Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
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10 Sulphotransferases

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Abbreviations

AAF, 2-acetylaminofluorene
AST, aryl sulphotransferase
BR-STL, brain sulphotransferase-like protein
DCNP, 2,6-dichloro-4-nitrophenol
DHEA, dehydroepiandrosterone
DHEA-S, dehydroepiandrosterone sulphate
E_1, oestrone
E_2, 17β-oestradiol
E_3, 16α,17β-oestriol
EST, oestrogen sulphotransferase
1-HEP, 1-(α-hydroxyethyl)pyrene
1-HMP, 1-hydroxymethylpyrene
HST, hydroxysteroid sulphotransferase
OH-AAF, *N*-hydroxy-2-acetylaminofluorene
PAP, 3′,5′-diphosphoadenosine
PAPS, 3′-phosphoadenosine-5′-phosphosulphate
PCP, pentachlorophenol
PST, phenol sulphotransferase
rT_3, reverse (3,5,5′) triiodothyronine
SMP-2, senescence marker protein 2
SNP, single-nucleotide polymorphism
SULT, member of the SULT gene/enzyme superfamily
T_3, 3,3′,5-triiodothyronine
T_4, thyroxine

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Introduction

REACTIONS, TERMINOLOGY

Sulphotransferases (EC 2.8.2), in the older literature also termed sulphokinases, transfer the sulpho (SO_3^-) moiety from a donor, usually 3′-phosphoadenosine-5′-phosphosulphate (PAPS), to a nucleophilic group of the acceptor molecule (Figure 10.1). The sulpho group has a pK_a value of smaller than 1.5 in most structures. The introduced negative charge affects important properties of the acceptor molecule, such as the interaction with receptors and transport proteins (including albumin), the water solubility and the penetration of cell membranes. Since the sulpho moiety as well as the sulphate, sulphamate and thiosulphate groups (formed by sulphonation of O, N and S atoms, respectively) are electron-withdrawing, certain conjugates are chemically reactive. They may spontaneously react with nucleophiles (such as DNA or water) or undergo elimination reactions. Therefore, sulphotransferases can activate various protoxicants and can also catalyse the dehydration and isomerisation of certain substrates.

Since sulphotransferases transfer the sulpho moiety (not the sulphate group), we term the reaction sulphonation rather than sulphation. The latter designation may be tolerable if the sulpho moiety is transferred to an oxygen atom, but is misleading in all other cases. For the products, the established names will be used, even if these are not correct.

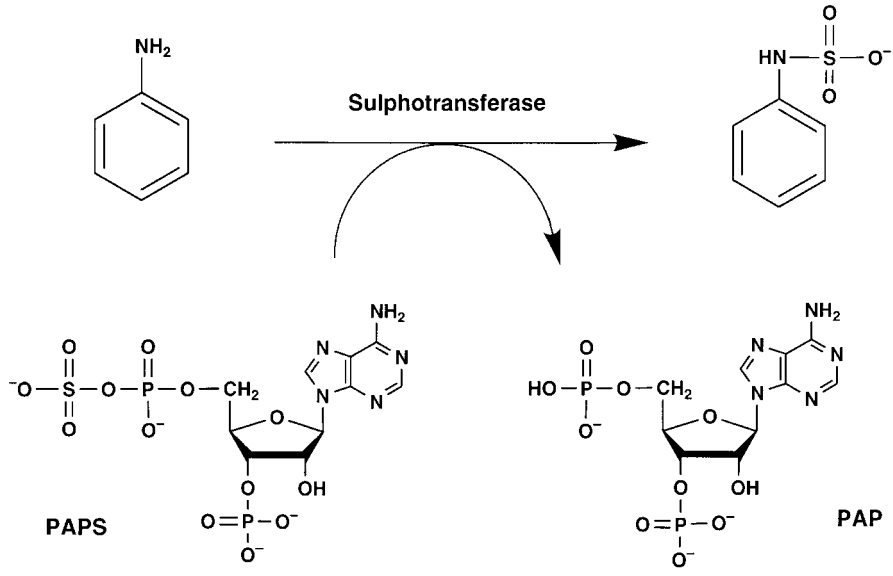

Figure 10.1 Scheme of the sulphotransferase-mediated sulpho transfer reaction shown for the substrate aniline. Enzyme, substrate and cofactor (PAPS) form a trimolecular complex. The sulpho group is directly transferred from PAPS to the substrate. In general, the reverse reaction is negligible.

The known sulphotransferases can be classified into three groups according to the principal acceptor molecules. Protein tyrosine sulphotransferases (Huttner 1987; Ouyang *et al.* 1998) and carbohydrate sulphotransferases (Bowman and Bertozzi 1999) sulphonate tyrosine residues of proteins and glycans attached to proteins and lipids, respectively. Since no xenobiotic-metabolising activities are known for these enzymes, only selected aspects are mentioned in the present review. The third group of enzymes sulphonates relatively small endogenous compounds (such as steroids and catecholamines) and xenobiotics. All enzymes of this group, investigated so far, belong to a common enzyme/gene superfamily. In the present review, the term SULT is used for any member of this superfamily, even if no sulphotransferase activity has been demonstrated so far, and the term sulphotransferase is used for any enzyme that transfers the sulpho group regardless of whether it belongs to the SULT superfamily. In particular, the SULT superfamily does not include the protein tyrosine sulphotransferases and carbohydrate sulphotransferases, as their structures and genetics are very distinct.

Various sulphoconjugates are substrates of sulphatases (also termed sulphohydrolases, EC 3.1.6). These enzymes transfer the sulpho group from the conjugate to water. They are presented in detail in Chapter 15 of this book.

HISTORY

In 1865, Baumann reported that urine of dogs, humans and horses contains sulphuric acid 'paired' with phenol, catechol and an indigo-forming compound (indoxyl) (Baumann 1865a,b). Heating in concentrated hydrochloric acid resulted in the hydrolysis of these conjugates to the phenols and inorganic sulphate. Application of phenol to the skin of a dog and feeding of catechol led to strong increases in the urinary levels of the corresponding sulphoconjugates (Baumann 1876). Half a century later, sulphoconjugates of the steroid hormones oestrone (E_1) (Schachter and Marrian 1938) and androsterone (Venning *et al.* 1942) were detected in mammalian urine. DeMeio and Tkacz (1950) were the first to demonstrate a sulphoconjugation in a subcellular preparation. Phenol was converted to phenyl sulphate in the presence of rat liver homogenate supplemented with α-ketoglutarate, adenylic acid and inorganic sulphate. Since all these components were necessary, it was concluded that oxidation reactions were needed to provide the energy for the conjugation reaction. Subsequently, this process could be subdivided into two steps, the activation of sulphate and the transfer of the sulpho group to the phenolic acceptor. Eventually, Robbins and Lipmann (1956, 1957) elucidated the structure of 'active sulphate', PAPS (Figure 10.1).

Phenol (or aryl) sulphotransferases (PSTs or ASTs) I, II and IV from rat liver were among the first sulphotransferases that were purified to apparent homogeneity (Sekura and Jakoby 1979, 1981). In particular, AST IV shows an extremely broad substrate tolerance. More than 100 substrates have been reported for this enzyme. In addition to many phenols, it sulphonates many other types of aryl compounds and even a few compounds that do not contain an aryl moiety, such as 2-nitropropane. AST IV is the sulphotransferase form that has been studied most thoroughly also in many other respects, such as kinetic parameters, mechanism of action and inhibition. However,

later it was found that AST IV, purified from male rat liver, comprised two distinct homodimers (most likely rSULT1A1 and rSULT1C1, see below) and the corresponding heterodimer and that these enzymes differ in their substrate specificity (Kiehlbauch *et al.* 1995). Since differing purification methods and enzyme sources have been used, the exact nature of the enzyme(s) investigated in various studies is elusive. This example illustrates two common situations: (a) The name of an individual xenobiotic-metabolising enzyme may not exactly reflect its function and substrate specificity. (b) A purified enzyme that appeared homogeneous in state-of-the-art analyses may demonstrate a relevant heterogeneity with the refinement of the analytical methods.

Chatterjee *et al.* (1987) were the first to clone the cDNA of a member of the SULT superfamily. This cDNA was reported to encode rat senescence marker protein 2 (SMP-2). This protein was found in the liver of senescent male rats, but was absent in young adult males. Its homology to sulphotransferases was detected only later, when the cDNAs of known sulphotransferases were cloned (Ogura *et al.* 1990b). SMP-2 is a member of the SULT2A subfamily, which encodes hydroxysteroid sulphotransferases (HSTs). Since SMP-2 was never studied for sulphotransferase activity, we wanted to perform such analyses using cDNA-expressed SMP-2. However, we failed to isolate the cDNA from the liver of senescent rats, but always ended up with the cDNA of ST-60 (another member of the SULT2A subfamily). Moreover, the cDNA sequences of rat SMP-2 and a mouse HST (STa-2) published in the GenBank (accession numbers J02643 and L27121, respectively) were gradually corrected and differ now only in one nucleotide of the available sequence of 970 nucleotides, including the entire coding region. Therefore, it is suspected that the SMP-2 cDNA of Chatterjee *et al.* (1987) has been cloned from a mouse rather than a rat cDNA bank or from a contaminated rat cDNA bank, and that the expressed SMP-2 protein (i.e. mouse STa-2) was so similar in its electrophoretic mobility and immunoreactivity to rat HSTs that the mistake was not noticed. It is probable that the true rat SMP-2 protein is one of the known rat HSTs, probably STa and/or ST-60 (described below). This account serves to remind us that cloning and sequencing errors were common in the pioneering period of that technology, although the error rate can be kept very low nowadays.

In a recent review, Nagata and Yamazoe (2000) have listed the cDNAs of 10 human and 31 other mammalian *SULT* genes. Many of these cDNAs have been expressed individually in bacteria or in mammalian cells, allowing the characterisation of precisely defined enzyme proteins. The comparison of their activities and levels with those in tissues of the corresponding species is very useful in the elucidation of the enzymic basis of sulphonation.

Elisabeth and James Miller (1966) pioneered the concept that most carcinogens are electrophiles or are metabolised to electrophiles. A sulphuric acid ester, *N*-sulphooxy-AAF, was the first electrophilic metabolite of a carcinogen to be discovered (DeBaun *et al.* 1968; King and Phillips 1968). Bioactivation via sulphonation is not taken into account in standard *in vitro* mutagenicity test systems, because the target cells used do not express sulphotransferases and externally generated reactive sulphuric acid esters do not readily penetrate into the cells. This problem can be solved by the expression of sulphotransferases in target cells of test systems. Initial results from such systems indicate that a substantial number of compounds can be activated to mutagens via metabolic sulphonation (Glatt 2000a).

SCOPE OF THIS REVIEW

This chapter focuses on the metabolic and toxicological aspects of the xenobiotic-metabolising sulphotransferases with special consideration of new molecular-biological findings. The following articles may be useful to the reader looking for more detailed information on other aspects of sulphonation. A comprehensive review written by Mulder and Jakoby (1990) presents in detail the sulphonation pharmacokinetics in intact human and animal organisms, isolated organs and cell cultures; studies on enzyme kinetics and enzymic mechanisms using subcellular preparations and purified enzymes are also presented in depth. Other recent reviews are focused on the genetics of *SULTs* (Weinshilboum *et al.* 1997; Nagata and Yamazoe 2000; Glatt and Meinl 2001), the enzymic properties of individual SULT forms (Falany 1997), steroid sulphotransferases (Hobkirk 1993; Strott 1996), the regulation of the expression of SULTs (Runge-Morris 1997), the cofactor supply of sulphotransferases (Klaassen and Boles 1997) and the sulphotransferase-mediated activation of carcinogens and mutagens (Miller and Surh 1994; Glatt 1997, 2000a). Purification procedures for sulphotransferases and characteristics of purified enzymes have been reviewed by Singer (1985). Furthermore, two volumes of the journal *Chemico-Biological Interactions* (Vol. 92, 1994; Vol. 109, 1998) are dedicated to the sulphotransferases. Older reviews on sulphoconjugation (Dodgson and Rose 1970; Roy 1971; DeMeio 1975) are not only of historical interest, but contain numerous remarkable and puzzling findings that now can be readily followed up using the actual molecular biology knowledge and techniques.

Functions and effects of sulphonation

FACILITATION OF EXCRETION

Numerous xenobiotics are excreted as sulpho- and glucuronic acid conjugates in the urine or faeces. The negative charge introduced with these moieties usually increases the water solubility and inhibits the passive penetration of cell membranes by the conjugates. The transmembrane transport of conjugates requires special mediators, e.g. the ATP-dependent multidrug-resistance proteins (Jedlitschky *et al.* 1996; Walle *et al.* 1999). In polar cells, such as the hepatocytes, the multidrug-resistance proteins are not uniformly distributed on the plasma membrane (König *et al.* 1999). Therefore, selective transport to the bile duct or the basolateral site (and from there to the blood) is possible. According to a traditional rule, small molecules are preferentially excreted in the urine, whereas large molecules prefer the biliary route. The critical size varies among species. In the rat, molecules with a mass of less than 325 ± 50 usually are not excreted into the bile (Hirom *et al.* 1972). The corresponding value in the human is 500 to 700. The molecular mass of sulphoconjugates is lower by 98 units than that of the corresponding glucuronides; often a higher percent of the sulphoconjugate than of the glucuronide is excreted in the urine (Mulder and Bleeker 1975; Møller and Sheikh 1982). However, the size-related rules are simplistic. A better knowledge of the distribution and substrate specificity of the various anion transmembrane transporters will help to better predict the excretion routes of individual metabolites. Probenecid, an inhibitor of the active tubular transport of organic anions, decreased the renal

clearance of certain sulphoconjugates, e.g. diflusinal sulphate (Macdonald *et al.* 1995) and *p*-hydroxytriamterene sulphate (Muirhead and Somogyi 1991).

Bile and urine are not the only excretion routes for conjugates. Enterocytes express transmembrane transporters on the luminal as well as the serosal surface and export conjugates in both directions (e.g. Sund and Lauterbach 1986, 1993; Walle *et al.* 1999). The human and the guinea pig, in contrast to the rat, express high levels of sulphotransferases in the intestinal mucosa (see below). Therefore, the direct excretion of sulphoconjugates into the gut lumen, without the loop via the liver, could be particularly important in the former species.

INACTIVATION OF BIOLOGICALLY ACTIVE MOLECULAR SPECIES

The predominant mechanism of pharmacological effects involves the non-covalent binding of the agent to specific receptors. The conjugation with the negatively charged sulpho group will abolish or drastically alter this interaction. Indeed, the sulphonated steroids do not show any relevant affinity to steroid receptors nor any hormonal activity as such (e.g. Hähnel *et al.* 1973). Likewise, catecholamine sulphates do not bind to adrenoreceptors (Lenz *et al.* 1991 and references cited therein) nor to dopamine receptors (Werle *et al.* 1988), and T_3 sulphate does not bind to the thyroid hormone receptor (Spaulding 1994). This metabolic inactivation of pharmacologically active species, complemented by the facilitation of the excretion, dominates the view of many pharmacologists on conjugation reactions so strongly that other important roles are often ignored.

FORMATION OF INACTIVE STORAGE AND TRANSPORT FORMS OF HORMONES

Dehydroepiandrosterone sulphate (DHEA-S) is the major circulating steroid in the human. In young adults, it reaches plasma levels of 10 μM, whereas unconjugated dehydroepiandrosterone (DHEA) is present at concentrations of about 10 nM (Baulieu 1996). Likewise, E_1 sulphate is the most abundant C18-steroid in human blood (Loriaux *et al.* 1971). Half-lives of several hours have been reported for DHEA-S and testosterone sulphate in human blood; their metabolic clearance rate was approximately 40 times lower than those of the respective free steroids (Wang *et al.* 1967). In rat and rabbit, this factor was only approximately 2. A high affinity to serum albumin (Puche and Nes 1962; Plager 1965) and the reabsorption from the renal tubules (Longcope 1995) are involved in the retention of steroid sulphates in the organism. Moreover, numerous tissues express steroid sulphatases (Chapter 15) that can release the free hormone from the conjugate (Martel *et al.* 1994; Dooley *et al.* 2000). Therefore, it is obvious that steroid sulphates are not metabolic end products destined only for excretion, but also serve as storage and transport forms of steroid hormones. DHEA-S and 16α-hydroxy-DHEA-S produced by the adrenal gland of the foetus are a major source of the maternal oestrogens during pregnancy in primates (Siiteri and MacDonald 1966; Parker *et al.* 1984; Kuss 1994); their conversion into the oestrogens occurs in the placenta. Manipulation of the activities of oestrogen sulphotransferase

and sulphatase is considered a very promising approach for altering the oestrogen levels in target tissues such as breast tumours (Pasqualini and Chetrite 1999).

The much higher plasma levels and longer half-lives of steroid sulphates in the human, in comparison with rodents, indicates that this storage function may vary substantially between species.

Regulation via sulphonation/desulphonation may not be restricted to steroids, since mammalian sulphatases also can hydrolyse the sulphoconjugates of iodothyronines (Santini *et al.* 1992; Spaulding 1994; Richard *et al.* 2001) and dopamine (Yoshizumi *et al.* 1992).

Vitamin D_3 3β-sulphate has been detected in human milk (Boulch *et al.* 1982). Treatment of vitamin D-depleted mother rats with vitamin D_3 3β-sulphate significantly improved the biochemical plasma parameters of pups (Cancela *et al.* 1987), but was inefficient in the mother rat (Cancela *et al.* 1985). Since administration of unconjugated vitamin D_3 to the mother rat was more effective even for the pup than administration of vitamin D_3 3β-sulphate, the role of the sulphoconjugation of vitamin D_3 is not understood.

Futile cycling between the sulphonated and deconjugated forms has been observed for some xenobiotics such as 4-methylumbelliferone (Kauffman *et al.* 1991; Ratna *et al.* 1993).

