutants for single positions (T73A, T202A, and S432G) and for double positions (T73A/T202A, T73A/S432G, and T202A/S432G) showed progressively less label and that for triple positions (T73A/T202A/S432G) exhibited no detectable labeling or activity. The graded effect based on number of sites remaining, not position, suggests each predicted PKC site is utilized for phosphorylation evidently by PKC isozyme(s) (21). Furthermore, calphostin-C, the PKC-specific inhibitor, inhibited 17β estradiol activity without detectable labeling in UGT1A10, as previously demonstrated for 1A1 unpublished data and (21). Glucuronidation of 17β-estradiol by other mutants is comparable to that shown in Fig. 5 and is discussed below. Complete inactivation of the triple mutant, but full 17β-estradiol activity for wild type UGT1A10, indicates activity is dependent upon phosphorylation.
It is also evident that a major radiolabeled species and a barely detectable one are present in the nascent UGT1A10 evidently reflecting on-going protein maturation due to glycosylation at 3 predicted glycosylation sites as previously seen for other UGTs (26). A minor band at the top is not distinguishable by Western blot analysis.

**In-vitro Glucuronidation by COS-1 Cells Transfected with Wild-type or UGT1A10 Mutants**

Since UGT1A10 exhibited high activity towards estrogens (Table 1B), phytoestrogens (12), and NSAIDs (Tables II and III), we examined the effect of independent, double and triple mutations on the capacity of UGT1A10 to glucuronidate 17β-estradiol, genistein, and flurbiprofen. The isozyme exhibited the greatest activity towards the phytoestrogen, genistein, followed by 17β-estradiol and flurbiprofen (Fig. 5). Its Km for genistein (not shown) is similar to that for 17β-estradiol and flurbiprofen (Fig. 2). Furthermore, the T73A, T202A, and their double mutant, T73A/T202A, exhibited null activity for each substrate. The S432G mutant caused an increase in activity for each of the substrates, and its double mutant with either T73A or T202A showed partial or additive activity with genistein and 17β-estradiol, but barely detectable activity with flurbiprofen. Finally, the triple mutant showed null activity for each of the test substrates (Fig. 5). There was no detectable difference in activity between S432G-1A10 and S432A-1A10 (unpublished data).

**Glucuronidation of [14C]-17β-Estradiol by COS-1 Cells Transfected with Wild-type or UGT1A10 Mutants**

To determine if phosphorylation of a recombinant isozyme controls glucuronidation in cells, we compared conversion of [14C]17β-estradiol by control COS-1 cells to UGT1A10-, T73A-1A10-, T202A-1A10-, or S432G-1A10-transfected cells. Before cell culture results are presented, we provide evidence [14C]17β-estradiol-glucuronide produced in-vitro is resolved by the TLC system (Fig. 6A). Lanes from left to right show migration of
\[^{14}\text{C}]17\beta\text{-estradiol (17}\beta\text{-est)}, \text{effect of control cell protein on migration of 17}\beta\text{-est, resolution of 17}\beta\text{-est and 17}\beta\text{-est-gluc, and hydrolysis of 17}\beta\text{-est-gluc by }\beta\text{-glucuronidase (}\beta\text{-G’dase) (Fig. 6A). Results in Fig. 6B show UGT1A10 and its S432G mutant formed comparable levels of }\[^{14}\text{C}-17\beta\text{-est-gluc, which was sensitive to }\beta\text{-glucuronidase (Lower panel). To the contrary, cells transfected with either T73A- or T202A-1A10 mutant failed to generate the glucuronide. The results demonstrate wild type UGT1A10 and its mutants at PKC sites exhibited glucuronidation patterns in cell culture similar to those in in-vitro assays.}

With duplicate cultures to those that synthesized \[^{14}\text{C}]17\beta\text{-estradiol-glucuronide (Fig. 6B), we demonstrate again that in-vitro results with 17\beta\text{-estradiol and genistein combined with }[^{14}\text{C}]\text{UDP-glucuronic acid replicated findings in cell culture (Fig. 6C). A scan of the Western blot (top panel) was used to normalize specific protein levels.}

