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Glucuronides in the Gut: Sugar-Driven Symbioses Between Microbe and Host

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The intestinal milieu is astonishingly complex, home to a constantly changing cocktail of small and large molecules, along with an abundance of bacteria, viral particles, and eukaryotic cells. Such complexity makes it difficult to develop testable molecular hypotheses regarding host-microbe interactions. Fortunately, mammals and their associated gastrointestinal (GI) microbes contain complementary systems that are ideally suited for mechanistic studies. Mammalian systems inactivate endobiotic and xenobiotic compounds by linking them to a glucuronic acid sugar for GI excretion. In the GI tract, the microbiota express β-glucuronidase enzymes that remove the glucuronic acid as a carbon source, effectively reversing the actions of mammalian inactivation. Thus, by probing the actions of microbial β-glucuronidases, and by understanding which substrate glucuronides they process, molecular insights into mammalian-microbial symbioses may be revealed amid the complexity of the intestinal tract. Here, we focus on glucuronides in the gut, and the microbial proteins that process them.

β-glucuronidase (GUS) enzymes expressed by the GI microbiota are at the interface of a metabolic symbiosis between microbe and host where they mediate the reactivation of molecules important in host health and disease. Microbial GUS enzymes regenerate toxic drugs and carcinogens in the mammalian GI (1), and their activities are associated with higher incidence of colon cancer and to diets that promote intestinal cancer (2). Endogenous molecules are also processed by GI GUS proteins, including glucuronides of hormones and neurotransmitters (3–5). These observations have led to hypotheses linking microbial GUS enzymes to the GI toxicity of drugs, the development of cancer, and increased incidence of Crohn's disease and colitis (2, 6–9). Thus, bacterial GUS enzymes appear to play an important role in health and disease by metabolizing glucuronides in the gut.

GUS proteins catalyze the hydrolysis of glycosidic bonds between glucuronic acid and either small molecules or the terminal ends of polysaccharides. For the purposes of this review, we will focus on small molecule glucuronides generated by Phase II drug metabolism to mark compounds for excretion. Glucuronides are produced by mammalian uridine diphosphate (UDP)-glucuronosyl transferase (UGT) enzymes that append glucuronic acid, derived from UDP-glucuronate, to hydroxyl, carboxylate, and other nucleophilic functional groups of aglycones (10). Glucuronidation almost exclusively inactivates and detoxifies molecules by increasing their water solubility, which promotes their removal from the body via the kidneys or GI tract (11). Once in the GI tract, these glucuronides serve as substrates for bacterial GUS proteins that remove the inactivating glucuronic acid moiety. Glucuronic acid then enters the Entner-Doudoroff pathway, a bacterial alternative to glycolysis that catabolizes sugar acids

and shunts the resulting pyruvate into the TCA cycle (12). Mammals also express a GUS enzyme ortholog that is localized to lysosomes in first-pass tissues like liver and intestines, and plays an essential role in degrading endogenous glycosaminoglycans (13). Germ-line mutations in human GUS cause Sly syndrome, a fatal lysosomal storage disease (14). Human GUS has also been shown to hydrolyze small-molecule glucuronides, a function that has been leveraged in drug design by attaching drugs to glucuronic acid such that they will be activated at a site of interest upon hydrolysis (15).

As a by-product of glucuronide hydrolysis, bacteria regenerate the original molecule that was eliminated by the host, facilitating reuptake by the GI epithelia and recirculation in the bloodstream (16). Glucuronidation in the liver, delivery to the GI lumen via the bile duct, reactivation and absorption via the intestinal epithelia, and transport back to the liver is termed enterohepatic circulation (Figure 1) (17), and it can significantly affect the pharmacokinetics of many drugs and also regulates the levels of endogenous compounds (4, 17, 18). Thus, GI microbial GUS enzymes have the capability of directly regulating local and systemic levels of exogenous and endogenous compounds involved in mammalian homeostasis.

