Critical Reviews in Biochemistry and Molecular Biology, 26(2): 129—150 (1991)

Roles of UDP-Glucuronosyltransferases in Chemical Carcinogenesis

Karl Walter Bock

Institute of Toxicology, University of Tübingen, Tübingen, Germany

Referee: T. R. Tephly, Department of Pharmacology, University of Iowa, Iowa City, IA 52242

ABSTRACT: UDP-glucuronosyltransferases (UGT) play a major role in the elimination of nucleophilic metabolites of carcinogens, such as phenols and quinols of polycyclic aromatic hydrocarbons. In this way they prevent their further oxidation to electrophiles, which may react with DNA, RNA, and protein. They also inactivate carcinogenic, N-oxidized metabolites of aromatic amines. Furthermore, glucuronides may be stable transport forms of proximate carcinogens excreted via the biliary or urinary tract, thereby liberating the ultimate carcinogen at the target of carcinogenicity. Isozymes of the UGT enzyme superfamily that control the glucuronidation of metabolites of aromatic hydrocarbons and of N-oxidized aromatic amines have been identified in rats and humans. Phenol UGT appears to be coinduced with other drug-metabolizing enzymes via the Ah or dioxin receptor. This isozyme probably controls various proximate carcinogens and contributes to the persistently altered enzyme pattern, leading to the "toxin-resistance phenotype" at cancer prestages. Knowledge about UGTs in different species, their regulation, and their tissue distribution will improve the risk assessment of carcinogens.

KEY WORDS: UDP-glucuronosyltransferases, carcinogenesis, aromatic amines, dioxin receptor, toxin-resistance phenotype

I. INTRODUCTION

The roles of UDP-glucuronosyltransferases (UGTs; EC 2.4.1.17) will be discussed in the context of other drug-metabolizing enzymes and of recent concepts of carcinogenesis. Two classes of carcinogens are used as examples, aromatic hydrocarbons and aromatic amines. Reviews are necessarily selective. Hence, a number of important contributions have not been included. The reader is referred to recent reviews on related drug-metabolizing enzymes (i.e., cytochromes P450 and glutathione *S*-transferases) and their role in carcinogenesis for further information.[1-4]

A. Role of Glucuronidation in the Control of Nucleophilic Metabolites and Thereby in Preventing their Conversion to Reactive Metabolites

A large number of exogenous and endogenous lipophilic chemicals are converted by phase I enzymes of drug metabolism to a variety of nucleophilic and electrophilic metabolites (Figure 1). It has been demonstrated that the interaction of the chemically reactive, electrophilic metabolites with critical cellular macromolecules often initiates toxicity and plays an essential role in the multistage carcinogenic process.[5] Electro-

1040-9238/91/$.50

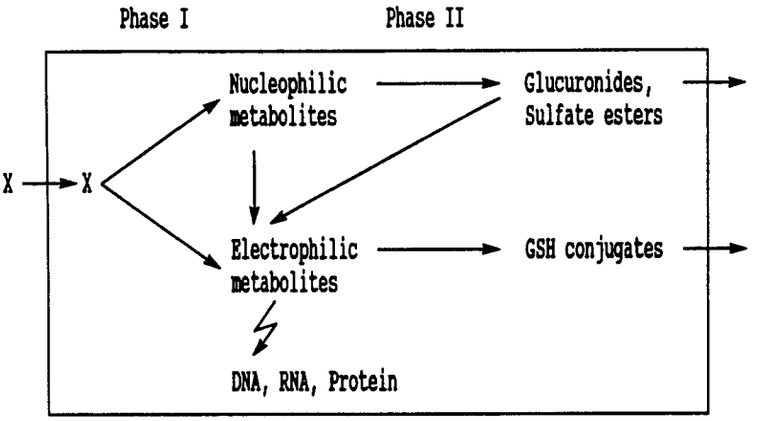

FIGURE 1. Scheme of cellular xenobiotic metabolism.

philic metabolites are largely controlled in phase II by a family of glutathione *S*-transferases (in the case of epoxides, together with epoxide hydrolases). However, the more stable and more abundant nucleophilic metabolites can also be converted to reactive metabolites. For example, phenols can be oxidized to radicals, polyphenols, semiquinones, and quinones. Quinones may undergo quinone/quinol redox cycles, with the generation of reactive oxygen species.[6-8] Therefore the control of nucleophilic phenols, quinols, and N-oxidized aromatic amines by both UGTs and sulfotransferases (the latter together with acetyltransferases/deacetylases)[9] may be as important as the control of electrophiles. It is the balance between phase I and phase II enzymes that is responsible for either detoxication or accumulation of toxic metabolites. In this context, it is to be noted that some conjugates, in particular sulfuric acid esters or acetic acid esters, are more reactive than the parent compound and represent ultimate carcinogens.[10] For example, the sulfuric acid ester of *N*-hydroxy-2-aminofluorene has been shown to bind to DNA and to activate critical genes, such as the *c-Ha-ras* protooncogene.[10,11]

B. Reactive Metabolites and Initiation of Carcinogenesis

Evidence is accumulating that in multistep carcinogenesis at least two stages can be distinguished, initiation and promotion. At the stage of initiation, protooncogenes such as *c-Ha-ras* (critical for cellular growth control) are altered by genotoxic lesions.[11] These lesions may lead to heritable changes in the base sequence of cellular DNA.[12-14] However, persistent alterations of at least two cooperating protooncogenes have been shown to be required for transformation.[15] Inactivation of antioncogenes may also be involved, as shown in the heritable predisposition to cancer.[16,17] Recently, it has been appreciated that, in addition to genotoxic lesions, cell proliferation markedly influences several steps in carcinogenesis (Figure 2).[18,19] In this model, a normal cell (N), possibly a stem cell, is converted by genotoxic lesions into a preneoplastic initiated cell (I). The rate of conversion of N to I is influenced by both the mitotic rate of normal cells (α_1) and by terminal differentiation or death of normal cells (β_1). Similarly, the size of the population of initiated cells (I) is determined by its mitotic rate (α_2) and its death rate (β_2). The size of the population of initiated cells will be a major determinant of its conversion to transformed cells (T). Proliferation of initiated cells can be markedly influenced by nongenotoxic agents, frequently termed *tumor promoters*. Since proliferation of initiated cells represents a critical stage of carcinogenesis that is affected by nongenotoxic agents, it is often termed the stage of *tumor promotion*. Hence the process of tumor promotion is mostly determined by the term α_2-β_2. It has to be distinguished from transformation, which is often determined by a second genotoxic lesion.

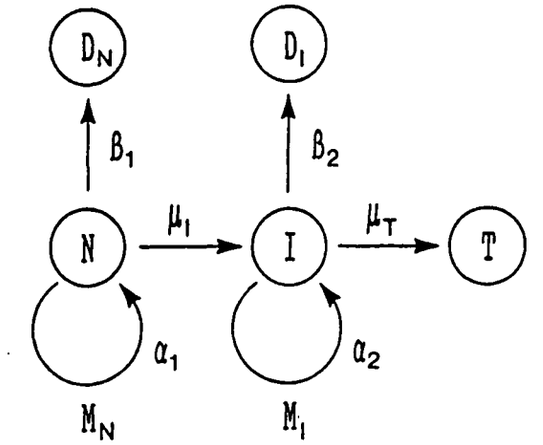

FIGURE 2. Two-stage model of carcinogenesis.[18,19]

C. Reactive Metabolites and Tumor Promotion

Despite the complexity of multiple processes leading to neoplasia, carcinogens can be classified into genotoxic and nongenotoxic agents (Figure 3). Nongenotoxic agents can be subdivided into (a) those compounds reacting with receptors that may directly affect growth control and (b) reactive metabolites that indirectly affect growth control through cytotoxicity and subsequent regenerative growth. Hence, the formation of reactive metabolites in cells not only determines initiation through formation of genotoxic lesions, but also tumor promotion through generation of cytotoxic metabolites, leading to cell death and regenerative growth. In fact, in the case of "complete carcinogens", the same ultimate toxins may be responsible for both genotoxicity and cytotoxicity. In the future, it may be advisable to characterize complete carcinogens on the basis of their relative genotoxic and cytotoxic activities.[20] This distinction may be important for low-dose extrapolation and risk assessment in toxicology.

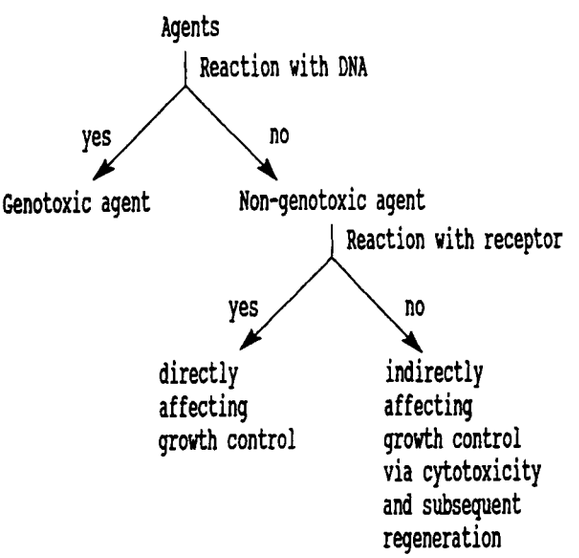

FIGURE 3. Classification of agents affecting carcinogenesis.

Regenerative proliferation due to cytotoxicity may be important for both initiation and promotion. At the stage of initiation, cell proliferation predisposes the cell to genotoxic actions of carcinogens, since it has been demonstrated that the liver is particularly vulnerable to genotoxic agents in the early S phase of the cell cycle.[21] At the stage of promotion, cytotoxicity may also be of importance. Initiated cells often show the "toxin-resistance phenotype".[22] Death of normal hepatocytes may lead to selective growth of toxin-resistant initiated hepatocytes, i.e., to their selective proliferation. In this context, it is interesting to note that carcinogens such as polycyclic aromatic hydrocarbons (in addition to being metabolized to genotoxic and cytotoxic reactive metabolites) directly stimulate a cellular receptor, the Ah receptor (see Section V).[23,24] This receptor controls, in addition to the regulation of proteins involved in drug metabolism, processes involved in cell growth and differentiation. Hence, polycyclic aromatic hydrocarbons represent tumor promoters, affecting cellular growth control by both direct and indirect mechanisms (Figure 3). The discussion of recent concepts of carcinogenesis is intended to emphasize the multiple ways in which the balance between activating and inactivating drug-metabolizing enzymes (including UGTs) influence neoplastic transformation.

Two roles of glucuronidation in the control of carcinogenesis warrant discussion: (1) the role of glucuronidation of metabolites of aromatic amines and aromatic hydrocarbons as transport forms of proximate carcinogens determining the site of carcinogenicity and (2) its major role in the detoxication of aromatic hydrocarbons and aromatic amines. After a brief discussion of var-

ious factors affecting glucuronide formation in the cell, the role of particular isozymes of UGT in the metabolism of carcinogens will be discussed, in particular the regulation of phenol UGT by the Ah receptor and its persistent alterations at cancer prestages. The contribution of these persistent enzyme alterations to the toxin-resistance phenotype will be summarized.