DIRECTION OF METABOLIC PROCESSING OF ENDOGENOUS COMPOUNDS

Sulphonation strongly accelerates the deiodination of thyroxine (T_4) and 3,3′,5-triiodothyronine (T_3) by the type I deiodinase (Visser 1994). Interestingly, T_4 sulphate is deiodinated exclusively in the inner ring to the sulphate of the hormonally inactive 3,5,5′-triiodothyronine (rT_3); the free prohormone T_4 is preferentially deiodinated in the outer ring, leading to the formation of the highly active hormone, T_3. Thus, sulphonation initiates the irreversible degradation of thyroid hormones by type I deiodinase. If type I deiodinase activity is low, as is the case in the foetus, T_3 sulphate may function as a reservoir from which active T_3 can be recovered by tissue sulphatase activity (Visser 1994).

In the same way, steroid sulphates do not require deconjugation in order to be further metabolised. They are substrates for various cytochromes P450 and dehydrogenases. For example, the following metabolic pathway has been established in the foetus: pregnenolone sulphate → 17-hydroxypregnenolone sulphate → DHEA-S → 16-hydroxy-DHEA-S (Pasqualini and Kinel 1985). Furthermore, testosterone sulphate can be converted directly to E_2 17-sulphate by human term placental microsomes (Satoh *et al.* 1992).

The loop sulphonation/further metabolism/desulphonation may lead to other products than the direct metabolism of the unconjugated species and avoids the formation of unconjugated intermediates.

BIOACTIVATION OF ENDOGENOUS MESSENGER MOLECULES

Not all biological effects of steroid hormones are mediated by the classical, intracellular steroid receptors. While sulphonation abolishes the interaction of steroids with steroid receptors, it appears to be important for other effects.

Various steroids, termed neurosteroids, are synthesised in the nervous system and exert a wide variety of diverse functions (Compagnone and Mellon 2000). Some of them are potent allosteric modulators of the γ-aminobutyric acid A ($GABA_A$) receptor (Paul and Purdy 1992; Park-Chung *et al.* 1999). Whereas various unconjugated steroids (including allopregnanolone and androsterone) are positive modulators, many steroid sulphates (such as DHEA-S and pregnenolone sulphate) negatively modulate the $GABA_A$ receptor. In addition, steroid sulphates can potentiate the activation of the *N*-methyl-D-aspartate (NMDA) receptor (Bowlby 1993) and are agonists of the $\sigma 1$ receptor (Noda *et al.* 2000; Zou *et al.* 2000). These molecular interactions of the steroid sulphates with receptors have been shown to lead to improved learning, enhanced memory performance, antidepressant effects, reduced conditioned-fear stress as well as to convulsions. Such effects were observed after the intracerebroventricular (Flood *et al.* 1995; Kokate *et al.* 1999) and subcutaneous (Reddy *et al.* 1998; Noda *et al.* 2000) administration of steroid sulphates to laboratory animals. Likewise, the administration of a steroid sulphatase inhibitor to rats for 15 days markedly increased the ratio of the plasma levels of DHEA-S to DHEA, and diminished a scopolamine-induced amnesia (Rhodes *et al.* 1997).

DHEA at pharmacological levels is a peroxisome proliferator in rats and mice; this effect appears to be mediated by DHEA-S rather than the unconjugated DHEA (Yamada *et al.* 1994; Peters *et al.* 1996). It has also been suggested that certain steroid sulphates, e.g. 2-hydroxy-E_2 17-sulphate, may act as antioxidants in the organism (Takanashi *et al.* 1995).

Cholesterol sulphate, formed in the differentiating keratinocytes, is essential for the keratinisation by activating the η isoform of protein kinase C (Kawabe *et al.* 1998). Also isoforms ε and ζ, and to a lower extent isoforms α and δ, are activated by cholesterol sulphate (Denning *et al.* 1995). Cholesterol sulphate is present at high levels in the acrosomes of spermatozoa and acts as a potent inhibitor of acrosin, a key proteolytic enzyme involved in the acrosome reaction; this inhibition appears to be reversed by sulphatases present in the female reproductive tract (Roberts 1987).

17,20β-Dihydroxy-4-pregnen-3-one 20-sulphate functions as a potent and specific olfactory stimulant with pheromonal actions in the goldfish (Sorensen *et al.* 1995).

Dopamine 4-sulphate demonstrated vasopressor activity in the peripheral and central nervous system, whereas dopamine 3-sulphate acted as a central depressor; these effects do not appear to be mediated via free dopamine formed by deconjugation (Minami *et al.* 1995a,b).

Various peptide hormones contain sulphonated tyrosine residues. In general, their sulphonation occurs by membrane-bound enzymes in the Golgi apparatus of the cells in which the peptides have been synthesised (Huttner 1987; Ouyang *et al.* 1998). However, there is evidence that some soluble enzymes are also capable of sulphonating certain peptide hormones. Hence, several peptides with an *N*-terminal tyrosine residue are substrates of rat AST IV (Sekura and Jakoby 1981). Furthermore, a soluble gastrin sulphotransferase has been detected in rat gastric mucosa, and sulphonated gastrin was a substrate of an aryl sulphatase (a commercial enzyme whose origin was not indicated) (Chen and Rosenquist 1990). Thus, reversible sulphonation may not only be used for the regulation of steroid and thyroid hormones, but also for the regulation of certain peptide hormones.

BIOACTIVATION OF PHARMACEUTICAL DRUGS

Minoxidil is an antihypertensive drug that also stimulates hair growth. In order to exert these effects, the *N*-oxide group of minoxidil has to be sulphonated (McCall *et al.* 1983; Buhl *et al.* 1990). The hypotensive effects of minoxidil were ablated in rats treated with the sulphotransferase inhibitor pentachlorophenol (PCP) and accentuated after sulphotransferase induction by the glucocorticoid triamcinolone acetonide (Duanmu *et al.* 2000). Minoxidil sulphate has the unique ability to non-enzymically sulphonate proteins, and it has been postulated that this mechanism is central to the pharmacological action of minoxidil (Groppi *et al.* 1990; Meisheri *et al.* 1991).

Triamterene, a potassium-sparing diuretic, is rapidly metabolised to *p*-sulphooxytriamterene (Mutschler *et al.* 1983). This sulphoconjugate is pharmacologically active and may be the primary mediator of diuretic and natriuretic effects observed after the administration of triamterene (Leilich *et al.* 1980). *p*-Sulphooxytriamterene as well as minoxidil sulphate are zwitterions and, therefore, are more lipophilic than their cationic parent drugs.

The salidiuretic effect of cicletanine is not caused by the parent drug but by its sulphoconjugate (Garay *et al.* 1991). In the rat, (+)-cicletanine was sulphonated to a fivefold higher extent than its antipode, and the resulting sulphoconjugate was three to four times more potent than that of (−)-cicletanine in stimulating the renal sodium excretion and in inhibiting the sodium-dependent Cl^-/HCO_3^- exchanger of erythrocytes (Garay *et al.* 1995).

The 3-sulphate and, in particular, the 6-sulphate of morphine are potent analgesics if injected intracerebroventricularly in mice (Brown *et al.* 1985). Under these conditions, morphine 6-sulphate is about 30 times more potent than unconjugated morphine. Morphine 6-sulphate also shows a high analgesic activity after subcutaneous and intravenous administration to rodents (Preechagoon *et al.* 1998). The analgesic activity of morphine 6-sulphate (and morphine 6-glucuronide) appears to be mediated by another isoform of the MOR-1 receptor (a μ receptor) than that of unconjugated morphine (Zuckerman *et al.* 1999). Codeine 6-sulphate also has analgesic activity in the mouse, but leads to seizures at doses below full analgesic activity (Zuckerman *et al.* 1999). Enkephalins, which are endogenous ligands of the opioid receptors, contain an *N*-terminal tyrosine residue that can be enzymically sulphonated (Sekura and Jakoby 1981). Structural similarities between this tyrosine residue and the hydroxybenzo moiety of morphine and codeine are obvious.

UNSTABLE SULPHOCONJUGATES

Spontaneous hydrolysis and displacement reactions of sulphoconjugates

Sulphoconjugates of strongly acidic phenols are unstable. The O–S bond is cleaved and the sulpho group is transferred to water. In some cases these conjugates can also be used as sulpho donors by sulphotransferases (see below).

Also, many benzylic and allylic sulphoconjugates are readily hydrolysed. However, in this case the C–O bond rather than the O–S bond is cleaved. For example, incubation of [^{18}O]-1-hydroxymethylpyrene (1-HMP) (Figure 10.4, compound **I**) with a rat HST and PAPS led to the rapid formation of [^{16}O]-1-HMP (Landsiedel 1998). The

intermediate formed, 1-sulphooxymethylpyrene, has been demonstrated, although it is short-lived in aqueous media ($t_{1/2} \sim 2.8$ min at 37°C). The heterolytic breakage of the C–O bond leads to the formation of a cation, which is stabilised by mesomerism. Such cations are strong electrophiles. In fact, 1-sulphooxymethylpyrene reacts in aqueous solution with nearly any nucleophile present (halogenide anions, thiols, amines, alcohols, amino acids) via an S_N1 mechanism (Landsiedel *et al.* 1996). Many of these displacement products are still reactive and eventually are converted to 1-HMP. In other cases, the displacement reactions of reactive sulphuric acid esters appear to follow an S_N2 mechanism. For example, the $t_{1/2}$ of 4-sulphooxycyclopenta[*def*]chrysene was strongly reduced in the presence of ethane thiolate (Landsiedel *et al.* 1996).

Acyl sulphates also are very unstable and alkylate the nucleophile 4-(*p*-nitrobenzyl)pyridine, but have not been reported as xenobiotic metabolites to date (van Breemen *et al.* 1985).

Sulphotransferase-mediated isomerisation reactions

If the benzylic/allylic hydroxyl group is attached to a chiral centre, its stereochemistry may be altered during a sulphonation/hydrolysis cycle. Thus, incubation of the individual enantiomers of 1-HEP with sulphotransferase *in vitro* led to their chiral inversion (Landsiedel *et al.* 1998). When an enantioselective sulphotransferase was used, such as human SULT1E1 (which has a 150-fold preference for (*S*)-1-HEP), a nearly complete chiral inversion was reached (reaction scheme in Figure 10.2).

Stiripentol is a chiral allylic alcohol that has been developed as an antiepileptic drug. After oral administration to rats, the (*R*)-enantiomer was extensively converted to its antipode, whereas little conversion was observed from the (*S*)-enantiomer (Zhang *et al.* 1994). This inversion was strongly reduced by PCP, an inhibitor of phenol sulphotransferases (see below), suggesting that the chiral inversion was mediated, at least in part, by enantioselective sulphonation. However, a final conclusion is not possible, since the inversion occurred only after oral but not after parenteral administration of the drug, and since a PAPS-dependent racemisation of (*R*)-stiripentol in hepatic cytosolic preparations was not observed (Zhang *et al.* 1994).

(*E*)-α-Hydroxytamoxifen was converted to its diastereomer, (*Z*)-α-hydroxytamoxifen, in the presence of a rat hydroxysteroid sulphotransferase (STa) and PAPS (Figure 10.4, compound **IV**) (Shibutani *et al.* 1998). It is probable that the Z/E-conversion occurred on the level of the carbo cation, when the double bond was less fixed due to mesomerism.

The mesomerism of benzylic and allylic carbenium ions and their nitrenium analogues not only may lead to *cis*/*trans*-isomerisations but also to positional isomerisations. For example, hydrolysis of 1′-sulphooxysafrole led not only to the formation of 1′-hydroxysafrole but also to 3′-hydroxyisosafrole (Figure 10.4, compounds **II** and **III**, respectively); likewise both alcohols were formed from 3′-sulphooxyisosafrole (Boberg *et al.* 1986). In the presence of rat liver cytosol, PAPS-dependent conversion of 3′-hydroxyisosafrole to 1′-hydroxysafrole was observed.

N-Sulphooxy-AAF, a reactive metabolite of the carcinogen AAF, has a half-life of approximately 4 s in aqueous solution at 37°C (Panda *et al.* 1989). The products formed include *N*-hydroxy-, 1-hydroxy-, 3-hydroxy-, 1-sulphooxy- and 3-sulphooxy-

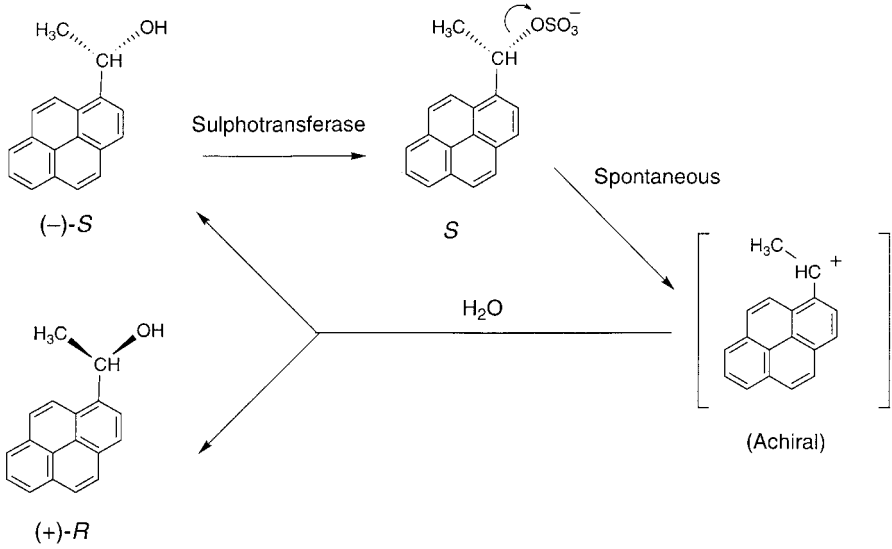

Figure 10.2 Sulphotransferase-mediated chiral inversion, shown for (-)-*S*-1-(α-hydroxyethyl) pyrene [(-)-*S*-1-HEP]. The sulphoconjugate of this substrate is unstable and hydrolyses via a carbonium intermediate to racemic 1-HEP. In the presence of a stereoselective sulphotransferase, such as human SULT1E1, a nearly complete inversion may occur after multiple cycles of sulphonation and hydrolysis (Landsiedel *et al.* 1998).

AAF (Panda *et al.* 1989; Smith *et al.* 1989; Kolanczyk *et al.* 1991; Novak and Rangappa 1992). Reaction of *N*-sulphooxy-AAF with glutathione yielded four conjugates which have been identified as 1-, 3-, 4- and 7-(glutathion-*S*-yl)-AAF (Beland *et al.* 1983). The formation of isomeric displacement products reflects the mesomeric properties of the compound. The isomerisation of *N*-sulphooxy-AAF to 1-sulphooxy- and 3-sulphooxy-AAF involves intramolecular substitution reactions.

Rat liver ASTs catalyse the Beckmann-like rearrangement of 9-fluorenone oxime to phenathridone (Mangold *et al.* 1986). While the sulphoconjugate of 9-fluorenone oxime was formed rapidly in the presence of ASTs, the subsequent conversion to phenathridone was slow and did not require the enzymes.

Sulphotransferase-mediated dehydration

The heterolytic cleavage of the sulphate group is not always associated with a substitution reaction (see above). Alternatively, a proton may be eliminated. In this case, the net effect of the sulphonation and the elimination reaction corresponds to a dehydration.

Cloning and sequencing of the cDNA of an insect retinol dehydratase indicated that it is a member of the SULT superfamily (Grün *et al.* 1996; Vakiani *et al.* 1998). Indeed, dehydration of retinol by this enzyme in subcellular systems required the presence of

PAPS, suggesting the reaction mechanism shown in Figure 10.3. The intermediate sulphate ester could not be demonstrated, probably because it is very short-lived.

Likewise, atropine (Figure 10.4, compound **V**) and scopolamine were dehydrated to apoatropine and aposcopolamine in the presence of guinea pig liver cytosol, ATP and sodium sulphate; the requirement of all three components strongly indicated that the reaction is mediated by a sulphotransferase (Wada *et al.* 1994). This notion was supported by the observation that DHEA, an alternate-substrate inhibitor of hydroxysteroid sulphotransferases, decreased the dehydration of atropine *in vitro* and *in vivo* (Wada *et al.* 1994). The dehydration of atropine and scopolamine is particularly remarkable, as these compounds are neither benzylic nor allylic alcohols. Their hydroxyl group is localised in the β-position of a side chain. The authors of that study

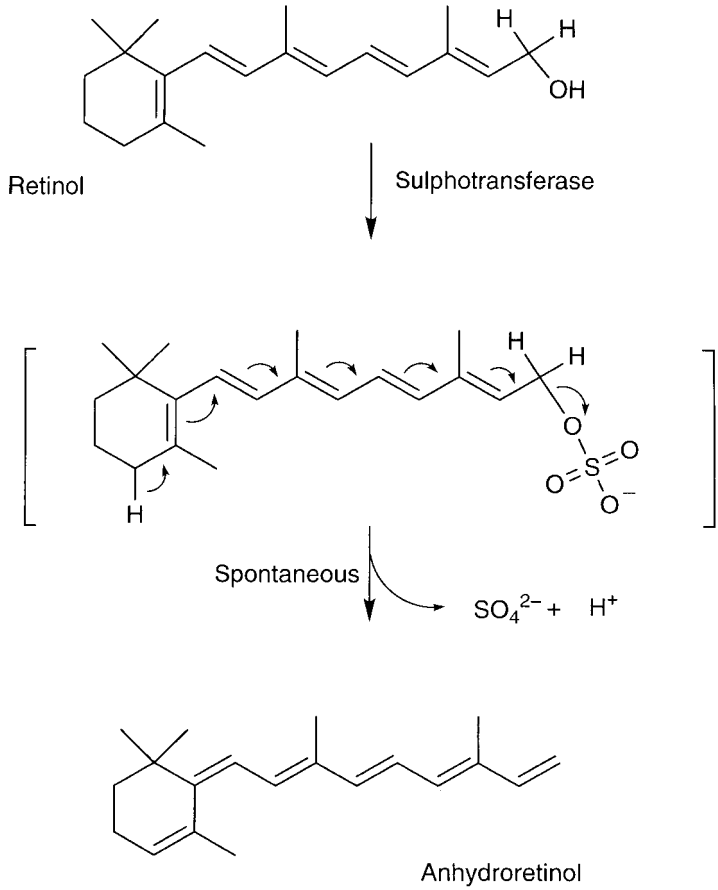

Figure 10.3 Sulphotransferase-mediated dehydration, shown for retinol. This reaction has been observed with an insect retinol dehydratase, which requires PAPS and is a member of the SULT superfamily (Vakiani *et al.* 1998).