\text{Relative Effectiveness of UGT1A10- versus UGT1A1-transfected COS-1 Cells toward Glucuronidation of }[^{14}\text{C}]17\beta\text{-Estradiol-} \text{ Despite the fact UGT1A10 (pH 6.4) showed more than 13-fold higher activity toward 17\beta\text{-estradiol than UGT1A1 (Table 1B and Fig. 3), 1A10 has, presumably, a high Km (estradiol) value as shown in Fig. 2 making it unclear which isozyme is likely to glucuronidate the estrogen under in-vivo conditions. Furthermore, the ineffectiveness of 20 }\mu\text{M 17\beta\text{-estradiol or lower on UGT1A1 activity, but substantial stimulation by 40 }\mu\text{M, complicated expectations for in-vivo glucuronidation by 1A1. Hence, we carried out glucuronidation of 20 and 40 }\mu\text{M }[^{14}\text{C}]17\beta\text{-estradiol in COS-1 cells transfected with UGT1A1 or 1A10 (Fig. 6D). After normalization for transfection efficiency, }[^{14}\text{C}]17\beta\text{-estradiol (20 or 40 }\mu\text{M) showed > 10-fold greater metabolism by UGT1A10 than by 1A1 (Fig. 6D).}

\text{DISCUSSION}
Among the isozymes encoded at the UGT1 complex locus, UGT1A10, distributed primarily in the GI tract (12) with significant levels in heart, placenta, spleen, pancreas, lymphnode, adrenal, and uterus, expressed more than 10 and 100-fold higher activity toward the primary estrogens, 17β-estradiol and estrone, respectively, than UGT1A1 (Table 1B). Moreover, UGT1A10 metabolized all estrogen derivatives tested at 16- to more than 10,000-fold higher rates than 1A1, except for 2,3-catechol estrogens. With an inverse pattern, UGT1A1 metabolized 2-OH-estradiol and 2-OH-estrone at rates 2- and 4.5-fold higher, respectively, than UGT1A10. Overall, this study shows that UGT1A10 metabolized estrogens at a higher level than other UGT1A isozymes and with an atypically broad pH range compared to its high pH-6.4 preference observed for a screen of 40 test chemicals (12). It is notable that UGT2B7 metabolized many estrogen derivatives (26-28), but not estrone or 17β-estradiol (data not shown here) (26), except 2B7 did show activity using microsomes isolated from a different expression system (24). The ineffectiveness of UGT1A1 at converting 17β-estradiol compared to the high level of conversion by 1A10 in cell culture suggests it is most likely responsible for glucuronidating endogenous estrogens, despite its high Km value. Thus, UGT1A10 appears to be unique in carrying out significant transformation of the primary estrogen, 17β-estradiol.

Interestingly, we discovered earlier that UGT1A10 also metabolized several categories of phytoestrogens (12): the isoflavone-type (daidzein, genistein, formononetin, and biochanin A), an isoflavan-type (equol), lactone-containing compounds (coumestrol and zearalenone) and estrogenic flavonoid precursors (5) [phloretin and others (12)] with a broad pH range and at a level comparable to the estrogens described in this study. Among 4 different categories of chemicals (12) and after eugenol, phytoestrogens were the most highly preferred class of substrates by UGT1A10. Phytoestrogens, like catechol estrogens (26, 27), are estrogenic due to structural similarities, which
allow competition at the level of the estrogen receptor (1,5). As UGT1A10 metabolized 17β-estradiol in vitro at low concentrations (2 to 20 μM) to a high extent, unlike 1A1, it is likely that 1A10 is adapted to convert both estrogens and dietary phytoestrogens, despite its Km (64 μM), but broad pH range (Fig. 2). The devastation that phytoestrogens can inflict on the reproductive systems of livestock that consume large quantities of clovers with high phytoestrogen content (2) underscores the potential damage these agents can impose via interaction with the estrogen receptor and the need to limit their GI-absorption. Predictably, GI-distributed UGT1A10, which is the most effective at converting these dietary phytoestrogens in vitro, is adapted to allow humans to limit their absorption to reduce the risks of reproductive failures.

A striking feature of ingested test-flavonoids was demonstrated by a lack of absorption even when taken in high levels (8, 9). The possibility existed that the chemicals undergo catabolism in the lower GI tract by microflora (9) as suggested by many aromatic acids derived from flavonoids found in urine of treated animals (9). Our examination of flavonoid- and catechin-derived catabolic acids uncovered their capacity to undergo glucuronidation. Unexpectedly UGT1A3, detectable in GI tissue by RT-PCR (23) and at moderate levels in liver by Northern blot analysis (unpublished data), showed significant glucuronidation of simple aromatic acids, such as pyruvic-, propionic-, isovaleric-, cinnamic-(cafeic, ferulic) and benzoic acid (vanillic), as well as a benzaldehyde-containing metabolite of flavonoids, produced by microflora in the lower gut (10). The two benzaldehydes were metabolized, at least, an order of magnitude better by UGT1A10, 1A8, or 1A7 than by UGT1A3. Generally, the evidence shows that acid-containing flavonoid metabolites are most effectively metabolized by UGT1A10 and 1A3.