Endogenous Glucuronides in the Gut

Endogenous glucuronides were clearly the driving force for the symbiotic evolution of host-associated bacterial GUS enzymes. Glucuronidated endogenous compounds include bilirubin, hormones, neurotransmitters, bile acids, and fatty acids, all of which influence host homeostasis. As such, GI microbial GUS enzymes participate in a nearly constant mutual symbiosis via the regulation of local and systemic levels of endogenous molecules.

One the most heavily glucuronidated endogenous molecules is bilirubin, a breakdown product of heme (19). While it is generally considered a waste product and toxin that contributes to hyperbilirubinemia and neonatal jaundice, normal levels of bilirubin have more recently been shown to have preventative antioxidant activities (20, 21). Approximately 16% and 80% of bilirubin exists as the monoglucuronide and diglucuronide conjugates, respectively, in the bile of healthy humans (19). Bilirubin glucuronides are generated in the liver by UGT1A1 and enter the GI tract from the bile duct. In the GI, bilirubin glucuronides are heavily metabolized by the intestinal microbiota into stercobilin, which gives feces its brown color, and urobilin, which is responsible for the yellow color of urine and the yellow complexion of jaundiced subjects (22). The deconjugated bilirubin that manages to escape further metabolism by bacteria is reabsorbed through the GI epithelia and undergoes enterohepatic circulation (18). However, enterohepatic circulation of bilirubin in healthy humans is relatively low due to bilirubin's nearly complete glucuronidation by the host and substantial subsequent metabolism to stercobilin and urobilin by the GI microbiota. In certain neonates or subjects with Gilbert's syndrome, though, bilirubin is significantly recycled, which contributes to CNS-toxic hyperbilirubinemia (19, 20). Thus, bacterial GUS and human UGT enzymes appear to have co-evolved a mutually symbiotic heme catabolism pathway to rid the host of high levels of toxic bilirubin and to provide the GI microbiota with a source of energy in the form of glucuronic acid.

Unconjugated bilirubin is also capable of forming insoluble calcium salts that contribute to the generation of brown pigment stones in the gallbladder and the biliary ductal system, which reduce bile flow and can impair liver function (23). Interestingly, the generation of these stones is concomitant with the presence of GUS-expressing Proteobacteria like *Escherichia coli* and *Klebsiella pneumoniae*, suggesting that bacterial GUS activity may promote the formation of the unconjugated bilirubin salts found in gall stones (23, 24). Bacteria of the family *Enterobacteriaceae*, which include *E. coli* and *K. pneumoniae*, are more abundant in the bile (25). The low affinity GUS inhibitor glucaro-1,4-lactone blocked calcium bilirubinate precipitation *in vitro* (23).

Hormones are also subject to glucuronidation. The estrogen metabolites estradiol, estrone, and estriol are glucuronidated by multiple UGT isoforms (26). *In vitro* studies have shown that *E. coli* GUS is capable of hydrolyzing a glucuronide metabolite of estriol, and does so with much greater activity than human GUS (27). Furthermore, estrogen metabolites exhibit significant enterohepatic circulation, suggesting that the regeneration of estrogen aglycones by the

GI microbiota may play an important role in regulating plasma levels of this hormone (28). Radiolabeling studies reveal that enterohepatic circulation of estrone and estriol varies by host species, which suggests that species differences in UGT expression or microbial composition may impact hormone metabolism (29). While bacterial GUS has been demonstrated *in vitro* to hydrolyze estrogenic glucuronides, a definitive role for the GI microbiota in the enterohepatic circulation of estrogenic metabolites *in vivo* has not been established. However, as posited recently, the reactivation of estrogenic metabolites by the GI microbiota may promote the enterohepatic circulation of estrogenic metabolites, which may subsequently foster the growth of estrogen-responsive tumors (30). It is important to note, though, that estrogen metabolites are also heavily sulfated through the action of mammalian sulfotransferases, another set of Phase 2 drug metabolizing enzymes that perform a role analogous to the glucuronidating UGTs (26). GI bacteria also harbor a variety of sulfatases to process highly sulfated polysaccharides and sulfated small molecules, including estrogen metabolites (26, 31). Overall, mammalian hormone inactivation is likely closely mirrored, and reversed, by enzymes in the GI microbiota.