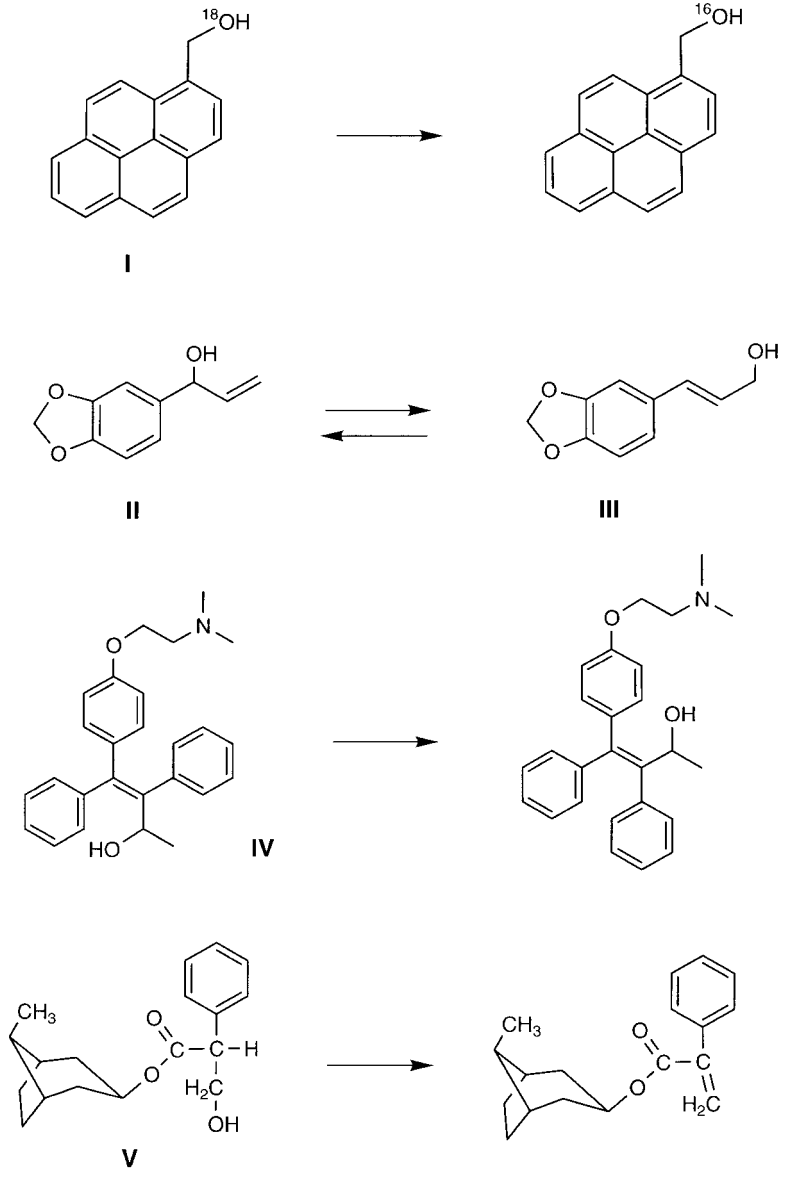

Figure 10.4 Sulphotransferase-mediated oxygen exchange in 1-hydroxymethylpyrene (1-HMP, **I**) (Landsiedel 1998), positional isomerisation of 1′-hydroxysafrole (**II**) and 3′-hydroxyisosafrole (**III**) (Boberg *et al.* 1986), *cis*/*trans* isomerisation of (*E*)-α-hydroxytamoxifen (**IV**) (Shibutani *et al.* 1998) and dehydration of atropine (**V**) (Wada *et al.* 1994).

suggested that the existence of a carboxyl group or a similar electron-attracting group at the benzylic position was pivotal to the dehydration reaction.

FORMATION OF REACTIVE SPECIES THAT COVALENTLY BIND TO MACROMOLECULES

As described above sulphate is a good leaving group in certain conjugates, leading to the formation of an electrophilic cation, which can react with water and numerous other nucleophiles. Besides small molecules, DNA and proteins are important nucleophilic reactants. *N*-Sulphooxy-AAF was the first electrophilic metabolite of a carcinogen to be discovered that reacted with cellular macromolecules (King and Phillips 1968; DeBaun *et al.* 1968). In the subsequent years, the research group of James and Elizabeth Miller demonstrated that various other carcinogens—including 4-aminoazobenzene, safrole, 2′,3′-estragole, and 6-hydroxymethylbenzo[*a*]pyrene—are also metabolised to reactive species via sulphonation (reviewed by Miller and Surh 1994). They showed that (a) the authentic sulphuric acid esters formed adducts with macromolecules, (b) the bioactivation of the precursors by subcellular preparations required the presence of PAPS, (c) inhibitors of sulphotransferases reduced the formation of macromolecular adducts *in vitro* and *in vivo* and (d) brachymorphic mice, which are genetically deficient in the synthesis of PAPS (see below), are resistant to the adduct formation and liver tumorigenicity by these compounds.

We extended these studies by expressing individual rodent and human SULTs in target cells of standard mutagenicity tests (Ames's *his*$^-$ *Salmonella typhimurium* strains and Chinese hamster V79 cells). These strains have been used for the detection of mutagens and the elucidation of activation pathways. Since we have reviewed this work recently (Glatt 1997, 2000a; Glatt *et al.* 2000), the findings are presented here only in a condensed form:

Using recombinant bacterial strains and mammalian cell lines, we have demonstrated SULT-dependent mutagenicity for approximately 100 compounds. Most of them belong to the following classes: benzylic alcohols, allylic alcohols, aromatic hydroxylamines, aromatic hydroxamic acids, secondary nitroalkanes and compounds that can be metabolised to any of these classes by other enzymes present in the test system.

For several compounds it was shown that activation within the target cell was important for the detection of a mutagenic effect; external activation using the same enzymes only led to a meagre effect in many cases.

Large differences in the substrate specificity towards these promutagens were observed between individual SULTs, e.g. between different forms from the same species, between orthologous enzymes of rat and human and between the allelic variants of human SULTs.

Nomenclature and substrate specificities of sulphotransferases

CLASSIFICATION SYSTEMS

After the metabolic sulphoconjugation was recognised as an enzymic reaction (DeMeio and Tkacz 1950), it was noticed that the activities towards different substrates

can differ in tissue distribution, sex dependence, ontogenetic development, pH optima, thermostability and sensitivity towards inhibitors. This strongly indicated the existence of multiple forms of sulphotransferases and required a specification of the investigated enzyme activities. The substrates were obvious parameters for a specification, even at a stage when little was known about the number and characteristics of the different enzyme forms. Therefore, many substrate-based names were used operationally. The creation of a name does not imply that a single enzyme form (or a group of similar enzymes) causes the sulphonation of the namesake nor that these enzymes do not sulphonate other substrates. In fact, it is usually difficult to judge the identity or non-identity of enzymes purified in different laboratories using different enzyme sources, purification procedures and/or substrates. Also the possible role of contamination is difficult to assess. Using precisely defined, cDNA-expressed enzymes, it is now evident that many SULTs have very broad, overlapping substrate specificities, that various sulphotransferases named after different substrates are identical and that sometimes the same name has been used by different authors for clearly distinct enzyme forms.

Moreover, many SULTs were detected from their cDNA and/or genomic DNA sequences; as yet, only minimal information is available on the substrate specificity of these forms. This would result in additional problems for a substrate-based nomenclature. On the other hand, the cloned nucleic acid sequences of xenobiotic-metabolising sulphotransferases demonstrated substantial similarities in the deduced amino acid sequences and in the gene structures of these forms (Yamazoe *et al.* 1994; Weinshilboum *et al.* 1997; Nagata and Yamazoe 2000). These findings strongly suggest that all these forms are derived from a common ancestral gene and thus form an enzyme/gene superfamily. The participants of the 3rd International Sulphation Workshop, held in Drymen (Scotland) in 1996, agreed to use SULT as the name of the superfamily. A systematic nomenclature, based on the structural/genetic similarity, analogous to that of the cytochromes P450 (Nelson *et al.* 1996), is in preparation but not yet finalised. Members of each family (indicated by the number after 'SULT') and subfamily (capital letter after subfamily number) show at least 45% and 60% amino acid sequence identity, respectively. Although not formally ratified, this nomenclature is already widely used, at least for human SULTs. The systematic designations as well as traditional names for the human SULTs are listed in Table 10.1. Orthologous enzymes from different species will receive the same systematic designation. If the species is not clear from the context, a prefix is used, such as h for human, r for rat, m for mouse, or gp for guinea pig. Moreover, in agreement with the practice for other gene families, only the first letter is usually capitalised for mouse and *Drosophila* forms, whereas all letters are capitalised for other species. Italics can be used to indicate genes, whereas names in Roman letters normally represent gene products (RNA, cDNA, and protein).

Some confusion may arise from the existence of an older, preliminary nomenclature proposed by Yamazoe and co-workers (Yamazoe *et al.* 1994; Nagata and Yamazoe 2000). In this nomenclature, ST is used as the abbreviation for the superfamily, and no attempts are made to identify orthologous forms. Each member of a subfamily receives a separate number in the order of the detection. It is important to realise this difference in the nomenclature. For example, ST1A3 is identical with human SULT1A1 (P-PST),

Table 10.1 Classification and characteristics of human SULTs[a]

Systematic name	Other names, characteristics properties, selected references
SULT1A1	Other names: phenol-sulphating phenol sulphotransferase (P-PST, P-PST-1), thermostable phenol sulphotransferase (TS PST, TS PST 1), hippocampus phenol sulphotransferase (H-PST) (refers to a specific transcript), ST1A3, human aryl sulphotransferase (HAST) 1 and 2 (indicating two transcripts that differ in the first, non-translated exon). State of knowledge: excellent compared to other hSULTs; the enzyme has been purified from various human tissues. Gene: located on 16p12.1-11.2; various SNPs, some of them involving an amino acid exchange; the most prominent polymorphism is in codon 213 (Arg/His exchange) (Raftogianis *et al.* 1999) RNA: two alternative first exons (in the non-coding region). Protein: dimer; the electrophoretic mobility of the subunit (apparent $M_r = 32$ kDa) differs from that of all other known hSULTs Substrates: numerous phenols (Campbell *et al.* 1987) and other aryl compounds, and also some small molecules that do not contain an aryl moiety; including drugs and drug metabolites (paracetamol, minoxidil, 4-hydroxytamoxifen) and many promutagens (Glatt 2000a); 4-nitrophenol at a low concentration (~ 4 μM) is often used to selectively detect 1A1 activity ($K_m \sim 1$ μM) in tissues that co-express the other major PST (1A3, $K_m \sim 2$ mM); 1A1 is more sensitive to inhibition by DCNP and more thermostable than 1A3 (however, smaller differences in thermostability are also found between different 1A1 alloenzymes) (Raftogianis *et al.* 1999). Tissue distribution: 1A1 is the dominating PST in liver; it is also expressed at lower levels in nearly all investigated extrahepatic tissues, including platelets, which have been used extensively for phenotyping (Weinshilboum 1990).
SULT1A2	Other names: phenol-sulphating phenol sulphotransferase 2 (P-PST-2), thermostable phenol sulphotransferase (TS PST 2), ST1A2, HAST4 and HAST4v (representing alloenzymes). State of knowledge: detected via its cDNA, only minute knowledge of function and distribution in the organism. Gene: located on 16p12.2 (close to *1A1* and *1A3*); various SNPs, some of them leading to an amino acid exchange (Raftogianis *et al.* 1999); the most prominent polymorphism involves codon 235 (Asn/Thr exchange) and usually is associated with polymorphisms in codon 7 (Ile/Thr) of 1A2 and in codon 213 (Arg/His) of the neighbouring *1A1* gene (Raftogianis *et al.* 1999; Engelke *et al.* 2000a). RNA: two alternative first exons (in the non-coding region); an incompletely spliced RNA has been detected in many tissues (Dooley *et al.* 2000). Protein: the wild-type alloenzymes of 1A1 and 1A2 differ in only six amino acid residues; the electrophoretic mobility of the subunit of 1A2 (apparent $M_r = 32.5$ kDa) differs from that of all other known hSULTs, including 1A1 (apparent $M_r = 32$ kDa). Substrates: 2-naphthol, minoxidil, 4 nitrophenol and 1-hydroxymethylpyrene are sulphonated by 1A2, although less efficiently than by 1A1; OH-AAF is much more efficiently activated by 1A2 than by any other hSULT; some aromatic hydroxylamines are also good substrates of 1A2 (Ozawa *et al.* 1994; Meinl *et al.* 2001a). Tissue distribution: 1A2 protein has not yet been demonstrated in any tissue, but is difficult to distinguish from 1A1 due to the high similarity (resolution in Western Blots is only possible if both forms are present at similar levels); 1A2

Table 10.1 (*continued*)

Systematic name	Other names, characteristics properties, selected references
	cDNA has been cloned from the liver; correctly spliced RNA has also been detected in the brain, lung, kidney and gut (Dooley *et al.* 2000).
SULT1A3	Other names: catecholamine-sulphating phenol sulphotransferase (M-PST), thermolabile phenol sulphotransferase (TL PST), human aryl sulphotransferase (HAST) 3 and 5 (indicating two transcripts that differ in the first, non-translated exon), placental oestrogen sulphotransferase (refers to a specific transcript; this name is misleading since E_1 and E_2 are only poor substrates of 1A3). State of knowledge: excellent compared to other hSULTs; the enzyme has been purified from various human tissues. Gene: located on 16p11.2. RNA: three alternative first exons (in the non-coding region). Protein: dimer; the electrophoretic mobility of the subunit ($M_r = 34$ kDa) is similar to that of 1C2 and 2A1; highly specific peptide antibodies detecting 1A3 are available (Rubin *et al.* 1996); 1A3 has been crystallised (Bidwell *et al.* 1999; Dajani *et al.* 1999b). Substrates: although numerous phenols are substrates for 1A1 and 1A3, their K_m values are often very different between these enzymes (Brix *et al.* 1999; Dajani *et al.* 1999b); 1A3 displays high affinity for monocyclic phenols containing hydrogen bond donors, e.g. an additional amino group (Dajani *et al.* 1999b) (as is the case with catecholamines); various β receptor agonists (Hartman *et al.* 1998), minoxidil, paracetamol and oxamniquine (which is activated to a mutagen) (Glatt 2000a) are among the drugs that are substrates of 1A3; moderate activity has been reported with E_1 by some research groups (Bernier *et al.* 1994; Suiko *et al.* 2000) but not by others; dopamine at a low concentration (~ 4 μM) is often used to selectively detect 1A3 activity ($K_m \sim 1$ μM) in tissues that co-express the other major PST (1A1, $K_m \sim 100$ μM). Tissue distribution: particularly high 1A3 protein and activity levels are found in jejunum and colon mucosa (Sundaram *et al.* 1989; Teubner *et al.* 1998; Eisenhofer *et al.* 1999); 1A3 is also expressed in many other tissues, including platelets, which have been used extensively for phenotyping (Weinshilboum 1990); expression is negligible in the liver (Heroux *et al.* 1989; Ozawa *et al.* 1998).
SULT1B1	Other names: thyroid hormone sulphotransferase (hST1B2), ST1B2. State of knowledge: detected via its cDNA; protein detected in various tissues. Gene: located on 4q13.1, close to *SULT1E1* and a *SULT1D* pseudogene (Meinl *et al.* 2001b); the sequence of Wang *et al.* (1998) encodes Glu in position 186, that of Fujita *et al.* (1997) encodes Gly in that position; we have sequenced the corresponding DNA region from 10 Chinese and 5 Caucasian subjects; all were homozygous for Glu (Meinl *et al.* 2001b). Protein: electrophoretic mobility of the subunit (apparent $M_r = 33$ kDa) similar to that of 1C1 and 4A1. Substrates: 4-nitrophenol, 1-naphthol and iodothyronines are normally used as substrates (Fujita *et al.* 1999; Wang *et al.* 1998); diethylstilboestrol; the promutagens 6-hydroxymethylbenzo[*a*]pyrene and 4-hydroxycyclopenta [*def*]chrysene are activated with high efficiency by this form (Glatt 2000a).

continued overleaf

Table 10.1 (*continued*)

Systematic name	Other names, characteristics properties, selected references
	Tissue distribution: the highest 1B1 protein levels have been detected in colon; 1B1 protein was also found in other parts of the gastrointestinal tract, liver and leukocytes (Teubner *et al.* 1998; Wang *et al.* 1998); high RNA levels were additionally detected in brain, ovary and kidney (Dooley *et al.* 2000).
SULT1C1	Other names: SULT1C sulphotransferase 1, ST1C2. The systematic name SULT1C1 may be changed to SULT1C4 because of the degree of the structural and functional similarities observed between various rodent and human SULT1C forms. State of knowledge: detected via genomic analysis and cDNA cloning (Her *et al.* 1997; Sakakibara *et al.* 1998a; Yoshinari *et al.* 1998a); only minute knowledge of function and distribution in the organism. Gene: located on 2q11.2 (Freimuth *et al.* 2000). RNA: An in-frame insert (leading to exon 3b instead of the regular exon 3a) has been found in some transcripts; however, no functional protein was produced from a cDNA containing exon 3b (Freimuth *et al.* 2000). Protein: the electrophoretic mobility of the subunit (apparent $M_r = 33.3$ kDa) is similar to that of 1B1, 1E1, 2A1 and 4A1 (33 to 33.5 kDa). Substrates: activity has been reported only with two substrates: 4-nitrophenol (requiring high substrate concentrations, 50 μM to 10 mM) and—in a single study (Yoshinari *et al.* 1998a)—with OH-AAF (50 μM); even with these substrates, the efficiency of 1C1 is very meagre compared to other hSULTs and rSULT1C1. For example, OH-AAF is not activated to a mutagen in *S. typhimurium* strains expressing 1C1, whereas 4 nM of OH-AAF was sufficient to double the number of revertants in the strain expressing 1A2 (Meinl *et al.* 2001a). Tissue distribution: 1C1 protein/activity has not yet been demonstrated in any tissue (the detection being complicated by the lack of specific antibodies and substrates); RNA has been detected in kidney, stomach, thyroid gland and foetal liver (Her *et al.* 1997), and also in ovary and some regions of the brain (Dooley *et al.* 2000).
SULT1C2	Other names: SULT1C sulphotransferase 2, ST1C3. State of knowledge: detected via genomic analysis and cDNA cloning (Sakakibara *et al.* 1998a); only minute knowledge of function and distribution in the organism. Gene: located on 2q11.2, close to *SULT1C1* (Freimuth *et al.* 2000) and other, not fully elucidated, *SULT1*C genes or pseudogenes. Protein: the electrophoretic mobility of the subunit (apparent $M_r = 34$ kDa) is similar to that of 1A3. Substrates: 4-nitrophenol, OH-AAF (Yoshinari *et al.* 1998a); E_1, bisphenol A, 4-octylphenol, 4-nonylphenol, diethylstilboestrol (Suiko *et al.* 2000); 1-hydroxymethylpyrene (Glatt *et al.* 2001); in all these studies, relatively high substrate concentrations were used. Tissue distribution: 1C2 protein/activity has not yet been demonstrated in any tissue (the detection being complicated by the lack of specific antibodies and substrates); the highest RNA levels were found in foetal lung and kidney; lower RNA levels were detected in foetal heart and in adult kidney, ovary and spinal cord (Sakakibara *et al.* 1998a).
SULT1E1	Other names: oestrogen sulphotransferase (EST, hEST-1), ST1E4.