Additionally, we compared the more complex carboxyl-containing therapeutic NSAIDs with the simple plant-derived acids. While UGT1A10 was superior to the other isozymes in converting
NSAIDs --as demonstrated by mefenamic acid and diflunisal (Table III)--, 1A8, 1A7, and 1A3 also avidly transformed mefenamic acid. Diclofenac was preferred by UGT1A3. UGT1A10 and 1A7 transformed the related acidic structure, furosemide. In summary, UGT1A10 appears to be the primary isozyme for converting both simple and polycyclic aromatic acids. Despite the essentially unique capacity of UGT1A1 to metabolize the endogenous acid, bilirubin (15), it was completely ineffective with exogenous acids in this study. Although catalysis by UGT1A3 did not reach the level observed for 1A7, 1A8 and 1A10, it is possible that over time its conversions under in-vivo conditions have an impact.

As biphenyl acetic acid, mefenamic acid, flurbiprofen, fenoprofen, ibuprofen, and indomethacin, and a list of other test-chemicals in this study had acidic groups but lack a hydroxyl group for glucuronide formation and were exceptional substrates for UGT1A10, the results demonstrate 1A10 is likely the major catalyst for forming carboxyl-linked glucuronides and not UGT1A3. Also, the high activity with methysalicylate and poor activity with salicylate indicates the absence of intramolecular hydrogen-bonding between hydroxyl and carboxyl substituent groups has a highly positive effect on metabolism by UGT1A10. Inability to form intramolecular hydrogen-bonding in the highly effective substrates, aldehyde-containing vanillin and o-vanillin compared to ferulic and vanillic acid (Table II), is further evidence hydrogen-bonding has a negative impact on conjugation (29).

Labeling of UGT1A10 expressed in COS-1 cells with $[^{33}P]$orthophosphate, which was not detected with T73A/T202A/S432G-1A10 triple PKC mutant, confirms the isozyme undergoes phosphorylation most likely by PKC. Further, the progressive decrease in labeling of single and double mutants suggests each predicted PKC site is phosphorylated (Fig. 4). As S432G mutant exhibited increased and apparently wild-type activity and T73A and T202A mutants were
completely inactive, one would expect S432G/T73A and S432G/T202A double mutants to be null. It is interesting, however, that the two double mutants show additive activity, indicating S432G is different and not the equivalent of wild-type. Furthermore, additive activity suggests phosphate groups are utilized in a hierarchical and/or combinatorial manner in controlling activity (21). Also, the complete loss of activity of both T73A and T202A demonstrates phosphorylation is required and that both threonines are critical for activity (Figs. 4 and 5). A comparison of PKC-site mutants in the amino terminus of UGT1A10 and UGT1A1 proved to have null activity, whereas mutants at the site located in the most carboxyl position (S432/S435) caused either an increase (this study) or decrease in activity (21). One can conclude that phosphorylation at specific sites (T73 and T202) is critical for activity, which will require structural studies to establish site-specific effects. Furthermore, the fact that cultures of COS-1 cells transfected with the wild-type or the active S432G mutant, but not the inactive mutants, glucuronidated 

While a natural polymorphism, T202I in UGT1A10, has been reported (30) that exhibited partial activity for 17β-estradiol and not null activity as our T202A mutant, it is not known what role phospho-threonine-202 plays in enzyme activity and how isoleucine-202 is able to support partial glucuronidation of 17β-estradiol. Structural studies of UGT or 1A10 are required to understand the role phospho-threonine/serine plays in catalysis. Partial activity by T202I-UGT1A10 is similar to our observation that T73A-UGT1A9 has 20% activity with propofol, whereas T202A/G and T73G mutants are null (Basu, N.K. et al, Manuscript in preparation). Also, our test with 17β-estradiol and S432G- versus S432A-1A10 mutants showed no detectable difference (unpublished data).