Other glucuronidated hormones include the androgen testosterone and the thyroid hormone thyroxine (32, 33). Both androgen and thyroxine glucuronides can be hydrolyzed by bacterial GUS enzymes (27, 34, 35). Androgens are key drivers of prostate cancer, resulting in therapies primarily focused on androgen deprivation in the form of surgical or chemical castration, although a more recent approach is the enhancement of androgen glucuronidation by UGTs (7). Thyroxine is a primary thyroid hormone that impacts a variety of processes including metabolic regulation (36). *In vivo* radiolabeling and *ex vivo* fecal assays indicate that bacteria play a key role in the enterohepatic circulation of thyroxine in mammals (34). As such, GI microbial GUS proteins could participate in the regulation of metabolism and development by promoting the enterohepatic circulation of thyroxine.

The neurotransmitters dopamine, norepinephrine, and serotonin are glucuronidated in the body and metabolized by bacterial GUS. Roughly 50% of all dopamine is generated in the GI (37), where it acts as a regulator of GI motility and water absorption (38, 39). Microbes were recently shown to have a significant role in the processing of dopamine glucuronide in the GI lumen of mice (5). This study utilized germ-free mouse models and GUS knockout strains of bacteria to demonstrate that microbial GUS activity is primarily responsible for dopamine glucuronide hydrolysis. The neurotransmitter norepinephrine, a chemical cousin of dopamine, is also glucuronidated and exhibited microbe-mediated glucuronide hydrolysis in the GI lumen (5). Similarly, serotonin is subject to glucuronidation, and plasma levels of serotonin in mice fluctuate based on the presence or absence of the microbiota (40, 41).

Bile acids are important to gut health and are significantly processed by the microbiota. Bile acids are primarily considered detergents that solubilize dietary components for digestion (42). Much like bilirubin, bile acids are heavily metabolized by the microbiota, which can dehydrate, oxidize, and deconjugate bile acid variants generated by the liver (43). In the liver, bile acids are conjugated to sulfate, taurine, and glycine moieties, all of which can be removed by GI microbial sulfatases and bile salt hydrolases. Bile acids are also glucuronidated in the liver (44), and the resulting conjugates account for between 12-36% of the bile acids excreted in the urine. By contrast, sulfate, glycine, and taurine conjugates make up 50-63%, 1.8-28%, and 4.1-8.3% of excreted bile acids in the urine, respectively (45). Thus, glucuronidated bile acids likely provide a significant energy source to bacteria capable of processing such compounds. Unraveling the connections between host and microbial bile acid metabolism pathways will likely reveal new insights into the co-evolution of mammals and microbes.

Fatty acids are another class of biological detergents processed by liver UGTs. Fatty acids play roles in mammalian biology that range from cell signaling to membrane integrity (46). *Ex vivo* and *in vitro* analyses show that a variety of fatty acids can be glucuronidated, including arachidonic acid, retinoic acid, prostaglandins, and derivatives of linoleic acid (47–49), although further studies are needed to determine whether fatty acid glucuronides are processed by bacterial GUS enzymes.

Finally, endogenous polysaccharides are a critical source of glucuronides in the gut. Chondroitin sulfate and hyaluronic acid are glucuronic acid-containing polysaccharides present in the GI tract (10). Bacteria express a wealth of endo- and exo-glycosidases that work in concert to break down complex polysaccharides. Analogous to human GUS, which catabolizes extracellular matrix polysaccharides in lysosomes, bacterial GI GUS enzymes play similar roles with substrates like chondroitin sulfate that enter the GI from host cells sloughed from the epithelia (50). An excellent review of microbial polysaccharide processing enzymes in the mammalian GI tract has recently been provided (51).