Table 10.1 (*continued*)

Systematic name	Other names, characteristics properties, selected references
	State of knowledge: detected via its cDNA; subsequently, the protein was also purified from human liver (Forbes-Bamforth and Coughtrie 1994). Gene: 4q13.1 Protein: dimer; the electrophoretic mobility of the subunit (apparent $M_r = 33.5$ kDa) is similar to that of 1C1 and 2A1. Substrates: high affinity for E_1 and E_2 ($K_m = 5$ nM, Zhang *et al.* 1998); for the selective detection of 1E1-mediated activities, they have to be used at a low concentration (5 to 20 nM), which is the physiological range; at micromolar concentrations, several other hSULTs show substantial activity towards E_1 and E_2 (Falany 1997); high activity towards iodothyronines (Kester *et al.* 1999); activity also with pregnenolone, DHEA, diethylstilboestrol, 1-naphthol and naringenin (Falany 1997); very efficient, stereoselective activation of the promutagen (-)-1-(α-hydroxyethyl)pyrene (Hagen *et al.* 1998; Landsiedel *et al.* 1998). Tissue distribution: 1E1 protein was detected in liver (Forbes-Bamforth and Coughtrie 1994), endometrium (Falany *et al.* 1998), jejunum (Her *et al.* 1996) and mammary epithelial cells in primary culture (Falany and Falany 1996a); RNA was detected by Northern Blot analysis in adult adrenal gland, liver and small intestine and in foetal kidney, lung and liver (Her *et al.* 1996) and by reverse transcription/polymerase chain reaction analysis also in skin, brain, vagina (Dooley *et al.* 2000).
SULT2A1	Other names: dehydroepiandrosterone sulphotransferase (DHEA-ST) hydroxysteroid sulphotransferase (HST, HSST), alcohol/hydroxysteroid sulphotransferase (hST_a), ST2A3. State of knowledge: excellent compared to other hSULTs; the enzyme has been purified from liver and adrenal gland. Gene: located on 19q13.3; SNPs involving amino acid exchanges (Met57Thr, Glu186Val) have been observed (with frequencies of 0.027 and 0.038) (Wood *et al.* 1996); two other published sequences also involve amino acid exchanges, Thr90Ser (Comer *et al.* 1993) and Leu159Val (Kong *et al.* 1992), but have not yet been corroborated in other investigations. Protein: dimer; the electrophoretic mobility of the subunit (apparent $M_r = 33.5$ kDa) is similar to that of some hSULT1 forms; however, antibodies with high selectivity for SULT2 forms are available. Substrates: the highest activity among the steroids has been found with DHEA; pregnenolone, testosterone, cortisol, and E_2 (Comer and Falany 1992; Forbes *et al.* 1995), bile acids (Radominska *et al.* 1990), cholesterol (Aksoy *et al.* 1994); digoxigenin (Schmoldt *et al.* 1992); 3α-hydroxycyproterone acetate and various benzylic alcohols are activated to mutagens by 2A1 (Glatt 2000a); activity has also been observed with 4-nitrophenol (Fujita *et al.* 1999), minoxidil (Kudlacek *et al.* 1997), iodothyronines (Li and Anderson 1999), E_1 and various xenoestrogens (Suiko *et al.* 2000) and OH-AAF (Lewis *et al.* 2000)—however, these substrates are conjugated much more efficiently by some SULT1 enzymes. Tissue distribution: in the adult, 2A1 protein has been detected in liver, adrenal gland and jejunum (in this order) but is absent or extremely low in other tissues investigated (Comer and Falany 1992; Her *et al.* 1996); the same expression pattern was observed on the RNA level, with the exception that moderate 2A1 RNA levels were detected , in addition, in ovary (Luu-The *et al.* 1995; Dooley *et al.* 2000); very high levels of SULT2A1 protein and activity have been

continued overleaf

Table 10.1 (*continued*)

Systematic name	Other names, characteristics properties, selected references
	detected in the foetal adrenal gland (Boström and Wengle 1967; Barker *et al.* 1994; Forbes *et al.* 1995).
SULT2B1	Other names: hydroxysteroid sulphotransferase State of knowledge: detected via its cDNA; only minute knowledge of function and distribution in the organism. Gene: 19q13.3 (close to *SULT2A1*). RNA: two alternative first exons (that contain coding sequences in contrast to the alternative first exons of the *SULT1A* genes) (Her *et al.* 1998). Protein: SULT2B1a and SULT2B1b differ in their *N*-terminal sequence and their electrophoretic mobility (apparent M_r of subunits: 39 and 41 kDa, respectively). Substrate: the only known substrate is DHEA. Tissue distribution: 2B1a and 2B1b proteins have not yet been detected in tissues; RNA was detected primarily in placenta, prostate and trachea (Her *et al.* 1998). Using reverse transcription/polymerase chain reaction, Dooley *et al.* (2000) detected high levels of 2B1a RNA in skin and high levels of 2B1b RNA in vagina, oral mucosa, colorectal mucosa and prostate.
SULT4A1	Other names: brain sulphotransferase-like cDNA/protein (BR-STL), ST5A2 State of knowledge: recently detected via its cDNA (Falany *et al.* 2000), function not known Gene: 22q13.2-.31 Protein: the electrophoretic mobility of the subunit (apparent $M_r = 33$ kDa) is similar to that of 1B1 and 1C1; the amino acid sequence of human 4A1 shows an unusually high degree of identity (98%) with the orthologous rat and mouse SULTs (that have identical amino acid sequences but markedly differ in their nucleotide sequences). Substrates: unknown. Tissue distribution: significant levels of 4A1 protein and RNA have been detected only in the brain.

[a] Genetic polymorphisms in *SULT* genes are mentioned under the keyword 'gene'. The electrophoretic mobilities were determined under uniform conditions in our laboratory using cDNA-expressed enzyme from *S. typhimurium*. SULT1A1, 1A3, 1B1, 1E1 and 2A1 showed the same migration as the cDNA-expressed enzymes; no standards from tissues were available for the other forms.

but not with human SULT1A3 (M-PST); in Yamazoe's system, M-PST is designated as ST1A5. The different names of the human SULTs are included in Table 10.1.

The genetic nomenclature differs in several principal aspects from the substrate-based nomenclature. Several RNAs may be formed from the same gene by differential splicing. The existence of multiple first exons has been observed for several human *SULT* genes (reviewed by Nagata and Yamazoe 2000). In most cases, the variable first exon is not translated. However, two different translation products (termed SULT2B1a and SULT2B1b) are formed from the human *SULT2B1* gene, as its first exon is variable and contains a part of the coding region (Her *et al.* 1998). Moreover, translation products may associate to homo- and hetero-oligomers and undergo other post-translational modifications (see below). Therefore, several distinct proteins may be formed from the same gene. Conversely, a protein may comprise products of more

than one gene. Besides, sequence similarities between genes do not automatically imply functional similarity of the gene products. Although human BR-STL (brain sulphotransferase-like protein, SULT4A1) (Falany *et al.* 2000), rat BR-STL (Falany *et al.* 2000), rat SMP-2 (Chatterjee *et al.* 1987), and rat ST-60 (Watabe *et al.* 1994) represent members of the SULT superfamily, enzyme activities have not yet been reported for these forms. It remains to be investigated whether they act as sulphotransferases or have adopted other functions.

In the following sections, an overview of the different forms of sulphotransferases is given. Both classification systems will be used, as none of these systems is comprehensive. For purified sulphotransferases the substrate-based classification is primarily employed. With the genetic classification, substrate specificities were normally studied using cDNA-expressed enzymes. The focus will be on sulphotransferases of the rat (from which the largest number of enzymes have been purified) and the human as well as special forms from other species. Whereas all four purified human forms can be associated unambiguously with genetically defined forms, this is not yet possible for most enzymes of the rat and other laboratory animals. The human sulphotransferases are presented in detail in Table 10.1.

SUBSTRATE-BASED NAMES OF SULPHOTRANSFERASES

Phenol (aryl) sulphotransferases (PSTs, ASTs)

A large number of phenols are metabolically sulphonated. The tissue distribution of phenol sulphotransferase activity substantially differs from that of the various steroid sulphotransferases, as already reported by Boström and Wengle (1967). Such observations led to the postulation of special PSTs. As discussed above, ASTs I to IV were purified from rat liver. The substrate specificity of these forms, in particular AST IV, is very broad. More than 100 substrates of this enzyme have been reported in the literature. They comprise not only numerous phenols (Sekura and Jakoby 1981; Guo *et al.* 1994; Parker *et al.* 1994) but also various benzylic alcohols (Rao and Duffel 1991a; Glatt *et al.* 1995), aromatic amines (Ozawa *et al.* 1994; Guo *et al.* 1994; Marshall *et al.* 2000), aromatic hydroxylamines and hydroxamic acids (Duffel *et al.* 1992; Gilissen *et al.* 1992; King *et al.* 2000), aryl oximes (Mangold *et al.* 1993) and the *N*-oxide, minoxidil (Hirshey and Falany 1990). For this reason, the name AST is often used instead of PST. However, some alkyl alcohols, nitroalkanes and steroids are also substrates of AST IV, at least under certain experimental conditions (Sodum *et al.* 1994; Andrae *et al.* 1999; Marshall *et al.* 2000). AST IV corresponds to rSULT1A1 or, in some cases, a mixture of rSULT1A1 and rSULT1C1. The presence of additional forms in some preparations of AST IV purified from tissues cannot be ruled out. For AST I to III, the correlates in the sequence-based nomenclature are not yet known. One of them may be rSULT1B1. It is also possible that the cDNA of some of these forms has not yet been cloned.

In the human, two different PSTs could be distinguished and were eventually purified (see below). One of them prefers simple phenols (a property that has led to the designation 'P-PST'), is relatively thermostable ('TS PST') and is highly sensitive to inhibition by 2,6-dichloro-4-nitrophenol (DCNP, see below and Table 10.3); the other

form has a high affinity for catecholamines (phenols with a positively charged group in the molecule) ('M-PST'), is relatively thermolabile ('TL PST'), and is inhibited only at relatively high concentrations of DCNP. The systematic names of P-PST and M-PST are hSULT1A1 and hSULT1A3, respectively. Several other human SULTs (1A2, 1B1, 1C1, 1C2, 1E1; see Table 10.1), detected via their cDNA, are also capable of sulphonating various phenols.

Dopamine sulphotransferases

Singer *et al.* (1988) have chromatographically resolved two dopamine sulphotransferases from rat liver cytosol. Dopamine sulphotransferase II (representing the second eluting peak) comprised 79% and 68% of hepatic dopamine sulphotransferase activity in untreated male and female rats, respectively, and appears to be identical to AST IV and minoxidil sulphotransferase 2 (see below). The relationship of dopamine sulphotransferase I with other purified and cDNA-expressed forms is not known.

In the human, dopamine is a diagnostic substrate for M-PST (SULT1A3) if used at low substrate concentrations. Human M-PST shows an apparent K_m value for dopamine of ~1 μM (Dajani *et al.* 1999a), much lower than rat liver cytosol (22.4 μM) and rat dopamine sulphotransferase II (47.5 μM) (Singer *et al.* 1988).

Dopamine is a good substrate for mouse St1d1, a recently detected member of a novel SULT subfamily (see below).

6-Hydroxymelatonin sulphotransferases

Melatonin, the main pineal gland hormone, is excreted mainly as 6-sulphooxymelatonin. Singer *et al.* (1995) have chromatographically resolved two 6-hydroxymelatonin sulphotransferases from rat liver cytosol. 6-Hydroxymelatonin sulphotransferase II comprised 80–90% of the recovered activity in untreated rats of both sexes. Although it co-eluted with dopamine sulphotransferase II/AST IV, it is not clear whether it is identical to these enzymes, as it has not been purified to homogeneity. The relationship of 6-hydroxymelatonin sulphotransferase I with other purified and cDNA-expressed forms is not known.

Minoxidil sulphotransferases

A minoxidil sulphotransferase from rat liver cytosol has been purified to apparent homogeneity (Hirshey and Falany 1990) and its cDNA has been cloned (Hirshey *et al.* 1992). The coding region of this cDNA is identical to that of PST-1 (ST1A1, rSULT1A1) (Ozawa *et al.* 1990).

Singer (1994) chromatographically resolved two minoxidil sulphotransferases from rat liver cytosol. Minoxidil sulphotransferases 1 and 2 comprised nearly 30% and 70% of the recovered enzyme activity from columns, and appear to be identical to dopamine sulphotransferases I and II, respectively. The author suspects that minoxidil sulphotransferase 2/dopamine sulphotransferase II is identical to AST IV.

Several human SULTs (e.g. 1A1, 1A2, 1E1, 2A1) display activity towards minoxidil

(Falany and Kerl 1990; Ozawa *et al.* 1994; Kudlacek *et al.* 1997; Anderson *et al.* 1998).

Oestrogen sulphotransferases (ESTs)

ESTs sulphonate the 3-hydroxy group of the physiological oestrogens E_1, E_2 and E_3. In general, but not always, the name is reserved for forms that have a high affinity for these substrates (apparent $K_m \ll 1$ μM) and are members of the SULT1E subfamily.

Although the physiological oestrogens are phenols, ESTs can be considered as a separate subgroup of enzymes, since they differ from PSTs in several properties. For example, E_1 and phenol sulphotransferase activities show different tissue distributions in the human; this is the case in the foetus (Wengle 1966) as well as in the adult (Boström and Wengle 1967). Likewise, the male/female ratio of E_3 sulphotransferase activity in adult rat liver was 21.3, whereas the same figure for 1-naphthol was only 2.2 (Borthwick *et al.* 1993). This observation led to the purification of a male-specific EST from rat liver (Borthwick *et al.* 1993). The purified enzyme is markedly unstable and has not been studied extensively. Bovine EST was the first mammalian sulphotransferase whose cDNA was cloned (Nash *et al.* 1988), apart from SMP-2. Mouse EST was the first sulphotransferase to be crystallised and subjected to X-ray analysis (Kakuta *et al.* 1997). In the guinea pig, four forms of EST exist that differ in their isoelectric point but have the same amino acid sequence (see below). All of these ESTs are members of the subfamily SULT1E.

Additional sulphotransferases, belonging to other subfamilies (e.g. human SULT1A1, 1A3, 1C2 and 2A1, Table 10.1) are capable of sulphonating physiological oestrogens and, therefore, have also been termed ESTs sporadically. While they can show substantial sulphonating activities towards E_1 and in particular towards E_2, high substrate concentrations (6 to 20 μM) are required for maximal activity, whereas maximal activity of human SULT1E1 is reached at 20 nM of these substrates (Falany 1997). Since human SULT1A1, 1A3 and/or 2A1 are expressed at much higher levels than SULT1E1 in most human tissues, they may dominate the sulphoconjugation of oestrogens in tissue preparations and cell cultures when high substrate concentrations are used. Nevertheless, these sulphotransferases are usually classified as PSTs and hydroxysteroid sulphotransferases (HSTs) rather than ESTs.

Several synthetic oestrogens are poor substrates of ESTs and are more efficiently conjugated by PSTs (Table 10.1) (Falany 1997; Suiko *et al.* 2000). This difference could be important since sulphotransferases appear to regulate the local oestrogen levels and ESTs and PSTs differ in their tissue distribution and regulation; therefore, synthetic oestrogens may be unable to exactly mimic physiological oestrogens (Kotov *et al.* 1999).

Hydroxysteroid, DHEA, cortisol, bile acid, cholesterol and alcohol sulphotransferases

The term hydroxysteroid sulphotransferase (HST) is normally reserved for enzymes that sulphonate alcoholic steroids. Phenolic steroids are not considered to be characteristic substrates of HSTs, although E_2 is metabolised by some HSTs and has even been used

in one study for monitoring the fractions in the purification of an HST (Lyon and Jakoby 1980). Whereas ESTs appear to sulphonate exclusively the phenolic 3-hydroxyl group of E_2, sulphonation of the alcoholic (17β) as well as the phenolic hydroxyl groups has been observed with certain HSTs. DHEA is a particularly good substrate of various HSTs and therefore has been used in many studies. The substrate specificity of HSTs is not limited to steroids; other alcohols and sometimes even alkyl amines (see below) are also included. All HSTs whose nucleotide sequences have been determined are members of the SULT2A or 2B subfamilies.

The complexity of HSTs and SULT2 forms appears to be low in the human but high in the rat. Only one HST has been purified from human tissues (human DHEA sulphotransferase, SULT2A1) (Falany *et al.* 1989). Its substrates include DHEA, cortisol, cholesterol and bile acids (Table 10.1). It appears to be responsible for all, or most, of the sulphotransferase activity towards hydroxysteroids in the liver and adrenal glands. Two other human HSTs have been identified via their cDNAs (SULT2B1a and 2B1b, Table 10.1). The cDNA-expressed enzymes sulphonate DHEA whereas other steroidal substrates have not yet been studied. The corresponding RNAs have not been detected in liver but, for example, in placenta, prostate and skin (Table 10.1).