II. GLUCURONIDES AS TRANSPORT FORMS OF CARCINOGENS DETERMINING THE TARGET OF CARCINOGENICITY

For more detailed information the reader is referred to previous reviews.[5,25] Aromatic amines found widespread use in the dye industry in the mid-19th century. Accordingly, aromatic amines were among the first chemicals to be recognized as human carcinogens. As early as 1895, a German physician, Ludwig Rehn,[26] suggested that cancers of the urinary bladder found in dyestuff workers were due to chemical exposure to certain aniline dyes. In 1938, Hueper and co-workers demonstrated the urinary bladder carcinogenicity of 2-naphthylamine in dogs (Figure 4).[27] Aromatic amines, such as 2-naphthylamine and 4-aminobiphenyl, are found in nanogram amounts in cigarette smoke.[28] These compounds, among others, may account for the positive correlation between cigarette smoking and the incidence of bladder cancer in humans.[29-31]

During high-temperature cooking processes, certain amino acids, such as tryptophan and glutamic acid, along with creatinine and glucose, are pyrolyzed to extremely mutagenic heterocyclic amines (Figure 4), such as Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole), Glu-P-1 (2-amino-3-methyldipyrido[1,2-a: 3′,2′-d]imidazole, and IQ (2-amino-3-methylimidazo-[4,5-f]quinoline). These derivates are carcinogenic at a number of sites in experimental animals and may contribute to the etiology of human cancer.[32] Recently, a number of nitroaromatic hydrocarbons, found, for example, in diesel emission, were found to be converted to *N*-hydroxy arylamines and to mutagens and animal carcinogens.[33] They may also be involved in the etiology of human urinary bladder cancer.[34]

In 1941, 2-acetylaminofluorene (2-AAF), a proposed insecticide, was shown to be carcinogenic to the liver, mammary gland, and urinary bladder of rats after dietary administration.[35] Subsequently, the *N*-hydroxy metabolite of 2-AAF was found to be more carcinogenic than the

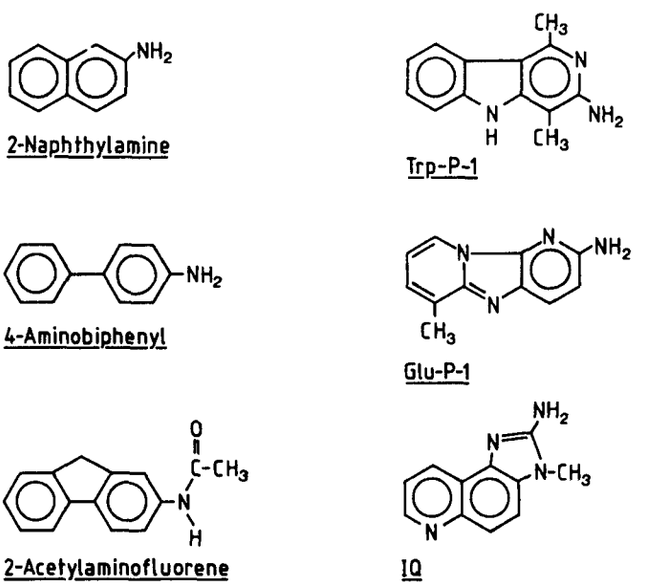

FIGURE 4. Structures of carcinogenic aromatic amines (for abbreviations see text).

parent compound.[5] This provided the first evidence for the concept of metabolic activation and of the formation of a more "proximate carcinogen". It was later found that sulfation (and acetylation/deactylation reactions) led to even more reactive intermediates, which formed covalent adducts with DNA (ultimate carcinogens). Glucuronidation led to the formation of the *N-O*-glucuronide of the corresponding *N*-hydroxy-2-AAF. Similarly, *N*-hydroxy-2-naphthylamine and *N*-hydroxy-4-aminobiphenyl have been shown to be converted to the corresponding *N*-glucuronides.[36-38] These *N*-glucuronides represent stable transport forms that are excreted either by the biliary system into the intestine or via the blood into the urinary system (Figure 5). In the case of 2-napthylamine, the corresponding *N*-hydroxy-*N*-glucuronide has been shown to decompose at the slightly acidic pH of urine to the hydroxylamine and to its protonated nitrenium ion, which readily reacts with DNA, and may initiate bladder cancer.[5,39] In the case of *N*-hydroxy-4-aminobiphenyl, both the glucuronide and the unconjugated hydroxylamine enter the bladder.[25] The resulting DNA adducts have been identified in exfoliated urothelial cells of the dog, a method that may be useful to monitor adduct levels in humans.[40]

In addition to being *N*-oxidized in the liver and transported to the bladder as the *N*-hydroxy-*N*-glucuronide, 2-naphthylamine is converted to 1-hydroxy-2-naphthylamine in the liver and can be further oxidized in the bladder epithelium to the corresponding electrophilic iminoquinone by peroxidases, such as prostaglandin H synthase.[25] This may be an alternative way to form DNA adducts. 1-Naphthylamine, in contrast to 2-naphthylamine, has not been found to be a bladder carcinogen in experimental animals and humans.[41,42] This is due to the lack of *N*-oxidation of 1-naphthylamine,[25] in addition to rapid glucuronidation to the *N*-glucuronide.[43]

In the case of 3,2′-dimethyl-4-aminobiphenyl, the *N*-glucuronide of the corresponding hydroxylamine is secreted via the bile into the intestine, and the hydroxylamine is liberated in the colon by bacterial β-glucuronidase, initiating colon carcinogenicity.[44] Similarly, glucuronides of *N*-oxidized heterocyclic amines (formed during the cooking process) may be secreted via the

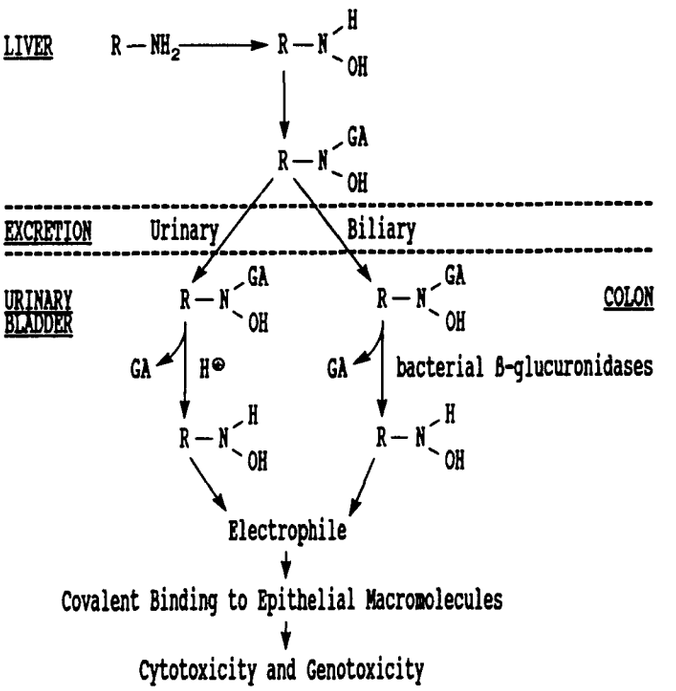

FIGURE 5. Hypothesis for arylamine-induced bladder and colon carcinogenesis. GA = glucuronic acid.

bile into the intestine, where the ultimate carcinogens may be liberated by β-glucuronidases.

Various glucuronides generated in the metabolism of heterocyclic amines have recently been identified.[45,46] In these instances, the *N*-glucuronides act as transport forms of proximate carcinogens and determine the target of carcinogenicity.

Similar to aromatic amines, glucuronides of benzo(a)pyrene phenols and polyphenols, known to undergo enterohepatic circulation,[47] are split in the gut by bacterial β-glucuronidases and could conceivably contribute to the initiation of colon cancer.[48] Glucuronides are generally regarded as detoxication products. However, it is to be noted that enzymatic hydrolysis of 3-hydroxybenzo(a)pyrene glucuronide yields a DNA-binding product.[49]

III. ROLE OF GLUCURONIDATION IN DETOXICATION OF CARCINOGENS

A. Aromatic Hydrocarbons

Since the discovery of polycyclic aromatic hydrocarbons in chimney soot and coal tar, extensive studies have been conducted on the metabolic activation of these compounds to their ultimate carcinogens.[50,51] In contrast to the previously discussed glucuronides acting as transport forms of proximal carcinogens, glucuronidation must be regarded mostly as a detoxication mechanism. Only selected aspects linking glucuronidation with detoxication of benzo(a)pyrene, benzene, and 2-hydroxybiphenyl will be discussed here.

1. Benzo(a)pyrene

The relationship between inhibition of glucuronidation (and sulfation) by salicylamide and increased covalent binding has been studied in isolated hepatocytes[52] and in perfused liver.[53] Circumstantial evidence for the role of glucuronidation in the inactivation of genotoxic benzo(a)pyrene metabolites also stems from mutagenicity studies. Several laboratories have shown that the addition of UDP-glucuronic acid to the Ames test reduces benzo(a)pyrene mutagenicity.[54-57] In particular, the mutagenicity of benzo(a)pyrene-3,6-quinone was found to be reduced when liver homogenates of 3-methyl-cholanthrene-treated rats were used as the enzyme source.[58] The mechanism leading to mutagenicity is still unclear. In contrast, increased benzo(a)pyrene mutagenicity has been observed at high benzo(a)pyrene concentrations in the presence of UDP-glucuronic acid.[55-57] The latter effect is probably due to the removal of quinones, which inhibit benzo(a)pyrene metabolism at high concentrations.[59] Removal of quinones by glucuronidation of quinols enhances benzo(a)pyrene monooxygenase activity[60] and DNA binding of metabolites.[59]

Two selected pathways of benzo(a)pyrene metabolism are shown in Figure 6; at the top of the figure is the pathway leading to one of the ultimate carcinogens, the bay region dihydrodiol epoxide[50]; at the bottom is the pathway leading to quinones and to quinone/quinol redox cycles. In contrast to dihydrodiols, phenols and quinols appear to be good substrates of 3-methylcholanthrene-inducible phenol UGT.[61] Benzo(a)pyrene-3,6-quinol is efficiently conjugated to its mono- and diglucuronide.[8,62] In this way, redox cycles with the generation of semiquinones and reactive oxygen species can be prevented. The quantitative contribution of various pathways to benzo(a)pyrene mutagenicity still remains to be elucidated.

2. Benzene

Earlier findings indicated that the addition of UDP-glucuronic acid to a benzene oxidizing system reduced covalent binding of metabolites to protein.[63] Studies of the influence of enzyme inducers on benzene-induced bone marrow toxicity demonstrated a temporary protective effect of treatment with 3,3′,4,4′-tetrachlorobiphenyl (a 3-methylcholanthrene-type inducer), which was paralleled by an induction of hepatic UGT.[64] There is growing evidence that the liver is the primary site of bioactivation of benzene (Figure 7). Metabolites are generated in liver, which may be transported to the bone marrow. Partial hepatectomy or inhibition of hepatic benzene metabolism

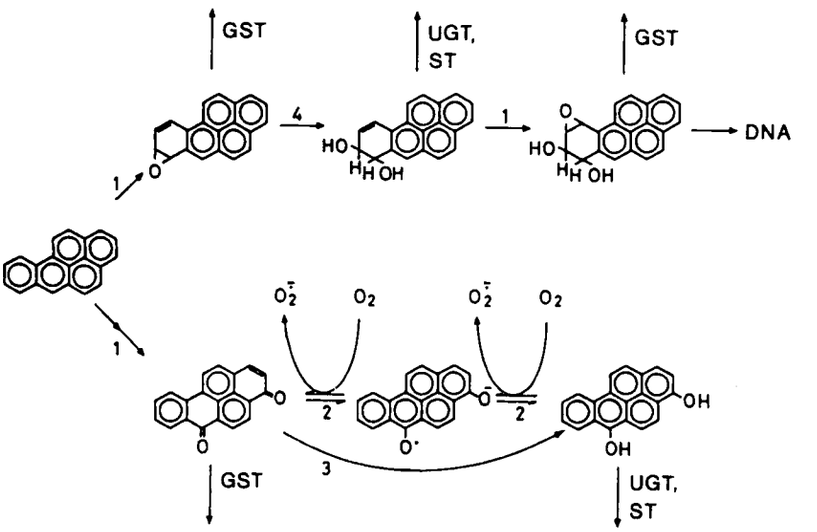

FIGURE 6. Selected pathways of benzo(a)pyrene metabolism. GST = glutathione *S*-transferases; ST = sulfotransferases. Numbers indicate phase I drug-metabolizing enzymes: (1) cytochrome P450-dependent monooxygenase; (2) NADPH-cytochrome P450 reductase; (3) NAD(P)H quinone reductase or DT diaphorase; (4) epoxide hydrolase.