Exogenous Glucuronides in the Gut

Glucuronides of drugs and other exogenous molecules have been a primary focus of research because of their potential importance to therapeutic efficacy and tolerance. Many drugs exhibit GI and liver toxicity that is mediated in part by bacterial GUS activity in the gut, resulting in a parasitic symbiosis in which bacteria receive sugar from drug glucuronides and the host retains toxic metabolites. Carcinogens and other dietary metabolites are also metabolized in the body via glucuronidation and processed by our microbial counterparts, providing a link between bacterial GUS enzymes and carcinogenesis. Exogenous glucuronides that reach the GI are diverse in chemical structure, suggesting that a proportional breadth of functional diversity may be present in the collection of microbial GUS enzymes in the GI.

The anticancer agent SN-38 is the archetype of how metabolism by bacterial GUS can lead to drug toxicity. SN-38 is the active form of the prodrug irinotecan, which is commonly used to treat colorectal and pancreatic cancers (52, 53). SN-38 is inactivated in the liver by conversion to SN-38-glucuronide (SN-38-G); in the GI lumen, however, microbial GUS enzymes recreate SN-38 and cause severe GI toxicity in the form of dose-limiting diarrhea. The authors' laboratory showed that potent, selective, and non-lethal inhibition of bacterial GUS enzymes reduces the GI toxicity of SN-38 in mice (54–56). This approach may improve the efficacy and tolerance of other anticancer drugs. Indeed, from a list of 155 anticancer agents, 24 are known to be glucuronidated, and of those that are glucuronidated, 21 (89% of 24) cause GI toxicity. Two such drugs are the histone deacetylase (HDAC) inhibitors belinostat and panobinostat, used to treat lymphoma (57, 58). Metabolites of lapatinib, a GI toxic drug used to treat hormone receptor positive breast cancer, are glucuronidated and their reactivation may damage the liver as well as the GI tract (59). GI microbial GUS enzymes contribute to hepatotoxicity via the enhancement of enterohepatic circulation, which leads to repeated liver exposure to toxic metabolites (17). Together, these examples highlight the role that bacterial GUS plays in cancer treatment, efficacy, and toxicity.

Non-steroidal anti-inflammatory drugs (NSAIDs), some of the most widely used therapeutics in the world, are also glucuronidated. NSAIDs inhibit cyclooxygenase enzymes and prostaglandin synthesis and contain a carboxylic acid group that is readily glucuronidated (60). The NSAID diclofenac is conjugated to glucuronic acid by UGT2B7 in the liver, delivered to the GI tract via the bile duct, and hydrolyzed by bacterial GUS enzymes in the GI (61, 62). The regeneration of diclofenac causes ulceration of the GI epithelia via an unclear mechanism that may involve disruption of mitochondrial function (63). Similar to SN-38, prevention of diclofenac regeneration by a selective bacterial GUS inhibitor reduced GI ulceration in mice (61). The GI damage of the NSAIDs ketoprofen and indomethacin can also be ameliorated by selective inhibition of bacterial GUS (62). Interestingly, the GI toxicity caused by NSAIDs is primarily localized in mice to the distal end of the small intestine, while the damage most often associated with irinotecan is located in the large intestine (64). It is possible that bacteria that thrive in the distal small intestine may have a greater capability to hydrolyze NSAID glucuronides than microbes in the proximal small intestine and colon (64).