The HSTs in rat liver have been studied extensively by Singer (1985). His group has separated three glucocorticoid sulphotransferases from female rat liver using anion exchange chromatography. These enzymes catalysed the sulphoconjugation of the 21-OH group of cortisol and corticosterone. One of these forms (STI) was female-specific, preferred DHEA over any other steroid substrate investigated, and showed a molecular weight of 160 000. STII and STIII were detected in the liver of both sexes, but their levels were higher in females. With STII, as with STI, DHEA was the preferred sulpho acceptor. STIII was a 66-kDa protein, composed of two electrophoretically identical subunits; glucocorticoids rather than DHEA served as the preferential substrates. Singer also studied the conversion of deoxycorticosterone to its 21-sulphate in rat liver. In males, this activity appeared to be due to STIII. However, in females, about half of the activity eluted between STI and STII, indicating the existence of additional HST(s), which preferentially metabolise mineralocorticoids. Bile acid sulphotransferase activity co-eluted with STI from the anion exchange column but unlike STI was detected in both sexes, thus demonstrating the existence of a further HST form, implying a minimum of five HSTs in rat liver.

Although E_2 was associated with ESTs rather than HSTs in later studies, Lyon and Jakoby (1980) used that substrate and butanol for the purification of an HST. They separated three peaks of activity towards both substrates by anion exchange chromatography using female rat liver cytosol; only two of these peaks were found in males. The female-specific form, termed HST 1, was purified to apparent homogeneity. The purified enzyme sulphonated various primary and secondary alcohols (including ethanol, 1-butanol, 1-hexanol, vitamin A, ascorbic acid, ephedrine and chloramphenicol), various alcoholic steroids and E_2. However, it was inactive towards E_1, 2-naphthol and 2-naphthylamine. The same research group used DHEA as the substrate during the purification of a second form (HST 2) from female rat liver (Marcus *et al.* 1980). Purified HST 2 conjugated various alcoholic steroids and E_2, but was inactive towards E_1, 2-naphthol, OH-AAF, deoxycorticosterone and cholesterol.

Barnes *et al.* (1989) separated three peaks of sulphotransferase activity towards a

bile acid, glycolithocholic acid, by anion exchange chromatography using female rat liver cytosol. An apparently homogenous enzyme (BAST I) was purified from one of the peaks. Its substrates included DHEA, testosterone, cortisol and E_2 in addition to various bile acids. The authors suggested that BAST I is the same protein as HST 2 of Marcus *et al.* (1980).

Ogura *et al.* (1990a) studied the enzymes involved in the sulphoconjugation of the benzylic alcohol, 5-hydroxymethylchrysene. They separated three peaks of enzyme activity, all of which were associated with HST activity, from female rat liver by anion exchange chromatography. An apparently homogeneous enzyme, termed STa, was purified to apparent homogeneity from the major peak. Later, apparently homogeneous proteins, termed STb and STc, were also isolated from the other peaks (Watabe *et al.* 1994). All three proteins showed equal subunit size (30.5 kDa) and cross-reacted with an antibody raised against STa. The form STa was also purified by Czich *et al.* (1994), who used the activation of 1-HMP to a bacterial mutagen for monitoring the chromatographic fractions. In that study, STa contributed nearly 70% to the activation of this promutagen by hepatic cytosol of female rats.

Using an antibody raised against STa and synthetic oligonucleotide probes, several cDNAs were isolated from a female rat liver cDNA library (Ogura *et al.* 1989, 1990a; Watabe *et al.* 1994). They were termed ST-20, ST-21, ST-40, ST-41 and ST-60. The encoded subunits consist of 284 or 285 amino acid residues. ST-40 and ST-41 differ by three nucleotides in the coding region involving only one amino acid substitution. ST-20 and ST-21 differ by eight nucleotides in the coding sequence, leading to six amino acid substitutions. One of these amino acid exchanges is localised in the *N*-terminus. Takahashi *et al.* (1998) have separated sulphotransferases from rat liver, whose *N*-terminal sequences corresponded to those of ST-20 and ST-21. Therefore, the sequence differences between their cDNAs appear to be real and can hardly be attributed to a genetic polymorphism. Apart from these findings, the precise relationships between ST-40 and ST-41 and between ST-20 and ST-21 are not known. Otherwise, the amino acid sequence identity between ST-20/21, ST-40/41 and ST-60 varies from 83% to 91% The differences are spread over the entire sequences, indicating that separate genes encode these three forms. The *N*-terminal and two internal peptide fragments of STa were contained in the amino acid sequence deduced from the ST-40/41 cDNAs, but not in those of the ST-20/21 and ST-60 cDNAs. Thus, ST-40/41 appears to be the cDNA that encodes STa, as corroborated by comparison of chromatographic, electrophoretic and functional characteristics of natural STa and cDNA-expressed ST-40 or ST-41 (Watabe *et al.* 1994). The *N*-terminal 24 amino acid residues of BAST I of Barnes *et al.* (1989) show the highest similarity (two substitutions) with the deduced amino acid sequence of ST-40/41. In conclusion, the precise relationship among the various purified rat HSTs, among some rat HST cDNAs, as well as between purified enzymes and cDNAs is not clear with the exception that STa is encoded by ST-40/41.

Two distinct HSTs that demonstrate substrate specificity with respect to the orientation of the 3-hydroxyl group have been isolated from the guinea pig adrenal gland (Driscoll *et al.* 1993). One form has a strong preference for 3α-hydroxysteroids (such as allopregnenolone and androsterone); the other form displays a similarly strong preference for 3β-hydroxysteroids (such as pregnenolone, 17-hydroxypregnenolone and DHEA). The corresponding cDNAs have been cloned (Lee *et al.* 1994a; Luu *et al.*

1995; Dufort *et al.* 1996). A distinction between 3α-HSTs and 3β-HSTs has not yet been reported for any other species.

Amine sulphotransferases

Ramaswamy and Jakoby (1987) have purified from guinea pig liver a sulphotransferase that showed broad substrate specificity towards primary and secondary aryl and alkyl amines. Although highly purified, this enzyme also sulphonated phenols, hydroxysteroids and even E_1. Two sulphotransferases with high amine *N*-sulphonating activity were purified from male rabbit liver cytosols (Shiraga *et al.* 1999b). One of these forms, designated AST-RB1, efficiently catalysed the sulphonation of alicyclic, alkyl, and aryl amines, but showed negligible activities towards typical substrates of PSTs and HSTs (Yoshinari *et al.* 1998b; Shiraga *et al.* 1999b). The second form, AST-RB2, efficiently catalysed the sulphoconjugation of desipramine; it showed also high activity towards DHEA, but was inactive towards 1-naphthol (Yoshinari *et al.* 1998c; Shiraga *et al.* 1999b). Cloning of the cDNA of these forms indicated that AST-RB2 is a member of the SULT2 family; therefore, it was given an additional name, ST2A8 (Yoshinari *et al.* 1998c). AST-RB1 differed markedly in its amino acid sequence from all known SULTs and therefore was considered to be the first representative of a new family, which led to the designation ST3A1 (Yoshinari *et al.* 1998b). The sequence of an orthologous cDNA from the mouse has been deposited in the GenBank (AF026075). Members of this family have not yet been detected in any other species, nor are human or rat sulphotransferases known that show a high selectivity for amines as substrates. However, certain PSTs and HSTs of these species have *N*-sulphonating activities.

A rat enzyme that *N*-sulphonates 4-phenyl-1,2,3,6-tetrahydropyridine, *N*-deethanolated tiaramide and desipramine was purified to apparent homogeneity from female liver (Naritomi *et al.* 1994). It also showed high activity towards DHEA; its *N*-terminal amino acid sequence was very similar, but not identical, to those of known rat liver HSTs. Therefore, the purified enzyme appeared to be a new form of the HSTs. Rat SULT1A1 (AST IV), a typical PST, *N*-sulphonates the heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (Ozawa *et al.* 1995a).

In human liver, the *N*-sulphonation of various alicyclic amines is mediated primarily by SULT2A1, which represents the prime HST in that tissue (Shiraga *et al.* 1999a). The heterocyclic amine 1,2,3,4-tetraisoquinoline is metabolised to its sulphamate by cytosolic fractions of human liver, duodenal mucosa and colon mucosa (Pacifici *et al.* 1997b). When hepatic cytosols from different subjects were compared, the rate of conjugation of 1,2,3,4-tetraisoquinoline correlated with that of testosterone, which is a diagnostic substrate of SULT2A1. In the colon, which does not express SULT2A1 (nor any other testosterone sulphotransferase activity), the conjugation of 1,2,3,4-tetraisoquinoline correlated with that of 4-nitrophenol, suggesting the involvement of a PST; among the three sulphotransferases detected in that tissue (SULT1A1, 1A3, 1B1) (Teubner *et al.* 1998), SULT1A1 shows clearly the highest activity towards 4-nitrophenol and therefore is likely to mediate the sulphonation of 1,2,3,4-tetraisoquinoline. Desipramine is *N*-sulphonated in the cytosolic fraction of human liver, lung, kidney,

ileum, colon and platelets (Romiti *et al.* 1992). In platelets, only SULT1A1 and 1A3 appear to be present, suggesting that one of these forms has *N*-sulphonating activity towards desipramine. In human liver, SULT1A1 appears to be the major enzyme catalysing the sulphonation of 2-naphthylamine (Hernandez *et al.* 1991).

N-Hydroxylamine sulphotransferases (HASTs)

An *N*-hydroxy-2-acetylaminofluorene (OH-AAF) sulphotransferase was purified to apparent homogeneity from male rat liver by Wu and Straub (1976). This enzyme is expressed nearly exclusively in males. Using the same substrate, Gong *et al.* (1991) purified a male-dominant and a male-specific sulphotransferase from male rat liver to homogeneity. They were termed HAST-I and HAST-II, respectively. (Note that the research group of McManus uses the abbreviation HAST in a totally different sense, namely for human aryl sulphotransferase—see Table 10.1.) It is possible that HAST-I and/or II correspond to the OH-AAF sulphotransferase of Wu and Straub. All these purified forms also showed high PST activities but differed from the known rat PST forms (AST I, II, III, and IV). The cDNA of HAST-I was identified, cloned and designated as ST1C1 (Nagata *et al.* 1993).

It was previously thought that OH-AAF is a good substrate of AST IV (e.g. Gilissen *et al.* 1992). However, Kiehlbauch *et al.* (1995) demonstrated that an AST IV, purified from male rat liver, contains several distinct components; one component encoded by *rSULT1A1* catalysed the sulphoconjugation of phenols, whereas another component (most likely encoded by *rSULT1C1*) dominated the conjugation of OH–AAF. Nevertheless, AST IV from rat liver, whose homogeneity and identity with rSULT1A1 was corroborated by peptide analysis, efficiently sulphonated various unsubstituted and *N*-alkylated *N*-hydroxyanilines (King and Duffel 1997; King *et al.* 2000).

In the human, two SULT1C forms (1C1 and 1C2) have been detected. They show only very low activity towards OH-AAF and other aromatic hydroxylamines/amides (references in Table 10.1). hSULT1A2 and 1A1 are much more active against OH-AAF and 2-hydroxylamino-5-phenylpyridine than are hSULT1C1 and 1C2 (Meinl *et al.* 2001a).

Dopa/tyrosine and thyroid hormone sulphotransferases

Sakakibara *et al.* (1995) purified a sulphotransferase from rat liver using tyrosine as the substrate. The purified enzyme also metabolised L-dopa, D-dopa, thyroid hormones and 4-nitrophenol. The author called it dopa/tyrosine sulphotransferase. It is now classified in the SULT1B family (ST1B1 or rSULT1B1). This rat enzyme and its human orthologue (ST1B2 or hSULT1B1) have been referred to as thyroid hormone sulphotransferases by Fujita *et al.* (1997; 1999). The high activity of ST1B2 (hSULT1B1) towards thyroid hormones has also been recognised by Wang *et al.* (1998). However, several other SULTs sulphonate thyroid hormones (for human SULTs see Table 10.1). High activity has been observed in particular with human EST (SULT1E1) (Kester *et al.* 1999). Moreover, in the rat, expression of EST but not of SULT1B1 is under the control of thyroid hormones.

Interestingly, as will be discussed later, the human SULT1A3 changed its substrate

specificity and displayed high dopa/tyrosine sulphotransferase activity in the presence of Mn^{2+} ions.

Eicosanoids as substrates of sulphotransferases

Liu *et al.* (1999) have recently cloned the cDNA (clone 679153) of a mouse SULT that efficiently catalyses the sulphonation of various prostaglandins, thromboxane B_2, and leukotriene E_4. Dopamine, serotonin, 4-nitrophenol, 2-naphthol, 2-naphthylamine and minoxidil are other substrates of this enzyme, whereas none of the investigated steroids was sulphonated (Sakakibara *et al.* 1998a; Liu *et al.* 1999). It is a member of a novel SULT subfamily. Nagata and Yamazoe (2000) have given the name St1d1 to this form. The sequence of an orthologous rat cDNA has been deposited in the GenBank under the accession number U32372. A homologous human pseudogene is located in the *SULT1E1/SULT1B1* cluster (Meinl *et al.* 2001b).

Protein tyrosine, tyrosine ester and gastrin sulphotransferases

Various plasma membrane and secretory proteins as well as peptide hormones contain sulphonated tyrosine residues. This modification is usually caused by membrane-bound protein tyrosine sulphotransferases, which differ genetically from the SULT superfamily (Huttner 1987; Ouyang *et al.* 1998). Nevertheless, xenobiotic-metabolising sulphotransferases were also studied for such activities. Rat AST IV sulphonates tyrosine methyl and ethyl esters as well as some peptides containing an *N*-terminal tyrosine residue (enkephalins and cholecystokinin heptapeptide) (Sekura and Jakoby 1981); free tyrosine and peptides with only internal tyrosine residues (cholecystokinin octapeptide, gastrin, angiotensin 1 and 2) were ineffective as sulpho acceptors. Due to this activity, rat AST IV has also been designated as tyrosine ester sulphotransferase (Chen *et al.* 1992). The same name has been given to a recently detected form (GenBank accession number U32372, see above). A soluble gastrin sulphotransferase has been detected in rat gastric mucosa (Chen and Rosenquist 1990). It is not yet known whether it is a member of the SULT superfamily.

Carbohydrate sulphotransferases

Carbohydrate sulphotransferases (Bowman and Bertozzi 1999) sulphonate glycans attached to proteins and lipids. They are localised in the Golgi membranes and in the serum. They are not members of the SULT superfamily but form a separate superfamily (http://www.gene.ucl.ac.uk/nomenclature/genefamily/sulfotrans.shtml). No xenobiotic-metabolising activity has been detected for these enzymes.

Arylsulphate sulphotransferases

Arylsulphate sulphotransferases transfer the sulpho group from arylsulphates to phenolic acceptors without the requirement of PAP (see below). They have been found in intestinal bacteria (Kim *et al.* 1986; Baek *et al.* 1998; Kwon *et al.* 1999), but not in mammals. They show high activity towards numerous phenolic substrates (Koizumi

et al. 1991; Konishi-Imamura *et al.* 1991; Kim *et al.* 1992). An arylsulphate sulphotransferase has also been detected in *Aspergillus orizae* (Burns *et al.* 1977).

SEQUENCE-BASED NAMES OF SULPHOTRANSFERASES

The classification principals for the sequence-based names have already been discussed. A complete list of the human members of the SULT family, together with their substrates, is given in Table 10.1. Therefore, only a few special forms require comment here.

Sakakibara *et al.* (1998a) have searched through a murine-expressed sequence tag bank and subsequently cloned two new SULT cDNAs. One of them (clone 679153 ST) is a member of the SULT1 family, but not of the established subfamilies (SULT1A, 1B, 1C and 1E). Therefore, Nagata and Yamazoe (2000) have classified it into a separate subfamily (ST1D) of their classification system. Its substrate specificity has already been described. The sequence of an orthologous rat cDNA (ST1D2, thyrosine–ester SULT) has been published in GenBank (accession number U32372). We have detected a human pseudogene belonging to the same subfamily (Meinl *et al.* 2001b).

The ST3 family (in Yamazoe's nomenclature) contains only two members, the rabbit amine ST3A1 and a mouse sequence, SULT-X2 (GenBank accession number AF026075). No further information (e.g. concerning enzymic activity) has been published for the latter form.

QUATERNARY STRUCTURE AND POST-TRANSLATIONAL MODIFICATIONS OF XENOBIOTIC-METABOLISING SULPHOTRANSFERASES

Four SULTs have been purified from human tissues: SULT1A1 from brain (Whittemore *et al.* 1986) and liver (Falany *et al.* 1990; Otterness *et al.* 1992), SULT1A3 from brain (Whittemore *et al.* 1985) and platelets (Heroux and Roth 1988), SULT2A1 from liver (Falany *et al.* 1989) and adrenal gland (Comer and Falany 1992), and SULT1E1 from liver (Forbes-Bamforth and Coughtrie 1994). All purified forms were homodimers, as deduced from size-exclusion chromatography under non-denaturing conditions and electrophoresis of the denatured proteins. The quaternary structure of other human SULTs has not been studied.

Many sulphotransferases from other species also are homodimers. However, ESTs purified from rat liver (Borthwick *et al.* 1993), mouse placenta (Hobkirk *et al.* 1985) and porcine endometrium (Brooks *et al.* 1987) exist as monomers. Substitution of two amino acid residues (Pro269Thr and Glu270Val) converts cDNA-expressed mouse EST to a dimer; conversely, the exchange of a single amino acid (Val269 Glu) was sufficient to transmute the normally dimeric human EST (SULT1E1) to a monomer (Petrotchenko *et al.* 2001).

Several rodent HSTs, including rat HST 1 (Lyon and Jakoby 1980), rat HST 2 (Marcus *et al.* 1980) and rat BAST I (Barnes *et al.* 1989), have a high tendency to aggregate; the enzymes found in freshly prepared subcellular preparations appear to consist of at least four subunits. Kiehlbauch *et al.* (1995) demonstrated that an AST IV purified from male rat liver comprised two distinct homodimers (most likely rSULT1A1 and 1C1) and the corresponding heterodimer. It is not known whether any functional characteristics are affected by heterodimer formation.