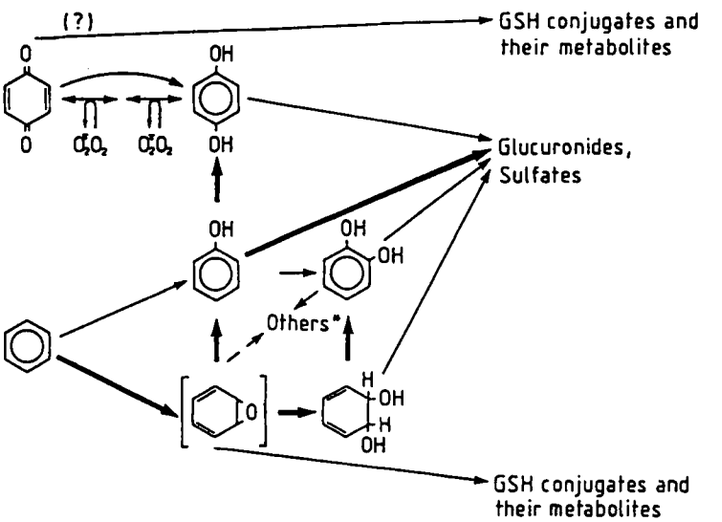

FIGURE 7. Selected pathways of benzene metabolism in hepatocytes.

resulted in a pronounced reduction of myelotoxicity.[65] In support of a role of glucuronidation in the inactivation of toxic intermediates, it has been shown that 3-methylcholanthrene treatment leads to a marked shift in the conjugation of phenol sulfation to glucuronidation in isolated hepatocytes.[66] The shift was shown to be due to the induction of phenol UGT. Phenol sulfate and sulfuric acid esters of other phenolic benzene metabolites are known to be less stable than the

corresponding glucuronides, and may liberate the parent compound by chemical or enzymatic hydrolysis at the target of toxicity.

3. 2-Hydroxybiphenyl

2-Hydroxybiphenyl is widely used as an antimicrobial agent to protect edible crop and hence the human population may be exposed. It is assumed, however, that this human exposure does not lead to a significant health hazard. At low doses, 2-hydroxybiphenyl is mostly excreted as the glucuronide and sulfate ester, and no toxicity is observed. At high doses, conjugation pathways are saturated and the compound is further oxidized to the corresponding hydroquinone and semiquinone, metabolites that are probably responsible for covalent binding, genotoxicity, and the initiation of bladder cancer.[67] This example demonstrates that saturation of glucuronidation leads to the accumulation of phenols and to their further oxidation to reactive metabolites.

B. Aromatic Amines

2-Acetylaminofluorene (2-AAF) is one of the most extensively studied chemical carcinogens (Figure 4). As with other aromatic amines, *N*-oxidation (catalyzed mainly by P4501A1 and P4501A2) represents the initial activation step, followed in liver by sulfuric acid conjugation. This unstable conjugate decomposes and leads (in long-term feeding studies) to the only DNA adduct, *N*-(deoxyguanosine-8-yl)-2-aminofluorene, which may be responsible for initiation of hepatocarcinogenesis by 2-AAF.[5,25] Glucuronidation competes with sulfuric acid conjugation. Conflicting results have been published about the reactivity of *N-O*-glucuronides of *N*-hydroxy-2-AAF. The *N-O*-glucuronide has been reported to react with DNA under *in vitro* conditions.[68,69] However, whereas *N*-glucuronides of arylhydroxylamines induce repair synthesis of DNA in cultured urothelial cells of several species, the *N-O*-glucuronide of *N*-hydroxy-AAF does not induce DNA repair synthesis in the absence of β-glucuronidase,[70] suggesting that no reactive metabolites are formed under *in vivo* conditions. These observations indicate that formation of *N-O*-glucuronides of arylhydroxylamines may represent a detoxication mechanism. Moreover, an inverse relationship was found between the carcinogenicity of 2-naphthylamine, 4-acetylaminobiphenyl, and 2-AAF and the ease of glucuronidation of their hydroxamic acid derivatives, suggesting that glucuronidation may play an important role in determining the carcinogenicity of arylamines and arylacetamides in the rat.[71] There are other examples that suggest a role of glucuronidation in detoxication. Enhanced glucuronidation of *N*-hydroxy-2-AAF in hepatocyte nodules may be involved in the "toxin-resistance phenotype" of these cells in the Solt-Farber model,[72] discussed in Section V.B. Further support for the role of glucuronidation in detoxication of carcinogens stems from studies of the mutagenicity of 2-naphthylamine.[73] The addition of UDP-glucuronic acid to the Ames test led to a marked reduction of 2-naphthylamine mutagenicity.

Knowledge about glucuronidation of *N*-hydroxy-2-AAF is scarce in human tissues. Studies with primary cultures of human hepatocytes demonstrated that the cells conjugated 2 to 52% (up to 51% glucuronides and 12% sulfates) of 2-AAF over a thousandfold concentration range. The C-hydroxylated AAF metabolites were conjugated to glucuronides more efficiently than *N*-hydroxy-2-AAF and deacetylated aminofluorene metabolites.[74]

The relationship between *N*-acetyltransferases and UGTs in determining the reactivity of *N*-oxidized arylamines is intriguing. Depending on the hepatic *N*-acetylation and *N*-deacetylation capacities, these metabolites appear mainly as the glucuronic acid conjugates of hydroxylamines or *N*-arylacetohydroxamic acids. The *N-O*-glucuronide of *N*-hydroxy-2-AAF is not carcinogenic when injected subcutaneously into the rat, and it does not induce DNA repair synthesis in cultured urothelial cells, unless in the presence of β-glucuronidase.[70] Depending on the species, the deglucuronidated hydroxamic acids can be activated by urothelial cells through *N*-deacetylation or *N,O*-acetyltransfer, and can produce DNA repair synthesis in cultured urothelial cells secondary to the modification of DNA. Because of the low level of β-glucuronidase in the urine, *N-O*-

glucuronides are subject to little metabolic activation in the bladder and are, therefore, considered to be detoxified metabolites. However, since both the *N*-glucuronides and free hydroxylamines induce DNA repair synthesis in cultured urothelial cells, the *N*-glucuronides of hydroxylamines are considered to be responsible for the induction of bladder tumors. This has been demonstrated by the instillation of the *N*-glucuronide of *N*-hydroxy-2-aminofluorene in the heterotopic bladder of rats.[75]

The formation of reactive glucuronides has been suggested in the case of *N*-hydroxyphenacetin, which has been proposed to be responsible for the kidney damage and carcinogenicity resulting from phenacetin.[76-79] However, the ultimate toxin responsible for phenacetin-induced kidney damage still needs to be elucidated. The complexity of glucuronidation in the formation of water-soluble excretory products has been demonstrated in studies of benzidine metabolism.[80]

IV. METABOLISM OF CARCINOGENS BY ISOZYMES OF THE UGT ENZYME SUPERFAMILY

A. Relationship between Patterns and Levels of UGT Isozymes and Glucuronide Formation in the Intact Cell

Comprehensive reviews on the various factors affecting glucuronide formation in cells have been published recently.[81,82] In addition to the patterns and levels of UGT isozymes, these factors include the level of the cofactor UDP-glucuronic acid and the "latency" of UGT activity. Although influenced by various pretreatments, the cellular level of UDP-glucuronic acid is generally held constant in hepatocytes (0.3 μmol/g tissue wet weight).[83,84] This is mainly due to the fact that, despite the varying demands for glucuronidation, regeneration of UDP-glucuronic acid appears to be quite high in the liver of fed rats.

Evidence derived from the gene structure of UGTs suggests that the active site of UGT is located on the luminal site of the endoplasmic reticulum (Figure 8).[85,86] This transmembrane topology implies that UDP-glucuronic acid, synthesized in the cytoplasm, has to be transported through the membrane to the active site. There is circumstantial evidence for a carrier of UDP-glucuronic acid.[87,88] Transport of UDP-glucuronic acid becomes rate limiting predominantly when high-turnover substrates are present at a high concentration. Maximal glucuronidation can be achieved, for example, by the addition of detergents to microsomes, leading to membrane perturbation. The ratio of UGT activities in disrupted vs. intact microsomes is called *latency*. Latency is less apparent at low substrate concentrations. For example, latency of UGT activity toward 1-naphthol is about 27-fold at 0.5 m*M* and only 3-fold at 0.002 m*M* (Lilienblum and Bock, unpublished results).

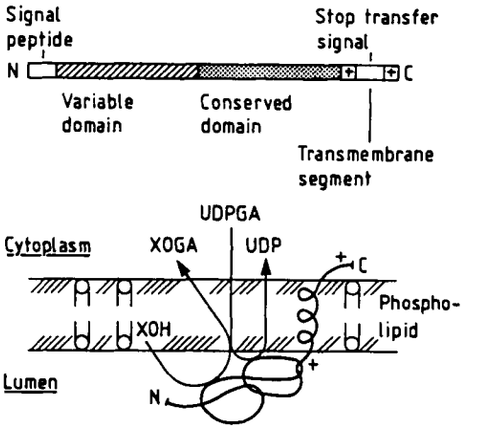

FIGURE 8. Common features deduced from UGT cDNA sequences and implication for the transmembrane topology of UGTs. GA = glucuronic acid; XOH = phenolic metabolite).

B. UGT Enzyme Superfamily

Many lines of evidence suggested multiplicity of UGTs in the past, for example, their differential inducibility by 3-methyl-cholanthrene or phenobarbital.[89-92] The existence of a supergene family of isozymes has been established recently by their purification to apparent homogeneity[93-95] and by cloning, sequencing, and expression of the cDNAs in cultured cells (Table 1). The presently used interim nomenclature is based on agreed substrates, preferably endogenous substrates.[114] This nomenclature is limited

TABLE 1
Hepatic UDP-Glucuronosyltransferases (UGTs)

Isozyme (substrates)	Overall similarity[a] (%)	Ref.
Rat		
Phenol UGT = UGT-1 (benzo(a)pyrene-3,6-quinol, 4-methylumbelliferone, 1-naphthol)	100	85[b], 93—95
Bilirubin UGT	67	96[b],97, 98
Digitoxigenin monodigitoxoside UGT	—	99
Morphine UGT = UGT-2a	—	93, 100
4-Hydroxybiphenyl UGT = UGT-2b (4-methylumbelliferone, chloramphenicol, testosterone, estradiol)	40	101[b], 102[b]
17β-Hydroxysteroid UGT (testosterone, estradiol at C-17, 1-naphthol)	39	103[b]
3α-Hydroxysteroid UGT (androsterone, lithocholic acid, 4-aminobiphenyl)	35	94, 104[b], 105[b]
Aromatic steroid UGT (esterone, estradiol at C-3)	—	98
Human		
Phenol UGT (4-methylumbelliferone, 1-naphthol, benzo(a)pyrene-3,6-quinol)	100	106[b], 107
Bilirubin UGT	—	108
Morphine UGT	—	109
Tertiary amine UGT (tripelennamine)	—	109
4-Aminobiphenyl UGT (pI 6.2)	—	110
Estriol UGT (pI 7.4) (estriol at C-16, 4-methylumbelliferone, 1-naphthylamine)	42	110, 111[b]
6-Hydroxysteroid UGT (hyodeoxycholic acid)	43	112[b]
3,4-Catechol estrogen UGT (4-hydroxyestrone, 4-methyl-umbelliferone)	43	113[b]

[a] Sequence alignment was achieved using the program ALIGN, Scientific and Educational Software, Stateline, PA, U.S.A.
[b] cDNA sequence.

because of the broad overlap of substrate specificity. A new nomenclature should be based upon the evolution of UGT amino acid sequences, similar to that adopted for the P450 enzyme family.[115] In contrast to the large P450 family, only two UGT families may have evolved, the UGT-1 family (including phenol UGT, the classic 3-methylcholanthrene-inducible UGT-1), and the larger UGT-2 family (which shows 45% overall similarity to members of the UGT-1 family, including the classic phenobarbital-inducible UGT-2b).