This study and our earlier results (12) support the conclusion that UGT1A isozymes have overlapping activities that collectively expand detoxification by having and using flexible properties
of different pH optima, which depend upon acceptor substrate. Since mucosal tissues of the GI tract that harbor different UGT isozymes face wide variations in chemical structures and chemical gradients in the GI, it is likely the differential properties represent adaptations that enhance the detoxification process. As UGT1A10 is located primarily in GI mucosa from duodenum through colon (12) positioned to restrict GI absorption of damaging chemicals, this represents the first demonstration that UGT1A10 is pivotal for detoxifying estrogens/phytoestrogens and aromatic acids with evident dependency upon phosphorylation. Why this critical function of limiting chemical absorption of potentially damaging dietary constituents toward the reproductive system and other endogenous targets is dependent upon phosphorylation warrants further investigations. Unfortunately, many medicinal drugs, such as NSAIDs, also encounter premature conversion by these isozymes compromising therapeutic efficacy. The requirement for phosphorylation allows a target for down-regulation to enhance absorption of orally administered therapeutic drugs, which undergo significant glucuronidation, as recently suggested via transient inhibition of UGT activity by curcumin treatment (12).

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REFERENCES


**FIGURE LEGENDS**
FIG. 1 Glucuronidation of epigallocatechin 3-0-gallate, 17β-estradiol, and flurbiprofen by human microsomes from GI tissues. Normal human GI tissues were collected (12) and stored at -80°C until microsomes were prepared (14), and glucuronidation of epigallocatechin 3-0-gallate (ECG), 17β-estradiol, and flurbiprofen at 200 µM each was carried out in 2-hr incubations at 37°C as described under Experimental Procedures. The images represent autoradiograms of the TLC plate that resolved the [14C]-glucuronides. Product is the mean ± S.E. A total of 2 esophagi, 3 stomachs, 3 duodena, 2 ilea, and 4 colons were analyzed thrice in triplicates (12).

FIG. 2 pH optimization and Km determination of 17β-estradiol and flurbiprofen glucuronidation by UGT1A10. (A and C) pH profiles were carried out with sodium phosphate from pH 5 to 7 and with triethanolamine from pH 7.0 to 9.0 (12) with 200 µM substrate in 2 hr incubations at 37°C as described under Experimental Procedures. (B and D) Similarly, Km values were established using 300 µg cellular protein in 2 hr incubation for glucuronidation of 17β-estradiol (pH 7.0) and flurbiprofen (pH 6.4). Assays were repeated thrice in triplicates; standard errors ranged from ± 1 to 3 %.

FIG. 3 Comparison of in-vitro glucuronidation of 17β-estradiol by UGT1A1 and UGT1A10.
Glucuronidation of 17β-estradiol was carried out at pH 6.4 in 2-hr incubations at 37°C as described under Experimental Procedures.

FIG. 4 Labeling of recombinant UGT1A10 and its mutants with [33P] orthophosphate. Cells were either mock-transfected or transfected with UGT1A10 or its single mutants (S432G, T73A, and T202A) or 3 double mutants (T73A/T202A, T73A/S432G, and T202A/S432G) or the triple mutant (T73A/T202A/S432G). Conditioned transfected-COS-1 cells, grown in 35 mm plates, were exposed to [33P] orthophosphate (5.0 mCi/mL)-containing medium for 8 hr as described under Experimental Procedures. With all samples containing equal cellular protein, duplicate
sets of unlabeled and radiolabeled samples for control, UGT1A10, and mutants were immunocomplexed and processed in a SDS-7.5 % PAGE system as described under Experimental Procedures. The radiolabeled gel was fixed and exposed to x-ray film for 48 hr (bottom panel), and the unlabeled gel was subjected to Western blot using anti-UGT (top panel). Glucuronidating activity was analyzed with 200 µM 17β-estradiol in a 2-hr incubation at 37°C. The experiment was repeated thrice. ND denotes not detectable.

FIG. 5 Effects of various mutations at predicted PKC phosphorylation sites in UGT1A10 on activity toward different substrates. (A) Western blot analysis with anti-UGT was carried out with 10 % SDS-PAGE as described under Experimental Procedures. (B) Glucuronidation assays were carried out using equal amounts of specific protein from COS-1 cells transfected with constructs having either single, double, or triple mutations at PKC sites in UGT1A10. Single mutants were T73A, T202A, and S432G; double mutants were T73A/T202A, T73A/S432G, and T202A/S432G; and the triple mutant was T73A/T202A/S432G. Flurbiprofen incubated 3 hr at 37°C, while 17β-estradiol and genistein (a phytoestrogen) incubated 2 hr. Each chemical was at 200 µM in the reactions. Experiments were repeated thrice in triplicates; standard errors ranged from ± 2 to 4 %.