No post-translational modification has been reported for any human SULT. However, an internal disulphide bridge is readily formed between Cys66 and Cys232 in rSULT1A1. The reduced and oxidised enzyme forms differ substantially in their pH optima and substrate specificities (Marshall *et al.* 1997). Furthermore, two distinct forms of rSULT1A1 (α and β) can be isolated from rat liver as well as from a recombinant *Escherichia coli* strain (Yang *et al.* 1996). Form α has one molecule of PAP tightly bound per enzyme dimer and can use certain phenolic sulphoconjugates (e.g. 4-nitrophenyl sulphate) but not PAPS as sulpho donors. Form β does not contain a tightly bound PAP; it can utilise PAPS as well as 4-nitrophenyl sulphate as sulpho donor. The use of 4-nitrophenyl sulphate by form β, but not by form α, requires the addition of PAP to the incubation mixture. However, it is clear that the β-PAP complex is different from the α form, most likely due to a slowly occurring change in conformation. The purified α and β forms have been interconverted into the other form by extended incubation with PAP-degrading enzymes and a high concentration of PAP, respectively.

Likewise, guinea pig EST, purified from adrenal gland (and also from recombinant CHO cells), exists in multiple forms which differ in the isoelectric point but appear to have the same primary amino acid sequence (Lee *et al.* 1994b). The isoformation is caused, at least in part, by the binding of PAP. An acidic form containing an endogenous PAP molecule showed a particularly high affinity for 17β-E_2 but no enzyme activity. It has been suggested that this form functions *in vivo* as an oestrogen-binding protein rather than an enzyme. This notion is supported by the observation that about two-thirds of the total oestrogen (E_2 and E_1) content of the cytosolic fraction of guinea pig adrenal gland is in an unconjugated form, although this tissue shows a very high EST activity (Lee *et al.* 1994b).

Sulpho transfer reactions and sulpho donors

REACTION SCHEME AND MECHANISM

All enzymic sulphoconjugations investigated in mammalian organisms involve the transfer of the sulpho moiety (SO_3^-) from the cofactor PAPS to a nucleophilic site of the substrate (A) (Figure 10.1) or, in the reverse reaction, from a donor (AS) to PAP. Both substrates must be present on the enzyme active site simultaneously to yield a ternary complex. In general, PAPS is the leading substrate and PAP is the following product in the forward reaction (Whittemore *et al.* 1985, 1986). However, a random order of binding (and leaving) has also been reported for some sulphotransferase forms, e.g. rat AST IV (Duffel and Jakoby 1981), human EST (SULT1E1) (Zhang *et al.* 1998) and human bile salt sulphotransferase (SULT2A1) (Chen and Segel 1985).

In agreement with this reaction mechanism, PAP is an inhibitor of many sulphotransferases (Table 10.2). It competes with PAPS. Sulphotransferases may be inhibited at high substrate concentrations, possibly via formation of the dead-end complex SULT-PAP-A. Marked substrate inhibition is commonly observed for substrates with a low K_m, such as 3-chlorophenol for rat AST I (Sekura and Jakoby 1979), 2-naphthol (Duffel and Jakoby 1981) and 4-nitrophenol (Hirshey and Falany 1990) for rSULT1A1

Table 10.2 K_m and K_i values of various sulphotransferases for PAPS and PAP, respectively[a]

Enzyme, enzyme source[b]	Sulpho acceptor	K_m (PAPS), μM	K_i (PAP), μM	References
hSULT2B1a, expressed in COS-1 cells (cytosol)	DHEA	0.0033		Her *et al.* (1998)
hSULT2A1 (DHEA sulphotransferase), expressed in COS-1 cells (cytosol)	DHEA	0.0067		Wood *et al.* (1996)
hSULT2A1, expressed in COS-1 cells (cytosol)	DHEA	0.01		Kudlacek *et al.* (1997)
hSULT2A1, expressed in COS-1 cells (cytosol)	T_3	0.12		Li and Anderson (1999)
hSULT2A1, expressed in COS-1 cells (cytosol)	Minoxidil	0.13		Kudlacek *et al.* (1997)
hSULT2A1 (bile salt sulphotransferase), purified from liver	Glycolithocholic acid	0.7	0.2	Chen and Segel (1985)
hSULT2A1 (DHEA sulphotransferase), purified from adrenal gland	DHEA	1.6		Comer and Falany (1992)
hSULT2A1 (HST), expressed in V79 cells (postmitochondrial supernatant)	DHEA	2.2		Forbes *et al.* (1995)
hSULT2A1 (ST2A3), expressed in *E. coli* (purified enzyme)	DHEA	4.81		Fujita *et al.* (1999)
hSULT1E1 (EST), expressed in *E. coli* (purified enzyme)	E_2	0.059	0.038[c]	Zhang *et al.* (1998)
5β-Scymnol sulphotransferase of *Heterodontus portusjacksoni* (partially purified enzyme)	5β-Scymnol	4.35	0.37	Pettigrew *et al.* (1998)
Rat AST I, purified from liver	2-Naphthol	6.5	0.89	Sekura and Jakoby (1979)
Rat AST II, purified from liver	2-Naphthol	12	1.2	Sekura and Jakoby (1979)
Rat HST 1, purified from liver	E_2	12	35	Lyon and Jakoby (1980)
Phenol (tyrosine) sulphotransferase of *Euglena gracilis* (purified enzyme)	Tyrosine	15	20	Saidha and Schiff (1994)
Rat AST IV (rSULT1A1), purified from liver	Tyrosine methyl ester	23	29 - 50[d]	Sekura and Jakoby (1981)
Rat HST 2, purified from liver	DHEA	47	14	Marcus *et al.* (1980)
Human liver cytosol (adult)	2-Naphthol	155		Pacifici *et al.* (1988)

[a] The K_m values presented are selected from nearly 100 values compiled from the literature. For hSULT2A1, figures from several studies are shown to illustrate the variation. In the other cases, data are preferentially presented from studies in which the K_i for PAP was also determined. In general, values represent apparent K_m and K_i.
[b] Name used by the authors and, for human enzymes, systematic name.
[c] K_m for reverse reaction.
[d] Under different conditions (pH).

(AST IV, minoxidil sulphotransferase) or E_2 for rat and human EST (Sugiyama *et al.* 1984; Zhang *et al.* 1998). In addition, allosteric inhibition of human EST by E_2 has been observed (Zhang *et al.* 1998). Therefore, it is important to vary the substrate concentration when new enzymes or substrates are investigated. Inhibition by the product AS rarely has been reported. DHEA-S (Lyon and Jakoby 1980) and glycolithocholic acid sulphate (Chen and Segel 1985) are relatively potent inhibitors of some HSTs. Furthermore, formation of the dead-end complex SULT-PAPS-AS has been observed for human EST and E_2 sulphate (Zhang *et al.* 1998).

The transfer of the sulpho moiety (SO_3^-) from the cofactor PAPS to most substrates is virtually irreversible. However, with a small number of acidic phenolic substrates [in particular, 4-nitrophenol ($pK_a = 7.2$), 2-chloro-4-nitrophenol and 3,5-dinitrophenol ($pK_a = 6.4$)], the reverse reaction (PAP + AS → PAPS + A) has been observed (Gregory and Lipmann 1957; Robbins and Lipmann 1957; Duffel and Jakoby 1981; Anhalt *et al.* 1982). Even in the case of 4-nitrophenyl sulphate and 3,5-dinitrophenyl sulphate, PAP and AS are thermodynamically strongly favoured over PAPS and A ($K_{app} = [AS] \times [PAP] \times [A]^{-1} \times [PAPS]^{-1} = 26.4$ and 4.1, respectively) (Gregory and Lipmann 1957; Robbins and Lipmann 1957). Therefore, the reverse reaction can occur *in vitro*, if only PAP and AS are added to the enzyme. It is likely that the reverse reaction is negligible *in vivo* due to the presence of PAPS and the normally very low levels of PAP.

A transfer of the sulpho moiety from one conjugate (e.g. 4-nitrophenyl sulphate) to a substrate (e.g. 2-naphthol) is possible (Sekura and Jakoby 1981; Yang *et al.* 1996; Marshall *et al.* 1997; Frame *et al.* 2000). However, this transfer is indirect and requires the presence of PAP. In some cases, PAP is tightly bound to the active centre of mammalian sulphotransferases; in these cases, the addition of external PAP is not required for enzyme activity.

In the absence of PAPS and PAP, rat AST IV catalysed the hydrolysis of 2-chloro-4-nitrophenyl sulphate, i.e. transferred its sulpho group to water (Duffel and Jakoby 1981). This reaction was inhibited in the presence of the sulphotransferase inhibitor PCP. Likewise, rat AST IV may hydrolyse PAPS in the absence of an other sulpho acceptor; this effect has been observed especially with the reduced form of the enzyme (Marshall *et al.* 2000) and under alkaline conditions (pH 8.0) (Lin and Yang 1998). However, such sulphatase activity of sulphotransferases has been observed only rarely and only under very special experimental conditions.

In bacteria, some sulphoconjugation reactions are not supported by PAPS, but require an arylsulphate as sulpho donor (Kim *et al.* 1994; Kwon *et al.* 1999). Studies with an arylsulphate sulphotransferase from *Eubacterium* A-44 demonstrated the formation of an intermediate in which the sulpho group is covalently bound to the enzyme (Kim *et al.* 1986). The sulpho acceptors in the enzyme are a tyrosine residue and probably, at other stages of the transfer reaction, a histidine residue. The sulpho group can then be transferred from the enzyme to a phenolic substrate. Thus, this bacterial aryl sulphotransferase operates via a ping-pong reaction mechanism.

PAPS, THE SULPHO DONOR

The sulpho donor PAPS was detected by Robbins and Lipmann (1956; 1957). It is a mixed anhydride of a phosphoric acid residue and sulphuric acid. Due to the high

potential for hydrolysis of this bond (dG° ~ −19 kcal/mol; cited in Zhang *et al.* 1998), PAPS has a high 'sulpho donor potential'. A similar situation is found in sulphoconjugates of acidic phenols (see above) and in sulphoconjugates of carboxylic acids (which apparently are not formed metabolically or are too unstable to be detected).

PAPS is normally present in rat liver at levels of 30–70 nmol/g tissue (Klaassen and Boles 1997); in some other studies somewhat higher values have been reported (e.g. 138 nmol/g tissue, Kim *et al.* 1995). In other tissues and species, lower levels (3.6 to 32.7 nmol/g tissue) have been detected (Brzeznicka *et al.* 1987; Klaassen and Boles 1997). Sulphonation is a high-affinity, low-capacity enzymic process in which the entire content of PAPS in the liver can be consumed in less than 1 min (Klaassen and Boles 1997). Therefore, the rate of PAPS synthesis can be pivotal for the rate of sulphonation reactions. Rates of biosynthesis of up to 100 nmol/min/g of liver have been observed (Pang *et al.* 1981). Nevertheless, compounds that are extensively sulphonated (e.g. paracetamol, salicylamide, phenol, 1-naphthol) can decrease tissue levels of PAPS and inorganic sulphate (Krijgsheld *et al.* 1981; Hjelle *et al.* 1985; Kim *et al.* 1995).

PAPS is formed in two steps from inorganic sulphate and ATP. In the first reaction, these substrates are converted by ATP sulphurylase (EC 2.7.7.4) to adenosine-5'-phosphosulphate (APS) and pyrophosphate. APS and ATP are then converted by APS kinase (EC 2.7.1.25) into PAPS and ADP. In the mammalian organism, ATP-sulphurylase and APS-kinase are fused to a common enzyme protein, termed PAPS synthetase (Lyle *et al.* 1995; Kurima *et al.* 1999). The apparent K_m for sulphate in the sulphonation of various substrates in isolated hepatocytes is 0.3 to 0.5 mM (Mulder and Jakoby 1990). Serum levels of inorganic sulphate in humans are approximately 0.3 mM and decrease after the administration of therapeutic doses of acetaminophen (paracetamol), a sulpho acceptor. In some cases, the availability of inorganic sulphate is limiting for the formation of PAPS and sulphoconjugation (Galinsky *et al.* 1979; Klaassen and Boles 1997). Structural analogues of sulphate (such as molybdate, chromate, perchlorate, and nitrate), adenosyl-trapping agents (ethionine), uncouplers of oxidative phosphorylation, inhibitors of the mitochondrial electron transport (menadione), some hepatotoxicants (cadmium) and some glutathione depletors (diethyl maleate) can decrease the rate of synthesis and the tissue levels of PAPS (reviewed by Klaassen and Boles 1997).

In the mouse and in the human, two different PAPS synthetases, encoded by different genes, have been detected (Kurima *et al.* 1998, 1999; Franzon *et al.* 1999; Xu *et al.* 2000). Brachymorphic mice have a genetic deficiency in the synthesis of PAPS, which leads to reduced PAPS levels and decreased rates of sulphonation of xenobiotics (e.g. 4-nitrophenol) *in vivo* (Lyman and Poland 1983). The deficiency is due to a missense mutation in PAPS synthetase 2; this mutation selectively destroys its APS kinase activity (Kurima *et al.* 1998). Various carcinogens, including OH-AAF (Lai *et al.* 1985), *N*-hydroxy-2-aminofluorene (Lai *et al.* 1987), 4-aminoazobenzene and *N,N*-dimethyl-4-aminoazobenzene (Delclos *et al.* 1984) and 1'-hydroxysafrole (Boberg *et al.* 1983) showed substantially reduced DNA adduct-forming and/or carcinogenic activities in brachymorphic mice.

Assays for sulphotransferase activities

COFACTOR SUPPLY

In early times, a cofactor-generating system (e.g. 10 mM $MgSO_4$ and 10 mM ATP) was often used if sulphotransferase activities were determined in subcellular preparations. Under these conditions, the amount of sulphoconjugate formed may depend not only on the level of the sulphotransferase but also on that of the PAPS synthetase. For precise studies of the sulphotransferase reaction, the cofactor PAPS should be added directly. A wide range of apparent K_m values for PAPS has been reported for different sulphotransferases (0.0033 to 155 μM) and even for a single form, as illustrated in Table 10.2 for human SULT2A1 (0.0067 to 4.81 μM). Commercial PAPS preparations often contain substantial levels of its hydrolysis product, PAP (e.g. Lin and Yang 1998). Hydrolysis also occurs during its storage in aqueous solution under inappropriate conditions (we therefore store our stock solutions at −80°C). PAP is a potent competitive inhibitor of all investigated sulphotransferases; often, the K_i value for PAP is lower than the K_m value of the corresponding enzyme for PAPS (Table 10.2).

If sulphoconjugation is studied in intact cells, tissue sections or perfused organs, addition of PAPS to the medium is not required (and is useless). However, inorganic sulphate (in mM concentrations) is required to sustain the endogenous synthesis of PAPS used as sulpho donor.

ASSAYS USING [^{35}S]-LABELLED PAPS OR INORGANIC SULPHATE

The use of [^{35}S]-PAPS as a sulpho donor has led to convenient and sensitive assays. In general, unused [^{35}S]-PAPS and [^{35}S]-SO_4^{2-} are precipitated after the incubation as barium salts. Most sulphoconjugates are not precipitated by barium, and therefore, the radioactivity remaining in the supernatant after centrifugation can be used to quantify the sulphoconjugate formed (Wengle 1964; Foldes and Meek 1973). A few points have to be considered to avoid pitfalls with this assay:

(1) Due to problems with the background radioactivity and for cost reasons, the PAPS concentration normally used in these assays (0.1 to 1 μM) is low compared to the K_m values for PAPS reported for some enzymes (Table 10.2) and with regard to the concentrations used in some other assays (up to 1.8 mM, e.g. Marcus *et al.* 1980).
(2) Although most sulphoconjugates are soluble in the presence of barium, others are precipitated to various degrees; specifically, sulphoconjugates containing carboxyl groups may form insoluble barium salts (Foldes and Meek 1973).
(3) In the standard version of this assay, the sulphoconjugate formed is not further analysed; in practice, the product determined may result from endogenous substrates present in the enzyme preparation (Spencer 1960) or impurities in the investigated compound (e.g. 2-naphthol in some commercial batches of 2-naphthylamine) rather than the putative substrate; therefore controls (enzyme plus [^{35}S]-PAPS, without substrate) are pivotal and analysis of the product may be required in some cases.

(4) The assay is further complicated when enzyme inhibitors that are alternate substrates are studied.
(5) Unstable sulphoconjugates may not be detected.

The use of [^{35}S]-PAPS—with or without barium precipitation—can be combined with other analytical procedures, such as thin-layer chromatography (Sekura and Jakoby 1979) or high-pressure liquid chromatography. Similar analytical procedures can be used in intact cell systems if [^{35}S]-SO_4^{2-} is used for the labelling of the products (Suiko *et al.* 2000).

ASSAYS USING RADIOLABELLED SULPHO ACCEPTORS

Most sulphoconjugates are much more water-soluble than the corresponding unconjugated molecules. This property has been exploited in partition assays using radiolabelled substrates, in particular steroid hormones (Singer 1985).

If reactive, unstable conjugates are formed, they may be detected by trapping with an appropriate nucleophile, such as methionine (Wu and Straub 1976), guanosine (Lai *et al.* 1985; Surh *et al.* 1991), DNA (Ozawa *et al.* 1994, 1995a; Chou *et al.* 1995a,b), RNA (King and Phillips 1968; Boberg *et al.* 1983; Fennell *et al.* 1985), or methionine agarose beads (Ringer *et al.* 1990). This assay has been used for studying the sulphonation of various radiolabelled procarcinogens. It allows the determination of relative activities, but not of absolute rates. Other reactions of reactive sulphoconjugates also have been exploited for the quantification of the sulphonation reaction. For example, *N*-sulphooxy-AAF has been converted to AAF in the presence of dithiothreitol (Yamazoe *et al.* 1987).

FORMATION OF PAP

Since the sulphotransferase reaction leads to the stoichiometric formation of sulphoconjugate and PAP, the quantification of the PAP formed may be used for monitoring the sulphoconjugation of any substrates, including those which form unstable conjugates (Duffel *et al.* 1989). The assay is primarily suited for purified enzymes because crude subcellular preparations often contain substantial PAP-degrading activity.

The availability of highly pure PAPS (free from PAP) is particularly important for the performance and sensitivity of this assay.