Several problems have to be solved before a new nomenclature can be adopted. For example, two different isozymes (phenol UGT and bilirubin UGT) may be derived from the same gene by alternative splicing.[96] This hypothesis is based on recent studies of phenol UGT cDNA from Gunn rat liver, which revealed a -1 frameshift mutation in the gene sequence. This mutation introduces a premature stop codon, leading to a truncated protein lacking the C-terminal anchor in the endoplasmic reticulum membrane.[116] Since at least two UGT isozymes appear to be lacking in Gunn rats, these isozymes may share the same C-terminal portion. Hence, the overall similarity of 67% between bilirubin UGT and phenol UGT should not obscure the fact that from residue 285 to the C-terminal there is 100% identity.

Only a few isozymes in a small number of species have been sequenced. This represents a major obstacle to the establishment of a new nomenclature. In addition to nine UGT sequences from rats and humans (Table 1), a mouse UGT has been sequenced with regulatory properties similar to bilirubin UGT.[117] Moreover, a novel UGT has been sequenced from bovine olfactory epithelium.[118]

Knowledge about glucuronidation of carcinogens and their metabolites by particular UGT isozymes is limited. In particular, there is insufficient knowledge about the tissue distribution of UGT isozymes. In the rat, phenol UGT activities appear to be ubiquitously distributed, whereas other UGT activities appear to be restricted to the liver and intestine.[61,119] Only UGT isozymes of rat and human liver will be discussed. Phenol UGT conjugates planar substrates, including carcinogenic aromatic hydrocarbons and aromatic amines and their metabolites. Although conjugated with higher affinity by phenol UGT, currently used standard phenols are overlapping substrates for several UGTs (Table 1). A more complete list of phenol UGT substrates is given in Table 2. Phenols of polycyclic aromatic hydrocarbons may be more selective substrates of phenol UGT. Induction factors for the latter substrates are higher than those obtained for standard phenols.[120] Interestingly, the enzyme conjugates benzo(a)pyrene-3,6-quinol to its mono- and diglucuronide.[8] Due to the high induction factor for diglucuronide formation (40-fold), this reaction may serve as a selective probe for phenol UGT. The proximal carcinogens *N*-hydroxy-2-naphthylamine[93] and *N*-hydroxy-2-AAF[72] are also conjugated efficiently by phenol UGT. Aromatic amines appear to be overlapping substrates for several UGTs. Interestingly, 4-aminobiphenyl appears to be a substrate for androsterone UGT[94,121] and for a human liver isozyme eluting at pI 6.2 from chromatofocusing columns (Table 1).[110]

TABLE 2
Substrate Specificity of Rat Liver Phenol UGT

Standard Phenols	Ref.
Phenol[a]	66
4-Nitrophenol	93, 94
1-Naphthol	94, 94
4-Methylumbelliferone	94
Metabolites of Aromatic Hydrocarbons	
3-Hydroxy-BP[b]	93, 120
7-Hydroxy-BP	120
5-Hydroxy-dibenz(a,h)anthracene	120
BP-3,6-quinol	8
BP-3,6-quinol monoglucuronide	8
Aromatic Amines and their Metabolites	
1-Naphthylamine	43, 121
2-Naphthylamine	43, 121
N-Hydroxy-2-naphthylamine	93
Drugs	
Mitoxanthrone[a]	122
(R)-Naproxen	123
Thyroxine[a]	124

[a] Substrate specificity has been studied with the purified isozyme. When indicated assignment was carried out from induction studies.
[b] BP, benzo(a)pyrene.

V. REGULATION OF UGTs

Similar to P450 isozymes, rat liver UGT isozymes are known to be differentially regulated by prototype inducers, such as phenobarbital and 3-methylcholanthrene,[85,89-95,125] pregnenolone 16α-carbonitril,[99,125] and clofibric acid.[92,97] It is interesting that similar prototype inducers are found in the UGT and the P450 enzyme families.[115] Some UGT isozymes, such as phenol UGT, are also induced by antioxidants.[126-128]

Regulation by antioxidants is of particular interest due to the discovery of an antioxidant-responsive element (ARE) upstream of the structural gene of glutathione *S*-transferase Ya.[129] It is likely that a similar ARE is found upstream of the structural gene of phenol UGT. Induction of these conjugating enzymes [and of NAD(P)H quinone reductase] by antioxidants without a concomitant induction of P450 isozymes is noteworthy, since they are coordinately induced with P450 isozymes by the Ah receptor, discussed below.[126-128] Two kinds of regulatory properties will be discussed in detail: (1) regulation of phenol UGT by the Ah receptor and (2) persistent increase of phenol UGT expression at cancer prestages.

A. Regulation of Phenol UGT by the Ah Receptor

The Ah receptor is a soluble protein that binds planar aromatic compounds (particularly halogenated aromatic compounds) with high affinity (k_D-TCDD $\approx 6 \times 10^{-12}$ *M*).[23,24,130] These ligands include polycyclic aromatic hydrocarbons, such as benzo(a)pyrene and 3-methylcholanthrene; polyhalogenated hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; and a large number of plant constituents, such as β-naphthoflavone, rutaecarpine, and related quinazolinecarboline alkaloids present in the Rutaceae plant family.[131] After ligand binding, the Ah receptor complexes with *cis*-acting xenobiotic responsive elements (XRE) upstream of the first exon of the gene for P4501A1.[132,133] An identical element has been identified for glutathione *S*-transferase Ya.[129] Probably there is a similar XRE associated with phenol UGT. The Ah receptor is presumed to be a member of the erb A superfamily, which includes the receptors for steroid hormones, vitamin D, retinoic acid, and thyroid hormone.[134]

Earlier genetic evidence indicated that murine liver phenol UGT is regulated by the Ah receptor.[135] This result in the mouse model was supported in rat liver by induction studies using Ah receptor ligands differing in induction potencies by a factor of more than 100,000-fold (Figure 9).[58,136] In these induction experiments, primary cultures of rat hepatocytes and cultures of rat hepatoma H4IIE cells were incubated for 48 h with various concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) or the nonhalogenated benz(a)anthracene. PCDDs were designated by the position and number of chlorine atoms. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is termed *2,3,7,8-Cl_4DD*. Incubation for 48 h resulted in maximal induction of a P4501A1-de-

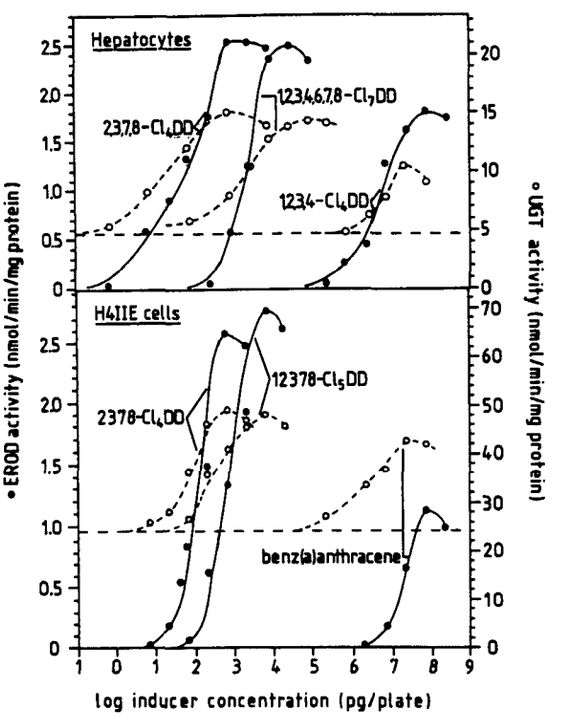

FIGURE 9. Dose-response curves of P4501A1-dependent 7-ethoxyresorufin *O*-deethylase (EROD) and of phenol UGT activity toward 4-methylumbelliferone. Cells were cultured as described.[136] Positions and numbers of chlorine substituents of the various polychlorinated dibenzo-*p*-dioxins are indicated by numbers.

pendent enzyme activity, 7-ethoxyresorufin *O*-deethylase (EROD) and of phenol UGT activity toward 4-methylumbelliferone. The data are shown as dose-response curves. Dashed lines indicate the levels of constitutive expression of phenol UGT in the two cell systems. More detailed data have been published.[136] As shown in Figure 9, both the P4501A1 and phenol UGT activities were induced at the same concentration of inducers, differing in potency by over 100,000-fold. Potencies of PCDDs have been calculated from the concentration of PCDD, leading to 50% of the induction maximum.

In humans, there is also some evidence for UGT isozymes responsive to 3-methylcholanthrene-type inducers. UGT activity toward paracetamol is increased in heavy smokers who are exposed to 3-methylcholanthrene-type inducers.[137,138] Paracetamol has been shown to be a substrate of human liver phenol UGT,[107] suggesting a similar regulation of this isozyme in humans as in rats. However, paracetamol probably represents an overlapping substrate for several isozymes.

At least six drug-metabolizing enzymes have been shown to be regulated by the Ah receptor, including P4501A1, P4501A2, NAD(P)H quinone reductase or DT diaphorase, aldehyde dehydrogenase, glutathione *S*-transferase Ya, and phenol UGT.[24] The physiological function of the receptor and its endogenous ligands remain unknown. It is conceivable, however, that in evolution the receptor has adopted the function to detoxify planar aromatic compounds, in particular those of plant origin.[24,58]

B. Persistent Increase of Phenol UGT at Cancer Prestages and its Contribution to the Toxin-Resistance Phenotype

The multiple mechanisms leading to drug resistance have been reviewed recently.[139] Similar to persistent alterations of other drug-metabolizing enzymes,[22,140] phenol UGT is increased at liver cancer prestages, such as hepatocyte foci[141,142] and hepatocyte nodules.[143,144] Alterations of UGT are heterogeneous, including both UGT-positive = UGT (+) and UGT-negative foci in mouse[145] and rat hepatocarcinogenesis models (A.- B. Kobusch and K. W. Bock, unpublished results). The present discussion only deals with UGT (+) nodules, which are preponderant in the rat. Investigation of a number of hepatocyte nodules and differentiated hepatocellular carcinomas, produced by feeding 2-AAF, suggested that phenol UGT was increased selectively. Bilirubin UGT and 17β-hydroxy-steroid UGT were not increased.[143] Northern blot analysis using a selective cDNA fragment of phenol UGT demonstrated increased expression of this isozyme under these conditions.[146] However, immunoblot analysis using antibodies to phenol UGT led to conflicting results. Whereas differentiated hepatocellular carcinomas showed increased expression of the M_r 55,000 phenol UGT polypeptide,[95] a new M_r 53,000 polypeptide was preponderant in hepatocyte nodules.[146] It has been demonstrated that phenol UGT represents a glycoprotein containing high mannose-type carbohydrate moieties of about M_r 2000.[85] In addition, it is known that glycoprotein synthesis is impaired in hepatocyte nodules produced by 2-AAF.[147] It is therefore conceivable that the phenol UGT polypeptide of Mr 53,000 found in hepatocyte nodules is produced by posttranscriptional modification, i.e., differential glycosylation, of phenol UGT (M_r 55,000).