Certain carcinogens are also glucuronidated. One of the most potent is the alkylating agent methylazoxymethanol (MAM), the active metabolite of azoxymethanol (AOM) that is used to model carcinogenesis in rodents (65). AOM is converted by cytochrome P450 2E1 and UGTs in the liver to generate MAM-glucuronide (MAM-G), and evidence exists that bacteria in the GI tract reactivate MAM-G to MAM and promote colon carcinogenesis (66, 67). The low-affinity bacterial

GUS inhibitor C-GAL has been shown to reduce colon carcinogenesis caused by AOM (68). Other carcinogens, like the polyaromatic hydrocarbons and heterocyclic aromatic amines, are also metabolized by the CYP-to-UGT pathway, and it has been suggested that microbes hydrolyze those glucuronide metabolites as well (69, 70). Interestingly, colon cancer patients exhibit higher fecal GUS activities than controls (2). Together, these results support the conclusion that the release of active carcinogens in the GI tract involves microbial GUS enzymes.

Two widely used lifestyle drugs metabolized by host UGTs and bacterial GUSs are ethanol and nicotine. While the majority of ingested ethanol is converted to acetaldehyde by alcohol dehydrogenase, a small fraction of ethanol is glucuronidated (71). In humans, ethanol glucuronide has been detected in the liver, bile and urine (72). *Escherichia coli* and *Clostridum sordellii* have both been shown to hydrolyze ethyl glucuronide *in vitro*, which may contribute to a greater retention of ethanol-derived metabolites in the body (73). Detection of ethyl glucuronide in hair has been employed as a biomarker to diagnose alcohol abuse (74). Nicotine and its metabolites are primarily processed in humans by oxidation, but they are also glucuronidated (75). Nicotine is unique among the aglycones discussed here in that it is conjugated to glucuronic acid through a nitrogen-linkage, and microbial GUS enzymes have been shown to cleave nicotine glucuronide (76). The glucuronides of ethanol and nicotine highlight the chemical diversity of exogenous compounds that serve as substrates for GUS proteins of the GI microbiota.

While not the primary focus of this review, a small number of plant polysaccharides that contain glucuronic acid are mentioned here. Gum Arabic is a plant-derived secretion that is predominantly composed of glucuronic-acid containing polysaccharides, and is widely utilized in the food and drug industry as a stabilizer (10, 77). This complex polysaccharide is indigestible to animals, but can be fermented by bacteria in the colon and is associated with weight loss in humans (78). The xylan hemicelluloses, which are heteropolymers of various sugars and components of the plant cell wall, also contain glucuronic acid (10). Like Gum Arabic, xylan polysaccharides are indigestible by human enzymes, but can be catabolized by GI microbes. Xylan complexity appears to require a diverse set of microbial xylanases to catabolize them to release smaller, glucuronic acid-containing sugars further processed by intestinal bacteria (79).

Microbial β-Glucuronidases in the Gut

Several investigations have detected *in vitro* GUS activity, *ex vivo* fecal GUS activity, and *in vivo* correlations between GI GUS enzyme activity and health. These studies have resulted in the identification of bacteria related to Crohn's disease, the discovery of increased GUS activity in patients with colorectal cancer and subjects on high fat diets, and the mechanistic elucidation of how bacterial GUS promotes drug toxicity (1, 2, 9, 55). To test the relationship between microbial GUS activity and disease, total fecal proteins have been extracted and GUS assays conducted (80). This approach yields an overall view of the fecal microbiota's GUS activity, but provides little granularity about the specific microbial enzymes involved. Other approaches involve culturing bacteria obtained from human fecal samples, and then assessing the GUS activity in these pure cultures (6, 81–88). A tabulation of strains analyzed in culture-based GUS activity assays reveals that bacteria from all the major phyla in the mammalian GI microbiota, including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, harbor enzymes that process glucuronides (Table 2). The conservation of GUS across all major GI bacterial phyla reinforces the hypothesis that GUS proteins may play key roles in chemical dynamics across the intestinal epithelium, and serve as a competitive growth advantage for bacteria in the crowded and unforgiving milieu of the mammalian gut.