PHOTOMETRIC AND FLUORIMETRIC ASSAYS

4-Nitrophenyl sulphate and 4-nitrophenol, in the phenolate form ($pK_a \sim 7$), differ in their spectral absorption. This property has been exploited for the continuous measurement of sulphonation reactions (Yang *et al.* 1996).

In the 'physiological assay' (4-nitrophenol + PAPS → 4-nitrophenyl sulphate + PAP), the decrease in the absorption ($\lambda = 400$ nm) is monitored.

In the 'transfer assay', 4-nitrophenyl sulphate, in the presence of a small amount of PAP, is used as the sulpho donor for another substrate, usually 2-naphthol. The increase in absorption ($\lambda = 400$ nm) is monitored to assess the overall reaction:

4-nitrophenyl sulphate + 2-naphthol → 4-nitrophenol + 2-naphthyl sulphate. Frame *et al.* (2000) have developed a microtitre plate version of this assay.

Various phenolic sulphuric acid esters can be extracted as ion pairs with methylene blue into chloroform. Nose and Lipmann (1958) have used this property to estimate the amount of sulphoconjugates formed from the absorption of the co-extracted methylene blue. Although this assay is not particularly sensitive and accurate, it is simple and has been used, for example, for monitoring the fractions during the purification of enzymes (Sekura and Jakoby 1979, 1981).

In continuous fluorimetric assays, resorufin (Beckmann 1991) or 7-hydroxycoumarin (Leach *et al.* 1999) have been used as the substrate. Because the decrease in the fluorescence is recorded, low substrate concentrations (usually much below the K_m value) have to be employed.

DETECTION OF SULPHOCONJUGATES BY LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY

Reverse-phase high-pressure liquid chromatography coupled with negative-ion electrospray mass spectrometry is well suited for the identification and quantification of sulphoconjugates (Engst *et al.* 1997). $(M\text{-}H)^-$ can be monitored using the selected-ion recording technique. The selectivity and sensitivity can be increased by usage of the multiple-reaction-monitoring technique. Structural information about the sulphoconjugates studied can be obtained from the HSO_4^- (m/z = 97) and/or SO_3^- (m/z = 80) fragments produced by higher cone voltages or by collision-induced dissociation processes using tandem mass spectrometry. In particular, the HSO_4^- fragment is only formed from alcoholic sulphoconjugates, but not from phenolic sulphoconjugates due to the lack of an available hydrogen atom. This differentiation is useful for the identification of the site of sulphonation in substrates (e.g. E_2) that contain phenolic as well as alcoholic hydroxyl groups.

UNUSUAL ASSAYS

If the sulphoconjugates formed are unstable and hydrolyse immediately, special conditions have been used for measuring reaction rates, such as the chiral inversion of enantiomerically pure 1-(α-hydroxyethyl)pyrenes (1-HEP) (Landsiedel *et al.* 1998) (*cf.* Figure 10.2) or the conversion of $[^{18}O]$-1-HMP to $[^{16}O]$-1-HMP (*cf.* Figure 10.4, compound **I**) (Landsiedel 1998).

A bacterial mutagenicity assay, a modification of the Ames test, can detect extremely low sulphotransferase levels. Varying concentrations of rat liver cytosol, or fractions obtained during the purification of rat HSTs, were used as the activating system for a promutagenic substrate, 1-HMP (Czich *et al.* 1994; Glatt *et al.* 1994). The mutagenic response was linear over a more than 100-fold range of the amount of enzyme source used. For the detection of HST activity with the standard substrate, (radiolabelled) DHEA, a 100-fold amount of enzyme source was required over that used to detect a mutagenic response with 1-HMP.

Occurrence of sulphotransferases in different species

NON-MAMMALIAN SPECIES

PAPS-dependent sulphotransferases are known from diverse living creatures. The nodulation (Nod) H protein of the bacterium *Rhizobium meliloti* transfers the sulpho group from PAPS to the nodulation factor core structure (Schultze and Kondorosi 1998). However, sulpho transfer from PAPS to small acceptor molecules has so far been observed only in eukaryotic organisms. A sulphotransferase purified from the macroalgae *Porphyra yezoensis* uses dithiothreitol and other thiols as sulpho acceptors (Kanno *et al.* 1996). Several flavonol sulphotransferases with different regio specificities are known from *Flaveria* species, plants belonging to the family of Asteraceae (Marsolais and Varin 1998). An enzyme that catalyses the sulphonation of brassinosteroids and mammalian oestrogenic steroids has been found *in Brassica napus* (Rouleau *et al.* 1999). Choline sulphotransferases occur in the halophytic genus *Limonium* and other Plubaginaceae (Rivoal and Hanson 1994) and in some fungi (Orsi and Spencer 1964; Renosto and Segel 1977). A membrane-bound mitochondrial sulphotransferase that transfers the sulpho group of PAPS to L-tyrosine and other phenols has been purified from *Euglena gracilis* (Saidha and Schiff 1994). The filamentous fungus *Cunninghamella elegans* displays PST activities (Cerniglia *et al.* 1982; Zhang *et al.* 1996). In marine sponges and echinoderms, approximately 90 and 350 sulphonated compounds have been detected, respectively, but very little is known about their biosynthesis (Kornprobst *et al.* 1998). The retinol dehydratase of the insect *Spodoptera frugiperda* is a sulphotransferase (Vakiani *et al.* 1998). The schistosomacidal drugs hycanthone and oxamniquine require bioactivation by an enzyme, probably a sulphotransferase, of the parasite (Pica-Mattoccia *et al.* 1997). Sulphotransferases and/or sulphoconjugates have also been detected in cnidarians (Cormier *et al.* 1970), snails (Takimoto *et al.* 1987b), crustaceans (Elmamlouk and Gessner 1978; Sanborn and Malins 1980; Takimoto *et al.* 1987a; Swevers *et al.* 1991; Li and James 2000), onychophors (Jordan *et al.* 1970), insects (Khoo and Wong 1993; Ngah and Smith 1983), sea urchins (Malins and Roubal 1982), sharks (Cuevas *et al.* 1992; Pettigrew *et al.* 1998), bony fishes (Layiwola and Linnecar 1981; Layiwola *et al.* 1983; Watkins and Klaassen 1986; Kasokat *et al.* 1987; James *et al.* 1997; Coldham *et al.* 1998; Finnson and Eales 1998; Tong and James 2000), frogs (DeMeio 1945; Bridgwater and Ryan 1957; Beyer and Frank 1985; Frank and Beyer 1986; Gorge *et al.* 1987), reptiles (Smith 1968; Huf *et al.* 1987) and birds (Raud and Hobkirk 1968; Collett and Ungkitchanukit 1979; Dickstein *et al.* 1980; Gregus *et al.* 1983; Singer *et al.* 1985; Yang *et al.* 1986; Watkins and Klaassen 1986; Short *et al.* 1988).

DIFFERENCES IN SULPHOTRANSFERASES BETWEEN MAMMALIAN SPECIES

Numerous xenobiotics and their metabolites can be conjugated by UDP-glucuronosyltransferases as well as sulphotransferases. This functional redundancy may be one of the reasons for large species-dependent differences in the preferred conjugation reaction of a chemical.

The house cat and other felines appear to be unable to form glucuronides from various phenols and, therefore, excrete them chiefly as sulphoconjugates (Capel *et al.*

1974; Williams 1974; Mulder and Bleeker 1975; Caldwell 1982). The reverse situation is found in the pig and the opossum (Roy 1963; Capel *et al.* 1974; Williams 1974). These deficiencies do not affect all phenols to the same extent. For example, *in vivo*, substantial amounts of phenolphthalein are glucuronidated in the cat, and sulphonation is a significant elimination pathway of 1-naphthol in the pig.

Watkins and Klaassen (1986) have determined the sulphotransferase activity towards three substrates (2-naphthol, E_2, taurolithocholate) in liver cytosol of eleven species (rat, mouse, guinea pig, rabbit, cat, dog, quail, trout, cattle, sheep and pig). Each substrate was conjugated in each species. Particularly high and low activities (percentage of the respective activity in the rat) were observed with taurolithocholate in dog (10), rabbit (290) and sheep (300); with E_2 in trout (20), quail (320) and cat (620); and with 2-naphthol in trout (5), pig (10) and cattle (380).

Due to the existence of a large number of sulphotransferase forms and competing enzymes, it is difficult to predict the importance of the sulphonation in the metabolism of a compound in a specific species from the known structure–activity relationships, which are very limited, and/or from investigations using subcellular preparations. The situation is further complicated, as homologous sulphotransferases may strongly differ in their tissue distribution between species.

Distribution of sulphotransferases

TISSUE DISTRIBUTION

Methodological aspects

The expression of sulphotransferases can be studied on various levels, i.e. those of enzyme activity, enzyme protein (usually with an antibody) and RNA (Northern Blotting, dot blots, or reverse transcription/polymerase chain reaction). Each method and each probe have their own limitations in specificity and accuracy. Furthermore, a high level of a SULT RNA is not always associated with a high level of protein and enzyme activity. Since the available data are quite fragmentary, many findings about the tissue distribution of SULTs are preliminary.

Tissue distribution of human SULTs

All SULT forms investigated show tissue-selective expression. Data for the human forms are contained in Table 10.1. In this species, only SULT1A1 and 1A3 are found in many different tissues. SULT1A1 displays its highest expression in the liver, but it is also found at lower levels in most other tissues. Expression of SULT1A3 is particularly high in the gut, significant in most other tissues, but negligible in the liver. The high intestinal expression of SULT1A3 appears to reflect an enzymic 'gut–blood barrier' for detoxification of dietary biogenic amines and the delimiting effects of endogenous dopamine generated in the 'third catecholamine system' in the gut (nearly 50% of the dopamine production of the body) (Eisenhofer *et al.* 1999).

The other human SULTs show narrower tissue distributions and often are absent in the liver. The tissue distribution of certain SULTs changes during ontogeny. For example, the liver is the tissue with the highest DHEA sulphotransferase activity

(primarily encoded by SULT2A1) in the adult; only half of this activity is detected in adult adrenal gland (Boström and Wengle 1967; Comer and Falany 1992). However, the activity in foetal adrenal gland is approximately thirty- and eight-fold higher than that in foetal and adult liver, respectively (Wengle 1966; Boström and Wengle 1967). Particularly high levels of SULT2A1 RNA have been detected in the foetal zone of the foetal human adrenal gland (Parker 1999). Other changes in the tissue distribution during ontogeny will be discussed later.

Tissue distribution of rat SULTs

The tissue distribution of SULTs in the rat strongly differs from that in the human and other species. In the rat, the expression of many SULTs is conspicuously focused on the liver (e.g. DeBaun *et al.* 1970; Wong and Yeo 1982; Glatt *et al.* 1990; Singer *et al.* 1995; Araki *et al.* 1997; Takahashi *et al.* 1998; Dunn II and Klaassen 1998). However, significant levels of minoxidil sulphotransferase (AST IV) have been detected in the outer root sheath of rat hair follicles (Dooley *et al.* 1991). Only recently, two rat SULT forms (SULT1C2 and 1C2A) were detected whose RNA is abundant in the kidney, at moderate levels in the stomach, very low in the liver and not detectable in the other investigated tissues, including intestine, brain and lung (Xiangrong *et al.* 2000).

It is important to realise that the enzyme level is not always the limiting factor for the sulphonation reactions occurring in the organism. For example, Mulder *et al.* (1984) studied the elimination of harmol in the rat and observed that its clearance rate via sulphonation substantially exceeded the hepatic blood flow rate, implying an important role of extrahepatic sulphonation. Likewise, phenol, at low levels, appears to be conjugated primarily in the lung, rather than in the liver, possibly because the perfusion rate of the lung is higher (nearly four-fold) than that of the liver (Cassidy and Houston 1980). Thus, even in the rat, the low extrahepatic levels of sulphotransferases can be sufficient for a substantial contribution to the sulphonation of certain substrates.

Distribution of SULTs in other species

Mouse olfactory mucosa shows higher sulphotransferase activity towards various phenolic odorants than hepatic tissue of this species (Tamura *et al.* 1997). At least part of this activity is mediated by mSult1c1, a form specifically expressed in the olfactory mucosa; no expression of this form was detected in any other tissue examined (liver, kidney, intestine, spleen, lung, brain, heart) (Tamura *et al.* 1998).

In the mouse, E_1 sulphotransferase activity is highest in the testis, followed by placenta and uterus, and very low in liver and adrenal gland (Hobkirk and Glasier 1992). However, this pattern may be changed in pathological states. For example, mouse EST RNA is dramatically induced in the livers of obese and diabetic C57BL/KsJ *db/db* mice, but its expression is unaffected in the testis of these animals (Song *et al.* 1995). In the guinea pig, the tissue distribution of E_1 sulphotransferase activity is very different from that in the mouse (Hobkirk and Glasier 1992). Highest levels are found in the adrenal gland and midgestational chorion, whereas activities are much lower in the liver and negligible in the gonads.

Substantial species differences in the tissue distribution of sulphotransferase activities were also observed with other substrates. Wong and Yeo (1982) studied the sulphonation of harmol and isoprenaline in cytosol preparations of four tissues (liver, kidney, small intestine, lung) from six species. In the rat and rabbit, both substrates were conjugated only in the liver. The dog showed activities in all tissues, with the highest levels occurring in liver and kidney. In the other species, the highest activities were detected in extrahepatic tissues: lung (monkey), kidney (mouse) and small intestine (guinea pig). Likewise, Schwenk and Locher (1985) observed higher 1-naphthol sulphotransferase activity in jejunal, ileal and colon cells than in hepatocytes of the guinea pig.

Thus, it appears that each SULT form shows a unique tissue distribution, and that orthologous SULT forms (as well as the activity towards a specific substrate) may fundamentally differ in their tissue distribution between species.

CELLULAR DISTRIBUTION

Sulphotransferases are not uniformly distributed among the different cell types of a tissue. For example, the levels of 1-HMP sulphotransferase activity in parenchymal, endothelial, and Kupffer cells of rat liver had a ratio of 216:40:1, respectively (Glatt *et al.* 1990). These differences are toxicologically important, as 1-HMP is activated to a DNA adduct-forming species by sulphotransferase; adduct levels in the different liver cell types of 1-HMP-treated rats were directly proportional to their respective 1-HMP sulphotransferase activities (Monnerjahn *et al.* 1993). Furthermore, sulphotransferases are not even distributed uniformly among the parenchymal liver cells, as demonstrated by immunohistochemical analysis of rat liver (Chen *et al.* 1995). In both sexes, centrilobular hepatocytes contained a higher level of AST IV than midzonal cells; even lower levels of AST IV were present in periportal cells (Chen *et al.* 1995). Rat STa (or sulphotransferases that cross-react with the antibody raised against STa) exhibited the opposite distribution. Similar results were obtained by Homma *et al.* (1997), who separated periportal and perivenous hepatocytes using the dual digitonin perfusion technique and then investigated enzyme activities in cytosol preparations of the harvested cells. Sulphotransferase activity towards 2-naphthol (a substrate of AST IV and other SULT1 forms) was approximately 1.5-fold higher in perivenous hepatocytes than in periportal cells. The activities towards DHEA and cortisol (substrates of STa and other SULT2 forms) were 1.6- to 5-fold higher in the periportal cells. However, chromatofocusing of cytosol of perivenous and periportal hepatocytes separated 2-naphthol sulphotransferase activity into three major fractions, which varied in their distribution between perivenous and periportal cells.

In adult male rat liver, only androgen-responsive hepatocytes located around the central vein contain immunoreactive EST protein and the corresponding RNA (Mancini *et al.* 1992).

Immunohistochemical analysis of human colon and ileum demonstrated high levels of SULT1A and 1B proteins in the differentiated enterocytes and negligible levels in the crypts, where the stem cells are localised (W. Teubner, M. Kretzschmar, C. N. Falany and H. R. Glatt, manuscript in preparation).

Antibodies raised against human SULT1A1 (P-PST) strongly stained neurones in

human hippocampus and thalamus, whereas they showed a weak reaction or none at all with the neighbouring glial cells (Zou *et al.* 1990).

SUBCELLULAR LOCALISATION

Sulphotransferase activities towards xenobiotics nearly always were associated with the soluble fraction of tissue and cell homogenates. However, Fernando *et al.* (1993) reported on the presence of a PST activity (towards 1-naphthol, 2-naphthol, 3-nitrophenol and 4-nitrophenol) in microsomal preparations of bovine liver.

Immunohistochemical analyses usually indicate that the sulphotransferases are present primarily in the cytoplasm. However, high levels of EST protein have been detected in cell nuclei of the adrenal cortex of guinea pigs (Whitnall *et al.* 1993) and in a small number of hepatocytes in rats (Mancini *et al.* 1992). In rat brain, a higher specific DHEA sulphotransferase activity was observed in the nuclei fraction than in the cytosol fraction; however, the absolute level of these activities was very low (Rajkowski *et al.* 1997). Besides, rat SULT1C2 and 1C2A appear to be present primarily in the lysosomes in gastric mucosa as well as in transiently transfected baby hamster kidney (BHK) cells (Xiangrong *et al.* 2000).

Tyrosine protein sulphotransferases are localised in the Golgi membranes (Huttner 1987; Ouyang *et al.* 1998). Likewise, the Golgi membranes are the primary location of the carbohydrate sulphotransferases (Bowman and Bertozzi 1999). However, some of them have also been detected in serum (Huynh *et al.* 1999; Nadanaka *et al.* 1999).

Regulation of sulphotransferase expression

ONTOGENETIC DEVELOPMENT, AGE- AND SEX-DEPENDENCE

Human

High levels of sulphotransferases have been detected in various foetal tissues. HST activity and protein level in the adrenal gland (Wengle 1966; Barker *et al.* 1994), HST activity in the kidney (Sharp *et al.* 1993), PST and HST protein levels in the lung (Hume *et al.* 1996) and 2-naphthol sulphotransferase activity in lung, kidney and gut (Pacifici *et al.* 1988) are much higher in the foetus than in the adult. Ritodrine sulphotransferase activity in various tissues is also higher in the foetus than in the adult (Pacifici *et al.* 1993b). SULT1C2 RNA was detected in the lung and heart of the foetus but not of the adult; likewise, its level was higher in foetal than in adult kidney (Sakakibara *et al.* 1998b). Approximately two-fold higher platelet 4-nitrophenol and dopamine sulphotransferase activities were observed in newborns than in adults (Pacifici and Marchi 1993).