Adaptive induction of phenol UGT by 3-methylcholanthrene-type inducers has to be clearly distinguished from the persistent alterations of this isozyme observed at cancer pre-stages. The persistent alterations of phenol UGT contribute to the "toxin-resistance phenotype" of preneoplastic cells (Table 3).[22] This phenotype denotes an altered pattern of drug-metabolizing enzymes observed in different carcinogenesis models. It includes decreased P4501A1 and decreased sulfotransferase (such as sulfotransferase IV),[149] increased glutathione *S*-transferase P, and increased phenol UGT, as well as increased expression of the multidrug-resistance gene product P-glycoprotein.[150] The toxin-resistance phenotype may explain selective growth of preneoplastic cells under the conditions of the Solt-Farber model.[72] In the evaluation of focal alterations, the progenitor cells of hepatocytes have to be considered. Mitoinhibitory agents such as 2-AAF are known to stimulate the growth of oval cells, which may be precursors of hepato-

TABLE 3
Permanent Alteration of Enzymes Involved in Drug Metabolism and Disposition in Rat Liver Nodules[a]

Enzyme	Relative nodular activity[a]
Phase I	
Cytochromes P-450	0.2
Aryl hydrocarbon hydroxylase	0.05—0.3
NAD(P)H quinone reductase (cytosol)	13
Aldehyde dehydrogenase (cytosol)	40
Epoxide hydrolase (microsomal)	5
Phase II	
Phenol sulfotransferase	0.06
Phenol UGT	5—10
Glutathione *S*-transferases	5
Glutathione *S*-transferase P	34
Others	
Gamma-glutamyltranspeptidase	100—170
Glycoprotein P (*mdr* gene product)	Increased

[a] Data are taken from references 143 and 148.

cytes.[151-154] Frequently studied rat liver epithelial cells show some characteristics of oval cells.[155] They also display a pattern of drug-metabolizing enzymes similar to the "toxin-resistance phenotype".[156] Whereas P450 activities were found to be lower in rat liver epithelial cells, phenol UGT activities were comparable to those found in differentiated hepatocytes. A "toxin-resistance phenotype", including increased UGT activity, is also found in tumor cells, in particular after treatment with chemotherapeutic agents,[157-159] and may explain therapeutic failure after long-term treatment with these agents.

C. Size Distribution of Phenol UGT-Positive Hepatocyte Foci in Relation to Additional Phenotypic Alterations (Complexity Level and Growth Characteristics)

As expected from the findings of others,[160,161] phenol UGT(+) foci were found to be phenotypically heterogeneous when four additional marker enzymes were analyzed comparatively in the foci (glutathione *S*-transferase P and gamma-glutamyltranspeptidase, which were increased, as well as ATPase and glucose-6-phosphatase, which were decreased). Phenotypic diversity was sorted into different complexity levels as follows. When foci were found to be only UGT(+), they were arbitrarily assigned to complexity level 1. When the above-mentioned one to four additional markers were also altered, they were ascribed to complexity levels 2 to 5. The frequency of size class diameters of foci was studied in relation to their different complexity levels (Figure 10). In this study hepatocyte foci were produced in a stop model[162] by the administration of *N*-nitrosomorpholine to male Wistar rats (180 g) in drinking water (80 mg/l) for 7 weeks. Stereologic analysis of foci was carried out at week 31 of the experiment. Focal intersections were sorted by different size classes according to their diameters. The size classes are defined by a log scale of maximum diameters according to Campbell et al.[163] It was found that the sizes of foci with higher complexity levels were larger than those with a lower complexity level. This finding suggests that foci with higher phenotypic complexity grow faster, extending the findings of others.[160,161] It is conceivable that the phenotypic diversity results from pleiotropic effects of initiation events (including regulation of genes that are critical for growth control), rather than from a collection of random, primary initiation events.[160] To elucidate the molecular mechanisms underlying the persistent enzyme alterations at cancer prestages, markers such as phenol UGT may be particularly useful, since much is already known about its regulatory properties.

VI. SUMMARY AND CONCLUSIONS

(1) UDP-glucuronosyltransferases (UGTs) play a major role in the elimination of nucleophilic metabolites of carcinogens, such as phenols and quinols of polycyclic aromatic hydrocarbons. In this way they prevent their further oxidation to electrophiles, which may react with DNA, RNA, and protein. They also inactivate carcinogenic, *N*-oxidized metabolites of aromatic amines. It has to be noted that electrophiles affect carcinogenesis in many ways, for example, by genotoxic alterations of protooncogenes, such as *c-Ha-ras* (initiation of carcinogenesis), or by cy-

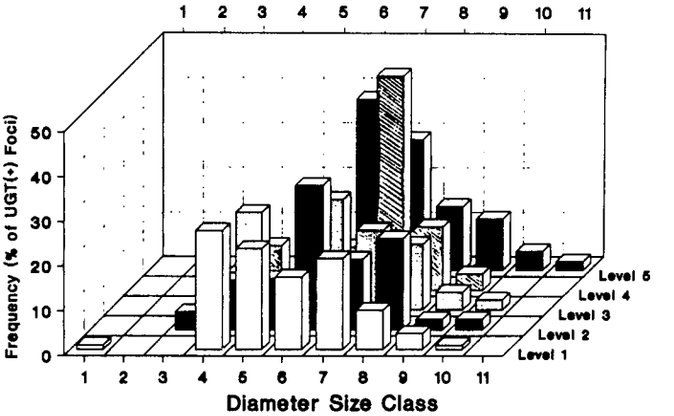

FIGURE 10. Size class distribution of phenol UGT-positive = UGT(+) foci in relation to additional phenotypic alterations (complexity level). Production of hepatocyte foci and assignment of complexity levels are described in the text. Diameter size classes and complexity levels of foci were calculated by stereologic analysis.[163]

totoxic damage of cells, stimulating regenerative growth of toxin-resistant, initiated cells (tumor promotion).

(2) Two roles of glucuronides are emphasized: (1) their role as transport forms excreted via the biliary or urinary tract, thereby liberating the ultimate carcinogen at the target of carcinogenicity; (2) their role in sequestering proximate carcinogens, leading to their detoxication. For example, glucuronidation of quinols is important, since they are readily autoxidized to reactive semiquinones and quinones, and undergo toxic redox cycles, with the generation of reactive oxygen species. Arylacetohydroxamic acids, are inactivated by glucuronidation, instead of being activated by sulfation.

(3) Isozymes of the UGT enzyme superfamily have been identified that control the glucuronidation of phenols and quinols of aromatic hydrocarbons and of carcinogenic *N*-oxidized aromatic amines. In particular, a phenol isozyme is coinduced with other drug-metabolizing enzymes via the Ah- or dioxin receptor, and plays a major role in the control of various proximate carcinogens. This isozyme is persistently increased at cancer prestages in rat hepatocarcinogenesis models and contributes to the altered enzyme pattern, leading to the "toxin-resistance phenotype".

(4) Knowledge about the isozymes of this enzyme superfamily in different species, and about their regulation and tissue distribution, will improve the extrapolation of drug and carcinogen metabolism data from experimental animals to humans, as well as risk assessment of carcinogens. In particular, regulatory properties of these isozymes, such as the control of phenol UGT by the Ah receptor and its persistent alterations at cancer prestages, will open a new avenue to understand higher order, pleiotropic regulatory programs.

ACKNOWLEDGMENTS

The authors work referred to in the review was supported by the Deutsche Forschungsgemeinschaft. Secretarial help by Mrs. A. von Bank and Mrs. E. Schenk is gratefully acknowledged.

Added in proof: Resistance of guinea pigs (compared with rats) to bladder carcinogens has been attributed to a unique UGT in this species (Dawley R. M. et al., *Cancer Res.*, 51, 514, 1991).

Note added in proof: A new nomenclature system for UGT isozymes has been proposed by an international group of experts on the basis of divergent evolution of the genes, similar to that

proposed for the P450 enzyme family.[115] For naming each gene it is proposed that the root symbol "UGT" for "*U*DP-*g*lucuronosyl*t*ransferase" be followed by an Arabic number denoting the family, a letter designating the subfamily, and an Arabic numeral representing the individual gene. According to this nomenclature, UGT isozymes listed in Table 1 are named as follows: rat or human phenol UGT (which are believed to be orthologues) and rat bilirubin UGT are named UGT1A1 and UGT1A2, respectively. Phenobarbital-inducible 4-hydroxybiphenyl UGT, 3α-hydroxysteroid UGT and 17β-hydroxysteroid UGT will be UGT2B1, UGT2B2, and UGT2B3, respectively. Human 6-hydroxysteroid UGT, 3,4-catechol estrogen UGT, and estriol UGT are called UGT2B4, UGT2B7, and UGT2B8, respectively.

REFERENCES

1. **Armstrong, R. N.,** Enzyme-catalyzed detoxication reactions: Mechanisms and stereochemistry, *Crit. Rev. Biochem.*, 22, 39, 1987.
2. **Mannervik, B. and Danielson, U. H.,** Glutathione transferases — structure and catalytic activity, *Crit. Rev. Biochem.*, 23, 283, 1988.
3. **Coles, B. and Ketterer, B.,** The role of glutathione and glutathione transferases in chemical carcinogenesis, *Crit. Rev. Biochem. Mol. Biol.*, 25, 47, 1990.
4. **Guengerich, F. P.,** Enzymatic oxidation of xenobiotic chemicals, *Crit. Rev. Biochem. Mol. Biol.*, 25, 97, 1990.
5. **Miller, E. C. and Miller, J. A.,** Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules, *Cancer*, 47, 2327, 1981.
6. **Lorentzen, R. J. and Ts'o P. O. P.,** Benzo(a)pyrenedione/benzo(a)pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen, *Biochemistry*, 16, 1467, 1977.
7. **Lorentzen, R. J., Lesko, S. A., McDonald, K., and Ts'o P. O. P.,** Toxicity of metabolic benzo(a)pyrenediones to cultured cells and the dependence upon molecular oxygen, *Cancer Res.*, 39, 3194, 1979.
8. **Lilienblum, W., Bock-Hennig, B. S., and Bock, K. W.,** Protection against toxic redox cycles between benzo(a)pyrene-3,6-quinone and its quinol by 3-methylcholanthrene-inducible formation of the quinol mono- and diglucuronide, *Mol. Pharmacol.*, 27, 451, 1985.
9. **Shinohara, A., Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R.,** Acetyl coenzyme A dependent activation of *N*-hydroxy derivates of carcinogenic arylamines: mechanism of activation, species difference, tissue distribution, and acetyl donor specificity, *Cancer Res.*, 46, 4362, 1989.
10. **Miller A. and Miller, E. C.,** Electrophilic sulfuric acid ester metabolites as ultimate carcinogens, in *Biological Reactive Intermediates III; Advances in Experimental Medicine and Biology*, 197, 583, 1986.
11. **Wiseman, R. W., Stowers, S. J., Miller, E. C., Anderson, M. W., and Miller, J. A.,** Activating mutations of the c-Ha-ras protooncogene in chemically induced hepatomas of the male B6C3F$_1$ mouse, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 5825, 1986.
12. **Bishop, J. M.,** Retroviruses and cancer genes, *Adv. Cancer Res.*, 37, 1, 1982.
13. **Weinberg, R. A.,** Oncogenes of spontaneous and chemically induced tumors, *Adv. Cancer Res.*, 36, 149, 1982.
14. **Zarbl, H., Sukumar, S., Arthur, A. V., Martin-Zanca, D., and Barbacid, M.,** Direct mutagenesis of Ha-*ras*-1 oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats, *Nature*, 315, 382, 1985.
15. **Land, H., Parada, L. F., and Weinberg, R. A.,** Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes, *Nature*, 304, 596, 1983.
16. **Knudson, A. G.,** Mutation and cancer, oncogenes, and antioncogenes, *Cancer Res.*, 45, 1437, 1985.
17. **Weinberg, R. A.,** Finding the anti-oncogene, *Sci. Amer.*, 34, 1988.
18. **Moolgavkar, S. H.,** Multistage models for cancer risk assessment, in *Biologically Based Methods for Cancer Risk Assessment*, Travis, C. T., Ed., Plenum Press, New York, 1989, 9.
19. **Cohen, S. M. and Ellwein, L. B.,** Cell proliferation in carcinogenesis, *Science*, 249, 1007, 1990.
20. **Schwarz, M., Pearson, D., Port, R., and Kunz, W.,** Promoting effect of 4-dimethylaminoazobenzene on enzyme altered foci induced in rat liver by *N*-nitrosodiethanolamine, *Carcinogenesis*, 5, 725, 1984.
21. **Rabes, H. M.,** Development and growth of early preneoplastic lesions induced in the liver by chemical carcinogens, *J. Cancer Res. Clin. Oncol.*, 106, 85, 1983.
22. **Farber, E.,** Cellular biochemistry of the stepwise development of cancer with chemicals, *Cancer Res.*, 44, 5463, 1984.
23. **Poland, A. and Knutson, J. C.,** 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated hydrocarbons: examination of the mechanism of toxicity, *Ann. Rev. Pharmacol. Toxicol.*, 22, 517, 1982.
24. **Nebert, D. W., Petersen, D. D., and Fornace, A. J., Jr.,** Cellular responses to oxidative stress: the [Ah] gene battery as a paradigm, *Env. Health Persp.*, 88, 13, 1990.
25. **Beland, F. A. and Kadlubar, F. F.,** Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons, in *Chemical Carcino-*