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FIGURE LEGENDS

TABLE 1. Examples of molecules subject to glucuronidation in mammals.
AOM, azoxymethane
PhIP, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
NSAID, non-steroidal anti-inflammatory drug

TABLE 2. Bacterial strains from the human microbiota that have been shown to exhibit GUS activity in culture

FIGURE 1. Enterohepatic circulation of chemically distinct molecules (denoted as **X**) is mediated by the host and microbiota. Glucuronides (e.g., **X**-glucuronide) are generated primarily in the liver by UGTs (but can also be produced by GI epithelial UGTs) and then delivered by biliary secretion to the GI. In the alimentary canal, glucuronides are either excreted or metabolized by the GI microbiota's GUS enzymes. Reactivated aglycones in the GI (**X**) can be excreted, or reabsorbed and returned to the liver via the enterohepatic cycle.

FIGURE 2. Examples of chemically diverse endogenous and exogenous glucuronides (glucuronic acid shown in green) generated by mammalian UGT enzymes and metabolized by GI microbial GUS enzymes.
MAM, methylazoxymethanol
PhIP, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

TABLES

TABLE 1
Examples of molecules subject to glucuronidation in mammals.

Aglycone	Aglycone's Effect	Disease/health	Ref.
ENDOGENOUS			
Arachidonic acid	Signaling molecule	Inflammation	(48)
Bilirubin	Neurotoxin, antioxidant	Gall stones, jaundice	(19)
Chenodeoxycholate	Detergent	Cholestasis	(89)
Chondroitin sulfate	Glycosaminoglycan	Cancer	(90)
Dopamine	GI motility, water absorption	IBD, constipation	(5)
Estradiol	Sex hormone	Breast cancer	(26)
Hyaluronic acid	Glycosaminoglycan	Cancer	(10)
Norepinephrine	GI motility	IBD	(5)
Serotonin	GI motility	IBD	(40)
Testosterone	Sex hormone	Prostate cancer	(32)
Thyroxine	Thyroid regulation	Metabolic disorder	(36)
EXOGENOUS			
AOM	Alkylating agent	Cancer	(66)
Belinostat	HDAC inhibitor	GI toxicity	(57)
Benzo[a]pyrene	DNA adduct formation	Cancer	(70)
Diclofenac	NSAID	GI toxicity	(62)
Ethanol	Depressant	Liver toxicity	(91)
Indomethacin	NSAID	GI toxicity	(64)
Ketoprofen	NSAID	GI toxicity	(62)
Nicotine	Stimulant	Addiction	(75)
Panobinostat	HDAC inhibitor	GI toxicity	(58)
PhIP	Alkylating agent	Cancer	(69)
SN-38	Topoisomerase I inhibitor	GI toxicity	(54)

TABLE 2
Bacterial strains from the human microbiota that have been shown to exhibit GUS activity in culture.

Strain	Ref.
Actinobacteria	
Bif. adolescentis JCM 1275	(87)
Bif. angulatum NCFB 2237	(86)
Bif. bifidum NCFB 2454	(86)
Bif. breve NCFB 2257	(86)
Bif. longum JCM 1217	(87)
Bif. pseudolongum NCFB 2244	(86)
Col. aerofaciens JCM 7790	(87)
Bacteroidetes	
Bac. capillosus ATCC 29799	(84)
Bac. fragilis NCFB 2217	(86)
Bac. ovatus ATCC 8483	(84)
Bac. thetaiotaomicron	(87)
Bac. uniformis JCM 5828	(87)
Bac. vulgatus DCNC 23	(86)
P. johnsonii DSM 18315	(84)
P. merdae ATCC 43184	(84)
Firmicutes	
Bry. formatexigens DSM 14469	(84)
C. bartlettii DSM 16795	(84)
C. bifermentans NCFB 2189	(86)
C. butyricum DCNC 19	(86)
C. clostridioforme JCM 1291	(87)
C. paraputrificum JCM 1293	(87)
C. perfringens NCTC 8679	(86)
Ent. faecalis DCNC 24	(86)
Ent. faecium DCNC 26	(86)
Eubacterium L-8	(85)
F. prausnitzii M21/2	(84)
L. acidophilus DCNC 1237	(86)
L. gasseri ADH	(88)
Ros. inulinivorans DSM 16841	(84)
Rum. gnavus E1	(81)
Sub. variabile DSM 15176	(84)
Streptococcus LJ-22	(85)
Proteobacteria	
E. coli HGU-3	(6)