FIG. 6 Glucuronidation of 17β-estradiol by UGT1A10 and its mutants is similar under in-vivo and in-vitro conditions (A-C). Comparison of glucuronidation of 17β-estradiol by UGT1A10- or UGT1A1-transfected COS-1 cell culture (D). (A) Replica of the TLC system used to separate [14C] 17β-est from its gluc. Lanes from left to right: migration of [14C]17β-est, effect of control COS-1-cell homogenate on [14C]17β-est, production of [14C]17β-est-gluc by homogenates containing UGT1A10, and loss of [14C]17β-est-gluc formed by 1A10 following β-glucuronidase treatment. (B) Production of [14C]17β-est-gluc by COS-1 cell cultures transfected with 1A10,
S432G-1A10, T73A-1A10, T202A-1A10, or control. Three hr after exposure to 40 µM $^{14}$C 17β-est, 500 µL of culture medium were prepared for TLC analysis as described under Experimental Procedures. Before samples were applied to plates, one half of certain samples was treated with β-gluc (Lower panel) as described under Experimental Procedures. (C) In-vitro production of 17β-est-gluc and genistein-gluc using duplicate cultures to those in (B). In-vitro glucuronidation of 17β-est or genistein used $^{[14}$C]UDP-glucuronic acid as co-substrate and is shown after normalization with the Western blots (top panel) as described under Experimental Procedures. (D) Comparison of $^{[14}$C]17β-est glucuronidation (Counts) by UGT1A1- and UGT1A10-transfected COS-1 cell cultures. $^{[14}$C]17β-Estradiol was added to COS-1 cell cultures expressing UGT1A1 or UGT1A10, which incubated 3 hr, and 500 µL were processed and quantitated as described above under (B). Western blots (designated protein) of each UGT in duplicate cultures were carried out, scanned, and used to normalize counts as described under Experimental Procedures.

Abbreviations are: 17β-Est for 17β-estradiol; 17β-Est-Gluc for 17β-estradiol-glucuronide; and β-G’dase for β-glucuronidase.

LEGENDS FOR TABLES

TABLE 1A  Western blot analysis to establish relative levels of UGT1A1, 1A3, 1A7, 1A8, and 1A10 used to generate activities shown in Tables 1B, II and III was determined as described under Experimental Procedures. Sufficient protein was generated to allow the completion of the study. Experiments were repeated thrice in triplicates.

TABLE 1B  Nine estrogens or their derivatives were screened for glucuronidation by the UGTs identified using 200 µM of each chemical at pH 6.4 and 7.6 with 300 µg UGT-transfected COS-1 cell protein. Reactions incubated 4 hr at 37°C and were processed for quantitation as
described under Experimental Procedure. While 1A4, 1A5, 1A6, 1A9, 2B7, and 2B15 showed no detectable glucuronidation of 17β-estradiol or other estrogens, 1A9 showed detectable conversion (pmol/mg prot/4 hr) of 2-0H estrone (30/15 at pH 6.4 and 7.6, respectively), 4-0H estrone (180/520, at pH 6.4/7.6), and 2-methoxyestradiol (30/210 at pH 6.4/7.6).

**TABLE II** Sixteen aromatic acids, which are either therapeutic NSAIDs, derived directly from plants, or catabolites from flavonoid-glycosides by intestinal microflora were analyzed for glucuronidation by UGTs shown as described in the legend to Table 1B. Also, glucuronidation of nonacidic aromatic acetaminophen and synthetic clofibric acid was compared. Each substrate was analyzed at 200 µM.

**TABLE III** Eight therapeutic aromatic acids were analyzed for glucuronidation by the UGTs identified as described in the legend to Table 1B; each substrate was analyzed at 200 µM. Also, results were compared to that for biphenyl acetic acid and anthralin.
Table IA  Western blot of UGT proteins

Table IB  Glucuronidation of steroids by human UGTs

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Table II. Glucuronidation of aromatic acids and aldehydes by human UGTs

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Table III  Glucuronidation of Nonsteroidal anti-inflammatory drugs and hydroxylated aromatic hydrocarbons by human UGTs

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### Relative to Biphenyl Rings