genesis and Mutagenesis I, Cooper, C. S. and Grover, P. L., Eds., Springer-Verlag, Berlin, 1990, 267.
26. **Rehn, L.,** Blasengeschwülste bei Fuchsin-Arbeitern, *Arch. Klin. Chir.*, 50, 588, 1895.
27. **Hueper, W. C., Wiley, F. H., Wolfe, H. D., Ranta, K. E., Leming, M. F., and Blood, F. R.,** Experimental production of bladder tumors in dogs by administration of β-naphthylamine, *J. Ind. Hyg. Toxicol.*, 20, 46, 1938.
28. **Patrianakos, C. and Hoffmann, D.,** Chemical studies on tobacco smoke. LXIV. On the analysis of aromatic amines in cigarette smoke, *J. Anal. Toxicol.*, 3, 150, 1979.
29. **Wynder, E. L. and Goldsmith, R.,** The epidemiology of bladder cancer. A second look, *Cancer*, 40, 1246, 1977.
30. **Moolgavkar, S. H. and Stevens, R. G.,** Smoking and cancers of bladder and pancreas: risks and temporal trends, *J. Natl. Cancer Inst.*, 67, 15, 1981.
31. **Mommsen, S. and Aagaard, J.,** Tobacco as a risk factor in bladder cancer, *Carcinogenesis*, 4, 335, 1983.
32. **Sugimura, T.,** Past, present, and future of mutagens in cooked foods, *Environ. Health Perspect.*, 67, 5, 1986.
33. **Tokiwa, H. and Ohnishi, Y.,** Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment, *CRC Crit. Rev. Toxicol.*, 17, 23, 1986.
34. **Silverman, D. T., Hoover, R. N., Albert, S., and Graff, K. M.,** Occupation and cancer of the lower urinary tract in Detroit, *J. Natl. Cancer Inst.*, 70, 237, 1983.
35. **Wilson, R. H., DeEds, F., and Cox, A. J., Jr.,** The toxicity and carcinogenic activity of 2-acetaminofluorene, *Cancer Res.*, 1, 595, 1941.
36. **Kadlubar, F. F., Miller, J. A., and Miller, E. C.,** Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis, *Cancer Res.*, 37, 805, 1977.
37. **Radomski, J. L., Hearn, W. L., Radomski, T., Moreno, H., and Scott, W. E.,** Isolation of the glucuronic acid conjugate of *N*-hydroxy-4-aminobiphenyl from dog urine and its mutagenic activity, *Cancer Res.*, 37, 1757, 1977.
38. **Poupko, J. M., Hearn, W. L., and Radomski, J. L.,** *N*-Glucuronidation of *N*-hydroxy aromatic amines: a mechanism for their transport and bladder-specific carcinogenicity, *Toxicol. Appl. Pharmacol.*, 50, 479, 1979.
39. **Kadlubar, F. F., Unruh, L. E., Flammang, T. J., Sparks, D., Mitchum, R. K., and Mulder, G. J.,** Alteration of urinary levels of the carcinogen, *N*-hydroxy-2-naphthylamine, and its *N*-glucuronide in the rat by control of urinary pH, inhibition of metabolic sulfation, and changes in biliary excretion, *Chem.-Biol. Interact.*, 33, 129, 1981.
40. **Talaska, G., Dooley, K. L., and Kadlubar, F. F.,** Detection and characterization of carcinogen-DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by ^{32}P-postlabeling and subsequent thin-layer and high-pressure liquid chromatography, *Carcinogenesis*, 11, 639, 1990.
41. **Radomski, J. L., Deichmann, W. B., Altman, N. H., and Radomski, T.,** Failure of pure 1-naphthylamine to induce bladder tumors in dogs, *Cancer Res.*, 40, 3537, 1980.
42. **Purchase, I. F. H., Kalinowski, A. E., Ishmael, J., Wilson, J., Gore, C. W., and Chart, I. S.,** Lifetime carcinogenicity study of 1- and 2-napthylamine in dogs, *Br. J. Cancer*, 44, 892, 1981.
43. **Lilienblum, W. and Bock, K. W.,** *N*-Glucuronide formation of carcinogenic aromatic amines in rat and human liver microsomes, *Biochem. Pharmacol.*, 33, 2041, 1984.
44. **Weisburger, J. H.,** Colon carcinogens: their metabolism and mode of action, *Cancer*, 28, 60, 1971.
45. **Luks, H. J., Spratt, T. E., Vavrek, M. T., Roland, S. F., and Weisburger, J. H.,** Identification of sulfate and glucuronic acid conjugates of the 5-hydroxy derivative as major metabolites of 2-amino-3-methylimidazo[4,5-f]quinoline in rats, *Cancer Res.*, 49, 4407, 1989.
46. **Turesky, R. J., Bracco-Hammer, I., Markovic, J., Richli, U., Kappeler, A.- M., and Welti, D. H.,** The contribution of N-oxidation to the metabolism of the food-borne carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in rat hepatocytes, *Chem. Res. Toxicol.*, 3, 524, 1990.
47. **Chipman, J. K., Frost, G. S., Hirom, P. C., and Millburn, P.,** Biliary excretion, systemic availability and reactivity of metabolites following intraportal infusion of [^{3}H]benzo[a]pyrene in the rat, *Carcinogenesis*, 2, 741, 1981.
48. **Renwick, A. G. and Drasar, B. S.,** Environmental carcinogens and large bowel cancer, *Nature*, 263, 234, 1976.
49. **Kinoshita, N. and Gelboin, H. V.,** β-Glucuronidase catalysed hydrolysis of benzo[a]pyrene-3-glucuronide and binding to DNA, *Science*, 199, 307, 1978.
50. **Conney, A. H.,** Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture, *Cancer Res.*, 42, 4875, 1982.
51. **Hall, M. and Grover, P. L.,** Polycyclic aromatic hydrocarbons: metabolism, activation and tumour initiation, in *Chemical Carcinogenesis and Mutagenesis I*, Cooper, C. S. and Grover, P. L., Eds., Springer-Verlag, Berlin, 1990, 327.
52. **Burke, M. D., Vadi, H., Jernstroöm, B., and Orrenius, S.,** Metabolism of benzo[a]pyrene with isolated hepatocytes and the formation and degradation of DNA-binding derivates, *J. Biol. Chem.*, 252, 6424, 1977.
53. **Bock, K. W., Bock-Hennig, B. S., Lilienblum, W., and Volp, R. F.,** Release of mutagenic metabolites of benzo(a)pyrene from the perfused rat liver after inhibition of glucuronidation and sulfation by

salicylamide, *Chem.- Biol. Interactions*, 36, 167, 1981.

54. **Owens, I. S., Koteen, G. M., and Legraverend, C.,** Mutagenesis of certain benzo[a]pyrene phenols in vitro following further metabolism by mouse liver, *Biochem. Pharmacol.*, 28, 1615, 1979.
55. **Bock, K. W., Bock-Hennig, B. S., Fischer, G., Lilienblum, W., and Ullrich, D.,** Role of glucuronidation and sulfation in the control of reactive metabolites, in *Biochemical Basis of Chemical Carcinogenesis*, Thirteenth Workshop Conference Hoechst, Greim, H., Jung, R., Kramer, M., Marquardt, H., and Oesch, F., Eds., Raven Press, New York, 1984, 13.
56. **Nemoto, N., Takayama, S., Nagao, M., and Umezawa, K.,** Modification of the mutagenicity of benzo(a)pyrene on bacteria by substrates of enzymes producing water soluble conjugates, *Toxicol. Lett.*, 2, 205, 1978.
57. **Malaveille, C., Brun, G., Hautefeuille, A., and Bartsch, H.,** Effect of glutathione and uridine 5′-diphosphoglucuronic acid on benzo(a)pyrene mutagenesis in the Salmonella/microsome assay, in *Mechanisms of Toxicity and Hazard Evaluation*, Holmstedt B., Lauwreys, R., Mercier, M., and Roberfroid, M., Eds., Elsevier, Amsterdam, 1980, 175.
58. **Bock, K. W., Lipp, H.-P., and Bock-Hennig, B. S.,** Induction of drug-metabolizing enzymes by xenobiotics, *Xenobiotica*, 20, 1101, 1990.
59. **Shen, A. L., Fahl, W. E., Wrighton, S. A., and Jefcoate, C. R.,** Inhibition of benzo[a]pyrene and benzo[a]pyrene quinones, *Cancer Res.*, 39, 4123, 1979.
60. **Bock, K. W.,** Increase of liver microsomal benzo(a)pyrene monooxygenase activity by subsequent glucuronidation, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 304, 77, 1978.
61. **Bock, K. W., v. Clausbruch, U. C., Kaufmann, R., Lilienblum, W., Oesch, F., Pfeil, H., and Platt, K. L.,** Functional heterogeneity of UDP-glucuronyltransferase in rat tissues, *Biochem. Pharmacol.*, 29, 495, 1980.
62. **Segura-Aquilar, J. E., Barreiro, V., and Lind, C.,** Dicoumarol-sensitive glucuronidation of benzo(a)pyrene metabolites in rat liver microsomes, *Arch. Biochem. Biophys.*, 251, 266, 1986.
63. **Tunek, A., Platt, K. L., Bentley, P., and Oesch, F.,** Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modifications of this metabolism, *Mol. Pharmacol.*, 14, 920, 1978.
64. **Greenlee, W. F. and Irons, R. D.,** Modulation of benzene-induced lymphocytopenia in the rat by 2,4,5,2′,4′,5′-hexachlorobiphenyl and 3,4,3′,4′-tetrachlorobiphenyl, *Chem.- Biol. Interact.*, 33, 345, 1981.
65. **Sammett, D., Lee, E. W., Kocsis, J. J., and Snyder, R.,** Partial hepatectomy reduces both metabolism and toxicity of benzene, *J. Toxicol. Environ. Health*, 5, 785, 1979.
66. **Schrenk, D. and Bock, K. W.,** Metabolism of benzene in rat hepatocytes: influence of inducers on phenol glucuronidation, *Drug Metab. Disp.*, 18, 720, 1990.
67. **Reitz, R. H., Fox, T. R., Quast, J. F., Hermann, E. A., and Watanabe, P. G.,** Molecular mechanisms involved in the toxicity of orthophenylphenol and its sodium salt, *Chem.- Biol. Interact.*, 43, 99, 1983.
68. **Cardona, R. A. and King, C. M.,** Activation of the *O*-glucuronide of the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide by enzymatic deacetylation in vitro: formation of fluorenylamine-tRNA adducts, *Biochem. Pharmacol.*, 25, 1051, 1975.
69. **Irving, C. C.,** Influence of the aryl group on the reaction of glucuronides of *N*-arylacethydroxamic acids with polynucleotides, *Cancer Res.*, 37, 524, 1977.
70. **Wang, C. Y., Christensen, B., Zukowski, K., Morton, K. C., and Lee, M. S.,** Induction of DNA repair synthesis in human urothelial cells by the *N*-hydroxy metabolites of carcinogenic arylamines, *J. Natl. Cancer Inst.*, 72, 847, 1984.
71. **Wang, C. Y., Zukowski, K., and Lee, M. S.,** Glucuronidation of carcinogenic arylamine metabolites by rat liver microsomes, *Biochem. Pharmacol.*, 34, 837, 1985.
72. **Spiewak Rinaudo, J. A., Eriksson, L. C., Roomi, M. W., and Farber, E.,** Kinetics of excretion of 2-acetylaminofluorene in normal and xenobiotic-treated rats and in rats with hepatocyte nodules, *Lab. Invest.*, 60, 399, 1989.
73. **Bock-Hennig, B. S., Ullrich, D., and Bock, K. W.,** Activating and inactivating reactions controlling 2-naphthylamine mutagenicity, *Arch. Toxicol.*, 50, 259, 1982.
74. **Monteith, D. K., Michalopoulos, G., and Strom, S. C.,** Conjugation of chemical carcinogens by primary cultures of human hepatocytes, *Xenobiotica*, 20, 753, 1990.
75. **Wang, C. Y., Zukowski, K., Lee, M.-S., and Imaida, K.,** Production of urothelial tumors in the heterotopic bladder of rats by installation of *N*-glucuronosyl or *N*-acetyl derivates of *N*-hydroxy-2-aminofluorene, *Cancer Res.*, 47, 3406, 1987.
76. **Mulder, G. J., Hinson, J. A., and Gillette, J. R.,** Conversion of the *N*-*O*-glucuronide and *N*-*O*-sulfate conjugates of *N*-hydroxyphenacetin to reactive intermediates, *Biochem. Pharmacol.*, 27, 1641, 1978.
77. **Hinson, J. A., Andrews, L. S., and Gillette, R.,** Kinetic evidence for multiple chemically reactive intermediates in the breakdown of phenacetin *N*-*O*-glucuronide, *Pharmacology*, 19, 237, 1979.
78. **Camus, A.-M., Friesen, M., Croisy, A., and Bartsch, H.,** Species-specific activation of phenacetin into bacterial mutagens by hamster liver enzymes and identification of *N*-hydroxyphenacetin