FIGURES

Figure 1

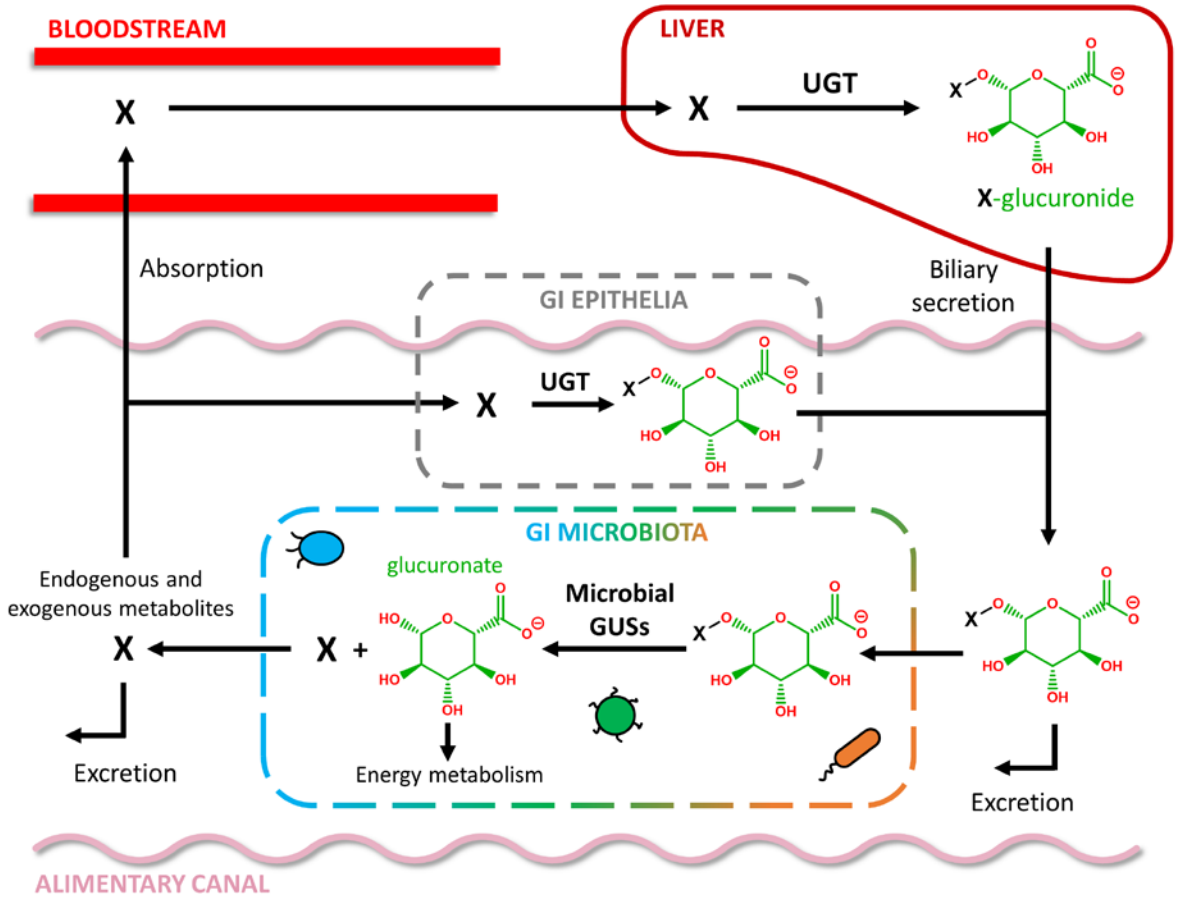
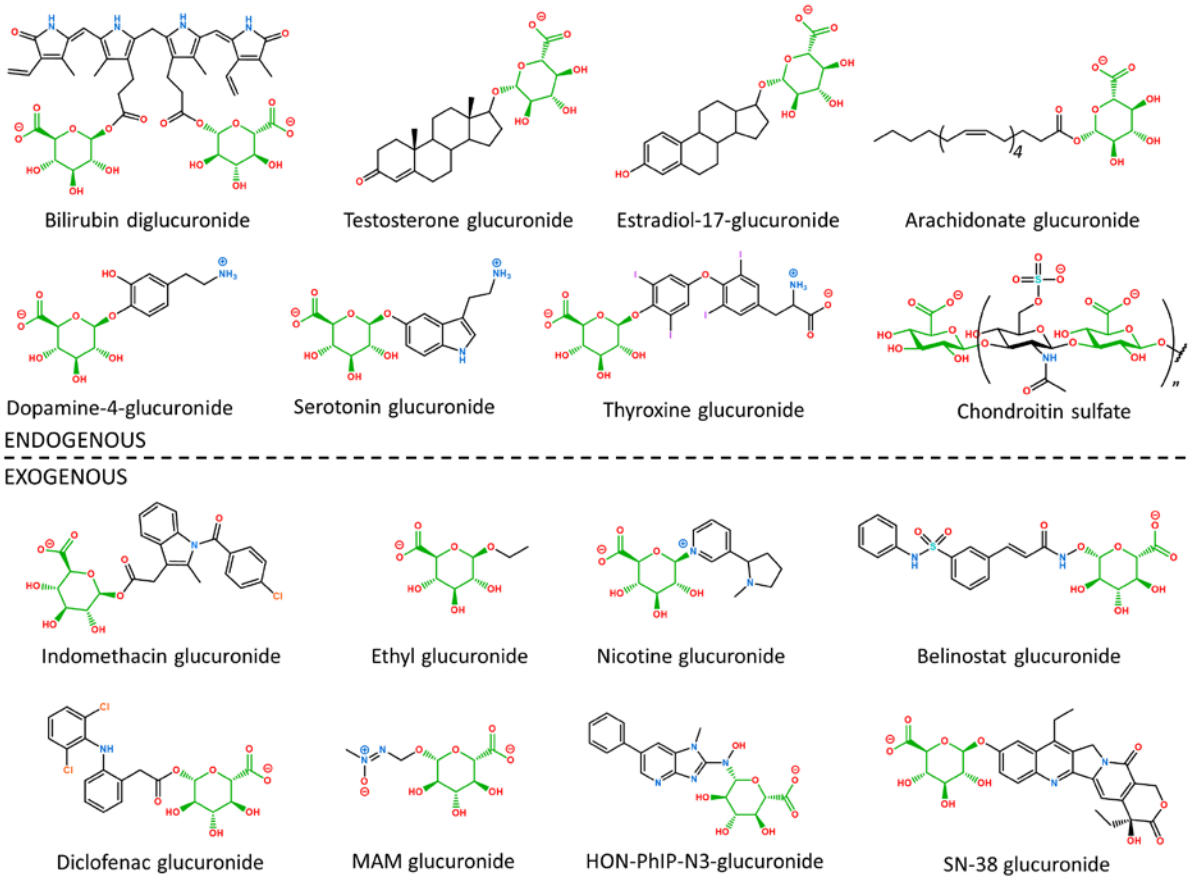


Figure 2



Glucuronides in the Gut: Sugar-Driven Symbioses Between Microbe and Host

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