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<th>Compound</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biphenyl acetic acid</td>
<td>4-H$_2$C-COOH</td>
<td>202±6</td>
<td>137±8</td>
<td>72±10</td>
<td>114±12</td>
<td>87±4</td>
<td>77±7</td>
<td>878±44</td>
</tr>
<tr>
<td>Diflunisal</td>
<td>2',4'-diF</td>
<td>3-COOH, 4-OH</td>
<td>189±19</td>
<td>142±17</td>
<td>450±18</td>
<td>33±6</td>
<td>76±4</td>
<td>43±6</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>2-F,4-CH-COOH, CH$_3$</td>
<td>391±6</td>
<td>169±10</td>
<td>21±1</td>
<td>52±4</td>
<td>36±4</td>
<td>108±12</td>
<td>211±20</td>
</tr>
</tbody>
</table>

### Relative to N-Biphenyl Rings

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenamic acid</td>
<td>2'-COOH</td>
<td>2,3-dimethyl</td>
<td>287±7</td>
<td>112±5</td>
<td>1365±18</td>
<td>1455±20</td>
<td>152±32</td>
<td>322±16</td>
<td>1053±226</td>
<td>4986±82</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2'-H$_2$C-COOH</td>
<td>2,6-diCl</td>
<td>289±46</td>
<td>196±12</td>
<td>131±2</td>
<td>175±3</td>
<td>134±1</td>
<td>112±8</td>
<td>172±4</td>
<td>136±12</td>
</tr>
</tbody>
</table>

### Relative to O-Biphenyl Rings

<table>
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<tr>
<th>Compound</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
<th>pH 6.4</th>
<th>pH 7.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenoprofen</td>
<td>CH$_3$</td>
<td>3-C-COOH</td>
<td>580±13</td>
<td>241±48</td>
<td>140±8</td>
<td>89±2</td>
<td>422±53</td>
<td>26±3</td>
<td>1670±62</td>
<td>278±2</td>
</tr>
<tr>
<td>Indoprofen</td>
<td>—</td>
<td>—</td>
<td>104±7</td>
<td>99±18</td>
<td>26±2</td>
<td>31±5</td>
<td>51±5</td>
<td>27±1</td>
<td>308±12</td>
<td>46±4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>—</td>
<td>—</td>
<td>326±39</td>
<td>113±4</td>
<td>219±1</td>
<td>109±5</td>
<td>255±14</td>
<td>20±5</td>
<td>946±42</td>
<td>150±10</td>
</tr>
<tr>
<td>Furosemide</td>
<td>NS$_2$C-COONH</td>
<td>104±13</td>
<td>52±16</td>
<td>315±11</td>
<td>370±27</td>
<td>49±10</td>
<td>48±6</td>
<td>492±14</td>
<td>228±10</td>
<td></td>
</tr>
<tr>
<td>Anthralin(Dithranol)</td>
<td>—</td>
<td>—</td>
<td>262±20</td>
<td>240±28</td>
<td>514±23</td>
<td>580±23</td>
<td>139±5</td>
<td>85±2</td>
<td>246±22</td>
<td>174±10</td>
</tr>
</tbody>
</table>
**Activity for Microsomes Isolated from Human GI-tissues**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Esophagus</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Ileum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG</td>
<td>89 ± 12</td>
<td>152 ± 18</td>
<td>795 ± 21</td>
<td>677 ± 16</td>
<td>272 ± 28</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>274 ± 17</td>
<td>1167 ± 24</td>
<td>7906 ± 46</td>
<td>4807 ± 84</td>
<td>1459 ± 39</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>62 ± 16</td>
<td>78 ± 13</td>
<td>685 ± 14</td>
<td>504 ± 26</td>
<td>142 ± 07</td>
</tr>
</tbody>
</table>

*pmol gluc/mg prot/hr*
Fig. 3

Product Formed In-vitro

[Graph and image showing the activity levels at different concentrations of 1A1 and 1A10.]
Fig. 6

A. Separation of 17β-Estradiol Glucuronide on TLC

B. Production of 17β-Estradiol-Glucuronide in Cell Culture

C. In Vitro UGT Activity

D. Product Formed in Cell Culture
Gastrointestinally-distributed UDP-glucuronosyltransferase1A10 which metabolizes estrogens and nonsteroidal anti-inflammatory drugs depends upon phosphorylation

Nikhil K. Basu, Shigeki Kubota, Meselhy R. Meselhy, Marco Ciotti, Bhabadeb Chowdhury, Masao Hartori and Ida S. Owens

*J. Biol. Chem.* published online April 26, 2004

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