O-glucuronide as a promutagen in the urine, *Cancer Res.*, 42, 3201, 1982.
79. **Feng, P. C. C., Fenselau, C., Colvin, M. E., and Hinson, J. A.,** Synthesis and analysis of glucuronides of *N*-hydroxyamides, *Drug Metab. Disp.*, 11, 103, 1983.
80. **Lynn, R. K., Garvie-Gould, C. T., Milam, D. F., Scott, K. F., Eastman, C. L., Ilias, A. M., and Rodgers, R. M.,** Disposition of the aromatic amine, benzidine, in the rat: characterization of mutagenic urinary and biliary metabolites, *Toxicol. Appl. Pharmacol.*, 72, 1, 1984.
81. **Siest, G., Magdalou, J., and Burchell, B., Eds.,** *Cellular and Molecular Aspects of Glucuronidation,* Colloque INSERM 173, John Libbey Eurotext, 1990.
82. **Mulder, G. J., Coughtrie, M. W. H., and Burchell, B.,** Glucuronidation, in *Conjugation Reactions in Drug Metabolism,* Mulder, G. J., Ed., Taylor and Francis, 1990, 51.
83. **Bock, K. W. and White,I. N. H.,** UDP-glucuronyltransferase in perfused rat liver and in microsomes: influence of phenobarbital and 3-methylcholanthrene, *Eur. J. Biochem.*, 46, 451, 1974.
84. **Ullrich, D. and Bock, K. W.,** Glucuronide formation of various drugs in liver microsomes and in isolated hepatocytes from phenobarbital- and 3-methylcholanthrene-treated rats, *Biochem. Pharmacol.*, 33, 97, 1984.
85. **Iyanagi, T., Haniu, M., Sogawa, K., Fujii-Kuriyama, Y., Watanabe, S., Shively, J. E., and Anan, K. F.,** Cloning and characterization of cDNA encoding 3-methylcholanthrene inducible rat mRNA for UDP-glucuronosyltransferase, *J. Biol. Chem.*, 261, 15607, 1986.
86. **Mackenzie, P. I., Chowdhury, N. R., and Chowdhury, J. R.,** Characterization and regulation of rat liver UDP glucuronosyltransferases, *Clin. Exp. Pharmacol. Physiol.*, 16, 501, 1989.
87. **Hauser, S. C., Ziurys, J. C., and Gollan, J. L.,** A membrane transporter mediates access of uridine 5′-diphosphoglucuronic acid from the cytosol into the endoplasmic reticulum of rat hepatocytes: implications for glucuronidation reactions, *Biochem. Biophys. Acta,* 967, 149, 1988.
88. **Vanstapel, F. and Blanckaert, N.,** Topology and regulation of bilirubin UDP-glucuronyltransferase in sealed native microsomes from rat liver, *Arch. Biochem. Biophys.*, 263, 216, 1988.
89. **Bock, K. W., Fröhling, W., Remmer, H., and Rexer, B.,** Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase, *Biochim. Biophys. Acta,* 327, 46, 1973.
90. **Lucier, G. W., McDaniel, O. S., and Hook, G. E. R.,** Nature of the enhancement of hepatic uridine diphosphate glucuronyl-transferase activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats, *Biochem. Pharmacol.*, 24, 325, 1975.
91. **Wishart, G. J.,** Demonstration of functional heterogeneity of hepatic uridine diphosphate glucuronosyltransferase activities after administration of 3-methylcholanthrene and phenobarbital to rats, *J. Biochem.*, 174, 671, 1978.
92. **Lilienblum, W., Walli, A. K., and Bock, K. W.,** Differential induction of rat liver microsomal UDP-glucuronosyltransferase activities by various inducing agents, *Biochem. Pharmacol.*, 31, 907, 1982.
93. **Bock, K. W., Josting, D., Lilienblum, W., and Pfeil, H.,** Purification of rat liver microsomal UDP-glucuronyl-transferase, *Eur. J. Biochem.*, 98, 19, 1979.
94. **Falany, C. N. and Tephly, T. R.,** Separation, purification and characterization of three isoenzymes of UDP-glucuronosyltransferase from rat liver microsomes, *Arch. Biochem. Biophys.*, 227, 248, 1983.
95. **Bock, K. W., Schirmer, G., Green, M. D., and Tephly, T. R.,** Properties of a 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase from rat liver, *Biochem. Pharmacol.*, 37, 1439, 1988.
96. **Sato, H., Koiwai, O., Tanabe, K., and Kashiwamata, S.,** Isolation and sequencing of rat liver bilirubin UDP-glucuronosyltransferase cDNA: possible alternate splicing of a common primary transcript, *Biochem. Biophys. Res. Commun.*, 169, 260, 1990.
97. **Scragg, I., Celier, C., and Burchell, B.,** Congenital jaundice in rats due to the absence of hepatic bilirubin UDP-glucuronyltransferase enzyme protein, *FEBS Lett.*, 183, 37, 1985.
98. **Roy Chowdhury, J., Roy Chowdhury, N., Falany, C. N., Tephly, T. R., and Arias, I. M.,** Isolation and characterization of multiple forms of rat liver UDP-glucuronate glucuronosyltransferase, *Biochem. J.*, 233, 827, 1986.
99. **Meyerinck von, L., Coffman, B. L., Green, M. D., Kirkpatrick, R. B., Schmoldt, A., and Tephly, T. R.,** Separation, purification, and characterization of digitoxigenin-monodigitoxoside UDP-glucuronosyltransferase activity, *Drug Metab. Dispos.*, 13, 700, 1985.
100. **Puik, J. F. and Tephly, T. R.,** Isolation and purification of rat liver morphine UDP-glucuronosyltransferase, *Mol. Pharmacol.*, 30, 558, 1986.
101. **Mackenzie, P. I.,** Rat liver UDP-glucuronosyltransferase. Identification of cDNAs encoding two enzymes which glucuronidate testosterone, dihydrotestosterone and β-estradiol, *J. Biol. Chem.*, 262, 9744, 1987.
102. **Mackenzie, P. I. and Rodbourn, L.,** Organization of the rat UDP-glucuronosyltransferase, UDPGTr-2, gene and characterization of its promoter, *J. Biol. Chem.*, 265, 11328, 1990.
103. **Harding, D., Wilson, S. M., Jackson, M. R., Burchell, B., Green, M. D., and Tephly, T. R.,** Nucleotide and deduced amino acid sequence of rat liver 17β-hydroxysteroid UDP-glucuronosyltransferase, *Nucl. Acids Res.*, 15, 3936, 1987.
104. **Jackson, M. R. and Burchell, B.,** The full length

coding sequence of rat liver androsterone UDP-glucuronyltransferase cDNA and comparison with other members of this gene family, *Nucl. Acids Res.*, 14, 779, 1986.

105. **Mackenzie, P. I.,** Rat liver UDP-glucuronosyltransferase. Sequence and expression of cDNA encoding a phenobarbital-inducible form, *J. Biol. Chem.*, 261, 14112, 1986.
106. **Harding, D., Fournel-Gigleux, S., Jackson, M. R., and Burchell, B.,** Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 8381, 1988.
107. **Forster, A., Brück, M., El Mouelhi, M., Fournel-Gigleux, S., Siest, G., Burchell, B., and Bock, K. W.,** Paracetamol glucuronidation by human liver phenol UDP-glucuronosyltransferase, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 343, R 11, 1991.
108. **Burchell, B., Coughtrie, M. W. H., Jackson, M. R., Shepherd, S. R. P., Harding, D., and Hume, R.,** Genetic deficiency of bilirubin glucuronidation in rats and humans, *Molec. Aspects Med.*, 9, 429, 1987.
109. **Thomassin, J., Styczynski, P., Coffman, B., Green, M., and Tephly, T.,** Studies on human liver microsomal morphine and tertiary amine UDP-glucuronosyltransferases: photoaffinity labelling and immunoinhibition, in *Hepatic Metabolism and Disposition of Endo- and Xenobiotics*, Bock, K. W., Gerok, W., Matern, S., and Schmid, R., Eds., Kluwer Academic Publishers, Dordrecht, 1991, in press.
110. **Irshaid, Y. M. and Tephly, T. R.,** Isolation and purification of two human liver UDP-glucuronosyltransferases, *Mol. Pharmacol.*, 31, 27, 1987.
111. **Coffman, B. L., Tephly, T. R., Irshaid, Y. M., Green, M. D., Smith, C., Jackson, M. R., Wooster, R., and Burchell, B.,** Characterization and primary sequence of a human hepatic microsomal estriol UDPglucuronosyltransferase, *Arch. Biochem. Biophys.*, 281, 170, 1990.
112. **Fournel-Gigleux, S., Jackson, M. R., Wooster, R., and Burchell, B.,** Expression of a human liver cDNA encoding a UDP-glucuronosyltransferase catalysing the glucuronidation of hyodeoxycholic acid in cell culture, *FEBS Lett.*, 243, 119, 1989.
113. **Ritter, J. K., Sheen, Y. Y., and Owens, I. S.,** Cloning and expression of human liver UDP-glucuronosyltransferase in COS-1 cells, *J. Biol. Chem.*, 265, 7900, 1990.
114. **Bock, K. W., Burchell, B., Dutton, G. J., Hänninen, O., Mulder, G. J., Owens, I. S., Siest, G., and Tephly,T. R.,** Commentary: UDP-glucuronosyltransferase activities: guidelines for consistent interim terminology and assay conditions, *Biochem. Pharmacol.*, 32, 953, 1983.
115. **Nebert, D. W. and Gonzalez, F. J.,** P450 genes: structure, evolution, and regulation, *Ann. Rev. Biochem.*, 56, 945, 1987.
116. **Iyanagi, T., Watanabe, T., and Uchiyama, Y.,** The 3-methylcholanthrene-inducible UDP-glucuronosyltransferase deficiency in the hyperbilirubinemic rat (Gunn rat) is caused by a −1 frameshift mutation, *J. Biol. Chem.*, 264, 21302, 1989.
117. **Kimura, T. and Owens, I. S.,** Mouse UDP-glucuronosyltransferase cDNA and complete amino acid sequence and regulation, *Eur. J. Biochem.*, 168, 515, 1987.
118. **Lazard, D., Tal, N., Rubinstein, M., Kehn, M., Lancet, D., and Zupko, K.,** Identification and biochemical analysis of novel olfactory-specific cytochrome P-450IIA and UDP-glucuronosyl transferase, *Biochemistry*, 29, 7433, 1990.
119. **Dutton, G. J.,** *Glucuronidation of Drugs and Other Compounds*, CRC Press, Boca Raton, FL, 1980.
120. **Lilienblum, W., Platt, K. L., Schirmer, G., Oesch, F., and Bock, K. W.,** Regioselectivity of rat liver microsomal UDP-glucuronosyltransferase activities towards phenols of benzo(a)pyrene and dibenzo(a,h)anthracene, *Mol. Pharmacol.*, 32, 173, 1987.
121. **Green, M. D. and Tephly, T. R.,** *N*-glucuronidation of carcinogenic aromatic amines catalyzed by rat hepatic microsomal preparations and purified rat liver uridine 5′-diphosphate-glucuronosyltransferases, *Cancer Res.*, 47, 2028, 1987.
122. **Wolf, C. R., Macpherson, J. S., and Smith, J. F.,** Evidence for the metabolism of mitozantrone by microsomal glutathione transferases and 3-methylcholanthrene-inducible glucuronosyl transferases, *Biochem. Pharmacol.*, 35, 1577, 1986.
123. **El Mouelhi, M. and Bock, K. W.,** Stereoselective (S)- and (R)-naproxen glucuronosyltransferase of rat liver, *Drug Metab. Disp.*, 19, 304, 1991.
124. **Bastomsky, C. H.,** Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetracholordibenzo-*p*-dioxin, *Endocrinology*, 101, 292, 1977.
125. **Watkins, J. B., Gregus, Z., Thompson, T. N., and Klaassen, C. D.,** Induction studies on the functional heterogeneity of rat liver UDP-glucuronosyltransferases, *Toxicol. Appl. Pharmacol.*, 64, 439, 1982.
126. **Bock, K. W., Kahl, R., and Lilienblum, W.,** Induction of rat hepatic UDP-glucuronosyltransferase by dietary ethoxyquin, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 310, 249, 1980.
127. **Prochaska, H. J. and Talalay, P.,** Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver, *Cancer Res.*, 48, 4776, 1988.
128. **De Long, M. J., Prochaska, H. J., and Talalay, P.,** Induction of NAD(P)H-quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: a model system for the study of anticarcinogens, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 787, 1986.
129. **Rushmore, T. H. and Pickett, C. B.,** Transcriptional regulation of the rat glutathione S-transferase

Ya subunit gene, *J. Biol. Chem.*, 265, 14648, 1990.
130. **Bradfield, C. A. and Poland, A.,** A competitive binding assay for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related ligands of the Ah receptor, *Mol. Pharmacol.*, 34, 682, 1988.
131. **Gillner, M., Bergman, J., Cambillau, C., and Gustafsson, J.-A.,** Interactions of rutaecarpine alkaloids with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver, *Carcinogenesis*, 10, 651, 1989.
132. **Denison, M. S., Fisher, J. M., and Whitlock, J.,** The DNA-recognition site for the dioxin-Ah receptor complex, *J. Biol. Chem.*, 263, 17221, 1988.
133. **Sogawa, K., Fujisawa-Sehara, A., Yamane, M., and Fujii-Kuriyama, Y.,** Location of regulatory elements responsible for drug induction in the rat cytochrome P-450c gene, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 8044, 1986.
134. **Evans, R. M.,** The steroid and thyroid hormone receptor superfamily, *Science*, 240, 889, 1988.
135. **Owens, I. D.,** Genetic regulation of UDP-glucuronosyl-transferase induction by polycyclic aromatic compounds in mice, *J. Biol. Chem.*, 252, 2827, 1977.
136. **Schrenk, D., Lipp, H.-P., Wiesmüller, T., Hagenmaier, H., and Bock, K. W.,** Assessment of biological activities of mixtures of polychlorinated dibenzo-*p*-dioxins: comparison between defined mixtures and their constituents, *Arch. Toxicol.*, 65, 114, 1991.
137. **Mucklow, J. C., Fraser, H. S., Bulpitt, C. J., Kahn, C., Mould, G., and Dolery, C. T.,** Environmental factors affecting paracetamol metabolism in London factory and office workers, *Br. J. Clin. Pharmacol.*, 10, 67, 1980.
138. **Bock, K. W., Wiltfang, J., Blume, R., Ullrich, D., and Bircher, J.,** Paracetamol as a test drug to determine glucuronide formation in man. Effects of inducers and of smoking, *Eur. J. Clin. Pharmacol.*, 31, 677, 1987.
139. **Hayes, J. D. and Wolf, C. R.,** Molecular mechanisms of drug resistance, *Biochem. J.*, 272, 281, 1990.
140. **Beer, D. G. and Pitot, H. C.,** Biological markers characterizing the development of preneoplastic and neoplastic lesions in rodent liver, *Arch. Toxicol.*, Suppl. 10, 68, 1987.
141. **Fischer, G., Ullrich, D., Katz, N., Bock, K. W., and Schauer, A.,** Immunohistochemical and biochemical detection of uridine-diphosphate-glucuronosyltransferase (UDP-GT) activity in putative preneoplastic liver foci, *Virchows. Arch.*, 42, 192, 1983.
142. **Fischer, G., Ullrich, D., and Bock, K. W.,** Effects of *N*-nitrosomorpholine and phenobarbital on UDP-glucuronosyl-transferase in putative preneoplastic foci of rat liver, *Carcinogenesis*, 6, 605, 1985.
143. **Bock, K. W., Lilienblum, W., Pfeil, H., and Eriksson, L. C.,** Increased uridine diphosphate-glucuronosyltransferase activity in preneoplastic liver nodules and Morris hepatomas, *Cancer Res.*, 42, 3747, 1982.
144. **Yin, Z., Sato, K., Tsuda, H., and Ito, N.,** Changes in activities of uridine diphosphate-glucuronosyltransferase during chemical hepatocarcinogenesis, *Gann*, 73, 239, 1982.
145. **Bock, K. W., Kobusch, A.-B., and Fischer, G.,** Heterogeneous alterations of UDP-glucuronosyltransferases in mouse hepatic foci, *J. Cancer Res. Clin. Oncol.*, 115, 285, 1989.
146. **Bock, K. W., Münzel, P. A., Röhrdanz, E., Schrenk, D., and Eriksson, L. C.,** Persistently increased expression of a 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase in rat hepatocyte nodules and hepatocellular carcinomas, *Cancer Res.*, 50, 3569, 1990.
147. **Eggens, I., Eriksson, L. C., Chojnacki, T., and Dallner, G.,** Role of dolichyl phosphate in regulation of protein glycosylation in 2-acetylaminofluorene-induced carcinogenesis in rat liver, *Cancer Res.*, 44, 799, 1984.
148. **Bock, K. W.,** Conjugation reactions in carcinogen metabolism and their permanent alterations after initiation of hepatocarcinogenesis, in *Liver Cell Carcinoma*, Bannasch, P., Keppler, D., and Weber, G., Eds., MTP Press, Lancaster, U.K., 1989, 251.
149. **Ringer, D. P., Norton, T. R., and Howell, B. A.,** 2-Acetylaminofluorene-mediated alteration in the level of liver arylsulfotransferase IV during rat hepatocarcinogenesis, *Cancer Res.*, 50, 5301, 1990.
150. **Thorgeirsson, S. S., Huber, B. E., Sorrell, S., Fojo, A., Pastan, I., and Gottesman, M. M.,** Expression of the multidrug-resistant gene in hepatocarcinogenesis and regenerating rat liver, *Science*, 236, 1120, 1987.
151. **Sell, S.,** Is there a liver stem cell?, *Cancer Res.*, 50, 3811, 1990.
152. **Evarts, R. P., Nagy, P., Nakatsukasa, H., Marsden, E., and Thorgeirsson, S. S.,** In vivo differentiation of rat liver oval cells into hepatocytes, *Cancer Res.*, 49, 1541, 1989.
153. **Hixson, D. C. and Allison, J. P.,** Monoclonal antibodies recognizing oval cells induced in the liver of rats by *N*-2-fluorenylacetamide or ethionine in a choline-deficient diet, *Cancer Res.*, 45, 3750, 1985.
154. **Germain, L., Noel, M., Gourdeau, H., and Marceau, N.,** Promotion of growth and differentiation of ductular oval cells in primary culture, *Cancer Res.*, 48, 368, 1988.
155. **McMahon, J. B., Richards, W. L., del Campo, A. A., Song, M. H., and Thorgeirsson, S. S.,** Differential effects of transforming growth factor-β on proliferation of normal and malignant rat liver epithelial cells in culture, *Cancer Res.*, 46, 4665, 1986.
156. **Schrenk, D., Eisenmann-Tappe, I., Gebhardt, R., Mayer, D., El Mouelhi, M., Röhrdanz, E., Münzel, P., and Bock, K. W.,** Drug metabolizing

enzyme activities in rat liver epithelial cells, hepatocytes and bile duct cells, *Biochem. Pharmacol.*, 41, 1751, 1991.

157. **Cowan, K. H., Batist, G., Tulpule, A., Sinha, B. K., and Myers, C. E.,** Similar biochemical changes associated with multidrug resistance in human breast cancer cells and carcinogen-induced resistance to xenobiotics in rats, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 9328, 1986.
158. **Akman, S. A., Forrest, G., Fong-Fong, C., Esworthy, R. C., and Doroshow, J. H.,** Antioxidant and xenobiotic-metabolizing enzyme gene expression in doxorubicin-resistant MCF-7 breast cancer cells, *Cancer Res.*, 50, 1397, 1990.
159. **Gessner, T., Vaughan, L. A., Beehler, B. C., Bartels, C. J., and Baker, R. M.,** Elevated pentose cycle and glucuronyl-transferase in daunorubicin-resistant P388 cells, *Cancer Res.*, 50, 3921, 1990.
160. **Peraino, C., Carnes, B. A., Stevens, F. J., Staffeldt, E. F., Russell, J. J., Prapuolenis, A., Blomquist, J. A., Vesselinovitch, S. D., and Maronpot, R. R.,** Comparative developmental and phenotypic properties of altered hepatocyte foci and hepatic tumors in rats, *Cancer Res.*, 48, 4171, 1988.
161. **Schwarz, M., Buchmann, A., Schulte, M., Pearson, D., and Kunz, W.,** Heterogeneity of enzyme-altered foci in rat liver, *Toxicol. Lett.*, 49, 297, 1989.
162. **Moore, M. A., Mayer, D., and Bannasch, P.,** The dose dependence and sequential appearance of putative preneoplastic populations induced in the rat liver by stop experiments with *N*-nitrosomorpholine, *Carcinogenesis*, 3, 1429, 1982.
163. **Campbell, H. A., Pitot, H. C., Potter, V. R., and Laishes, B. A.,** Application of quantitative stereology to evaluation of enzyme-altered foci in rat liver, *Cancer Res.*, 42, 465, 1982.