The situation is somewhat different in the liver. Whereas sulphotransferase activities towards numerous substrates have been detected in foetal liver, these were usually lower than the corresponding activities in the adult (Wengle 1966; Boström and Wengle 1967; Pacifici *et al.* 1988; Barker *et al.* 1994). However, the hepatic rates of the sulphonation of dopamine and ritodrine (β_2 agonist), which are mediated by SULT1A3, are higher in the foetus than in the adult (Cappiello *et al.* 1991; Pacifici *et al.* 1993b). In contrast, hepatic glucuronidation of ritodrine is hardly developed at

the midgestational stage. In newborns, the ratio of urinary sulpho- and glucuronic acid conjugates of ritodrine was markedly higher than in the mothers (Brashear *et al.* 1988).

Age-dependent expression of human sulphotransferases has not been observed in a large number of studies, apart from the foetal and early postnatal periods, nor was sex dependence detected in most tissues and with most substrates. The hepatic sulphotransferase activities towards testosterone, budesonide and 1,2,3,4-tetrahydroisoquinoline are slightly higher (18–28%) in males (Pacifici *et al.* 1997a,b). A pronounced sexual dimorphism in the platelet 4-nitrophenol sulphotransferase activity was detected in Finns but not in Italians; the median activity was nearly three-fold higher in Finnish men than in Finnish women and in Italians of either sex (Brittelli *et al.* 1999).

Rat

Little is known about the expression of sulphotransferases in the foetal rat. 2-Naphthol sulphotransferase activity was identified in several tissues on day 21 of gestation, although the activities in the extrahepatic tissues were low compared to those found in the human foetus (Pacifici *et al.* 1988). Between birth and 10 weeks of age, increases in PST activity were observed in liver and brain (approximately six-fold) but not in kidney (Maus *et al.* 1982). The hepatic level of SULT1B1 RNA was approximately four times higher at the age of 90 days than at birth in either sex; however, whereas the increase was continuous in females, it reached a peak in males between 30 and 45 days of age (Dunn II *et al.* 1999b). In the adult rat, SULT1B1 RNA and protein levels are similar in either sex.

In rat liver, the expression of all other SULT forms investigated is sex-dependent. SULT1A1 (AST IV) is moderately higher in males; EST (ST1E2) and SULT1C1 (ST1C1) are constitutively expressed only in males (Borthwick *et al.* 1993; Nagata *et al.* 1993; Liu and Klaassen 1996a). In contrast, expression of various HSTs is female-dominant or female-specific (Singer *et al.* 1976; Lyon and Jakoby 1980; Liu and Klaassen 1996b).

The hepatic RNA level of SULT1A1 increases several-fold between birth and puberty in either sex; in the same period, EST (ST1E2) and SULT1C1 RNA rise dramatically from a very low starting level but only in males (Liu and Klaassen 1996a). Hepatic cortisol sulphotransferase activity is very low at birth, develops in parallel in both sexes until 30 days after birth, then rises in females and drops in males until 50 days after birth (Singer *et al.* 1976). Among the different cortisol sulphotransferase forms, STII predominates in immature animals, STIII is the major form in adult males, and STI is essentially restricted to adult females (which also express high levels of STII and STIII). In senescent male rats, certain hepatic HSTs (initially termed SMP-2) are derepressed (Chatterjee *et al.* 1987; Song *et al.* 1990). Liu and Klaassen (1996b) studied the ontogeny of the hepatic expression of the RNAs of the following HSTs: ST-20/21, ST-40/41 and ST-60. The level of ST-40/41 RNA was highest in immature animals of either sex. In mature males, only ST-20/21 RNA was found, whereas in adult females all three RNA types were detected. The ontogenetic development and the sexual dimorphisms associate STI protein with ST-60 RNA, STII with ST-40/41 (which is

identical to STa), and STIII with ST-20/21. However, it is not known whether the associated forms are identical or only show similarities in their regulation.

Castration or oestrogen treatment of male rats leads to increases in various hepatic hydroxysteroid (cortisol, glycolithocholic acid) sulphotransferase activities and decreases in hepatic phenol (dopamine) sulphotransferase activity (Singer *et al.* 1976, 1988; Kirkpatrick *et al.* 1985). The anti-oestrogen tamoxifen, which is a partial agonist for some oestrogen receptors, also induces certain HSTs in male but not female rats (Kirkpatrick *et al.* 1985; Hellriegel *et al.* 1996; Nuwaysir *et al.* 1996). This induction is toxicologically significant, since tamoxifen is activated to a carcinogen by STa, an enzyme that is constitutively expressed only in females. This sex-dependent expression could explain why a short treatment with tamoxifen led to a high level of hepatic DNA adducts in female rats but only to a very low level of adducts in males (Davis *et al.* 2000). Treatment of rats with tamoxifen for a few weeks led to the induction of STa in males and, eventually, to the formation of similar levels of DNA adducts and to similar carcinogenic activities in both sexes (Davis *et al.* 2000).

Ovariectomy led to a decrease in hydroxysteroid (cortisol) sulphotransferase activity and an increase in phenol (dopamine) sulphotransferase activity in the liver, whereas treatment with testosterone had only minor effects on these activities (Singer *et al.* 1976, 1988).

The sex-dependent expression is primarily controlled by the pituitary gland via the pattern of growth hormone secretion (Gong *et al.* 1991, 1992; Borthwick *et al.* 1995b; Liu and Klaassen 1996a,b).

The expression of SULTs in extrahepatic tissues does not reflect the sexual dimorphism observed in the liver; in our experience, the extrahepatic expression is, in general, sex-independent.

Mouse

Similar to rats, substantial sex-dependent differences in hepatic sulphotransferase activities have also been found in mice. However, these dimorphisms differ fundamentally from those observed in the rat. In murine liver, most sulphotransferase activities are higher in females than in males. For example, hepatic T_3 sulphotransferase activity was five-fold higher in the females (Gong *et al.* 1992). Likewise, Borthwick *et al.* (1995a) found 13-, 5-, 60- and 4-fold higher hepatic E_1, E_3, DHEA and 1-naphthol sulphotransferase activities, respectively, in females than in males. Thus, hepatic EST expression is female-dominant in the mouse, but male-specific in the rat. The male mouse may not need EST in the liver, as it is highly expressed in the testis (Hobkirk and Glasier 1992), whereas no EST RNA is expressed in rat testis (Dunn II and Klaassen 1998).

DIFFERENTIATION OF CELLS AND TISSUES

In epidermal cells of murine skin, cholesterol sulphotransferase activity reached a sharp peak around day 16 of gestation; four months after birth, the activity decreased by a factor of 46 (Kagehara *et al.* 1994). Likewise, a strong increase in cholesterol sulphotransferase activity was observed during *in vitro* squamous differentiation of

rabbit tracheal epithelial cells (Rearick *et al.* 1987b), human bronchial epithelial cells (Rearick *et al.* 1987a) and human epidermal keratinocytes (Jetten *et al.* 1989). As already described, cholesterol sulphate is an important factor in the squamous differentiation. Minoxidil sulphotransferase activity also increased during the differentiation of human keratinocytes, but with a time course differing from that of cholesterol sulphotransferase activity, suggesting the involvement of distinct enzyme forms (Johnson *et al.* 1992).

In the uterus of the guinea pig (Freeman *et al.* 1983) and the mouse (Hobkirk *et al.* 1983), EST activity was detected in the second half of gestation, but not in the non-pregnant state. Treatment of pigs with progesterone led to an induction of EST activity in the proliferative endometrium (Meyers *et al.* 1983; Brooks *et al.* 1987). Expression of SULT1E1 in the human endometrium is low during the luteal phase, but high during the follicular phase (Rubin *et al.* 1999). It was strongly reduced in women using oral contraceptives, and no expression was detected during early pregnancy. The levels of the other sulphotransferases that have been established in the human endometrium, SULT1A1 and 1A3, were altered only moderately during these physiological stages. Progesterone induced SULT1E1 protein and activity in endometrial adenoma cells in culture (Falany and Falany 1996b), and thus the progesterone level may be important for the endometrial SULT1E1 expression during the menstrual cycle and in women using contraceptives. However, other factors are required to explain the lack of SULT1E1 expression during early pregnancy.

Pseudopregnancy, which was induced by treatment with E_2 followed by chorionic gonadotropin, led to a 30-fold increase in endometrial cholesterol sulphotransferase activity in the rabbit (Momoeda *et al.* 1994).

After partial hepatectomy, a marked decrease in sulphotransferase activity towards OH-AAF (but not towards 4-nitrophenol) was observed in the liver of male rats during the first days (Gilissen and Meerman 1992). During the period of rapid liver growth, the level of various SULT RNAs was decreased (Dunn II *et al.* 1999a). Particularly marked losses were observed for the male-specific forms SULT1C1 and EST (SULT1E2) in males and for the female-dominant form ST-20/21 in females.

CORTICOID AND THYROID HORMONES

Adrenalectomy led to a decrease in hepatic dopamine sulphotransferase II (AST IV) in male rats (Singer *et al.* 1988). Under non-hypertensive regimens, glucocorticoids induce AST IV, repress ST1C1, and have only minor effects on HSTs and ESTs in the liver of male rats (Kirkpatrick *et al.* 1985; Runge-Morris *et al.* 1996; Liu and Klaassen 1996d; Duanmu *et al.* 2000). Dexamethasone induced PST activity strongly in the kidney (6.6-fold), moderately in the liver (1.3-fold) and not at all in the brain of male rats (Maus *et al.* 1982). In females, dexamethasone induced ST-40/41 (STa) in addition to ST1A1 (AST IV) (Liu and Klaassen 1996d).

At hypertensive regimens of cortisol (Singer *et al.* 1988), the induction of dopamine sulphotransferase (AST IV) in the male rat liver was enhanced and associated with a clear increase in cortisol sulphotransferase activity (in particular. form STIII) (Singer *et al.* 1977). However, these effects are not specific for cortisol, but also occur in

hypertension produced in other ways (see below), e.g. induced by mineralocorticoids (Singer *et al.* 1977).

In the C57BL/Ks/J mouse (wild-type and *fat/fat* mutant), dexamethasone led to an approximately 10-fold induction of hepatic E_1 sulphotransferase activity (Leiter *et al.* 1999).

Thyroidectomy and drug-induced hypothyroidism led to a decrease in hepatic T_3 sulphotransferase activity in male rats but not in females (Gong *et al.* 1992; Kaptein *et al.* 1997). Administration of T_3 restored the activity. It also led to a 2-fold induction of T_3 sulphotransferase activity in hypophysectomised male and female rats (Gong *et al.* 1992). Treatment of hypophysectomised male rats with T_4 virtually abolished hepatic EST activity and immunoreactive protein, but had no effect on PST and HST activity and on protein levels (Borthwick *et al.* 1995b). Dunn II and Klaassen (2000) observed an increase in hepatic EST RNA in thyroidectomised rats of either sex and partial reversal of this effect by the infusion of T_3 and T_4. These findings indicate that EST is negatively regulated by thyroid hormones. Thyroidectomy had no effect on the hepatic levels of SULT1B1 and 1A1 RNA but altered the pattern of HST RNAs (with sex-dependent decreases in some forms and increases in other forms). Expression of the male-specific form SULT1C1, which exhibits T_3 sulphotransferase activity (Visser 1994), tended to be decreased in the liver of thyroidectomised rats (Dunn II and Klaassen 2000). All these effects of thyroidectomy were reversed, at least partially, by the infusion of thyroid hormone.

INFLUENCE OF PHYSIOLOGICAL STATE, DISEASE AND NUTRITIONAL FACTORS

Leiter *et al.* (1991) observed a dramatic increase in a high-affinity E_1 sulphotransferase activity and a concomitant strong decrease in DHEA sulphotransferase activity in the liver of genetically obese and diabetic female mice (*ob/ob* or *db/db*) compared to normal mice and *fat/fat* mice (which were obese and hyperinsulinaemic but not hyerglycaemic). Borthwick *et al.* (1995a) detected that the increase in E_1 and E_3 sulphotransferase activities is even stronger (nearly 100-fold) in male *ob/ob* mice than in females due to a lower constitutive activity in lean males. Interestingly, HST and PST activities were diminished in females but enhanced in males; these differential effects resulted in a strong mitigation of the sexual dimorphism in the *ob/ob* mouse compared to the controls. The increase in the EST activities was associated with the induction of a novel SULT RNA (Leiter and Chapman 1994) and a novel immunoreactive protein (Borthwick *et al.* 1995a), which were not detected in the liver of control animals of either sex.

Genetically, surgically as well as drug-induced hypertension leads to an induction of hepatic cortisol sulphotransferase activity, in particular of form STIII, in male rats (Turcotte and Silah 1970; Singer *et al.* 1977). Hypertension also induces hepatic dopamine sulphotransferase activity, primarily the form II (AST IV); conversely, anti-hypertensive treatments with spironolactone or hydralazine reduced dopamine sulphotransferase activity (Singer *et al.* 1988).

In Parkinson's disease, strong decreases in dopamine sulphotransferase activity were reported for various regions of the human brain, in particular for hypothalamus, frontal

and temporal cortex, amygdaloid nucleus, and occipital and frontal cortex (Baran and Jellinger 1992).

Selenium deficiency led to a drastic decrease (by 92.7%) in the expression of EST (SULT1E) RNA in male rat liver (Yang and Christensen 1998). A moderate decrease (by 38%) was also observed for the 4-nitrophenol sulphotransferase activity, along with various other alterations in the xenobiotic-metabolising system, in male mice receiving a selenium-deficient diet (Reiter and Wendel 1984). The mechanism and functional significance of these down-regulations are not known.

Protein-free diet enhanced hepatic UDP-glucuronosyltransferase activities in immature and adult rats but did not affect the sulphotransferase activities measured with 4-nitrophenol and DHEA (Woodcock and Wood 1971). Food restriction may lead to moderate alterations in the levels of hepatic sulphotransferase activities and proteins (Witzmann *et al.* 1996; Kaptein *et al.* 1997).

XENOBIOTIC INDUCERS

Induction of sulphotransferases by hormones and hormonally-active drugs has been presented in the preceding sections. Classical inducers of cytochromes P450 (e.g. phenobarbital and 3-methylcholanthrene) often co-induce conjugating enzymes, such as glutathione transferases and UDP-glucuronosyltransferases. These treatments usually had little or no effect on the sulphotransferase activities studied (Gram *et al.* 1974; Nemoto and Takayama 1978; Thompson *et al.* 1982; Watkins 1991; further references in Mulder and Jakoby, 1990). Under some conditions, certain sulphotransferase activities were moderately induced by 3-methylcholanthrene. However, most of these studies were conducted using standard substrates whose conjugation is catalysed by one or usually several major sulphotransferase forms. Moreover, most investigations were restricted to the liver. In light of the molecular complexity of the sulphotransferases, a re-examination is required, since minor forms and extrahepatic enzymes could be pivotal for the metabolism of specific xenobiotics and in particular the activation of procarcinogens in target tissues.

Runge-Morris *et al.* (1998) observed that treatment of male rats with phenobarbital did not significantly affect the hepatic RNA levels of SULT1C1 and EST (SULT1E2), increased those of SULT1B1 (SULT-Dopa/tyrosine, 4.2-fold), ST-20/21 (SULT-20/21, 1.6-fold, starting from a low level) and ST-60 (SULT-60, 4.2-fold, starting from a low level), and decreased those of SULT1A1 (by a factor of 2.4) and ST-40/41 (SULT-40/41, by a factor of 3.3). Hellriegel *et al.* (1996) reported on moderate (statistically not verified) changes in the levels of several hepatic SULT RNAs in phenobarbital-treated rats of either sex. Garcia-Allan *et al.* (2000) searched for phenobarbital-modulated genes in mouse liver using the differential cDNA display technique. The most strongly elevated RNA, detected as cDNA, was shown to encode a sulphotransferase, SULT-N (St1d1), rather than a cytochrome P450, as might have been expected. St1d1 shows a unique catalytic activity for the sulphoconjugation of eicosanoids.

Phenobarbital treatment of pregnant rats produced a 5-fold increase in the hepatic bile salt sulphotransferase activity of the neonate; an even stronger increase in this activity (17-fold) was observed after intrauterine exposure to lithocholate or maternal bile duct ligation (Chen *et al.* 1982).

Administration of subcarcinogenic doses of AAF to male rats leads to a transient down-regulation of the hepatic OH-AAF sulphotransferase activity (attributed now to SULT1C1) (Ringer *et al.* 1990, 1994). Similar effects were detected with other genotoxic hepatocarcinogens, e.g. benzidine, aflatoxin B_1, ethionine and thiacetamide (Ringer *et al.* 1985).

Treatment of human hepatocytes in culture with rifampicin led to an induction of 17α-ethinyl-E_2 sulphotransferase activity (Li *et al.* 1999). Other typical inducers of cytochromes P450, such as 3-methylcholanthrene, phenobarbital, dexamethasone and omeprazole, did not induce 17α-ethinyl-E_2 sulphotransferase activity. Rifampicin also induced 4-nitrophenol sulphotransferase activity in rat and human hepatocytes in culture (Kern *et al.* 1997).

A number of other compounds that are not prototypic inducers of cytochromes P450 caused induction of sulphotransferases. For example, Singer *et al.* (1984) reported that PCP and acetylsalicylic acid preferentially induced glucocorticoid sulphotransferase form STIII, whereas propranolol, metyrapone and aminoglutethimide selectively enhanced the levels of forms STI and/or STII in male rat liver.

SULPHOTRANSFERASES IN PRENEOPLASTIC AND NEOPLASTIC LIVER TISSUE

Expression of immunoreactive HST proteins is decreased in preneoplastic, ATPase-deficient foci in female rat liver (Werle-Schneider *et al.* 1993). In male rats, decreased levels of OH-AAF sulphotransferase activity and immunoreactive AST IV protein (SULT1A1 and/or 1C1) were detected in hepatic nodules and tumours (Ringer *et al.* 1994; Malejka-Giganti *et al.* 1997). Thus, the regulation of sulphotransferases in preneoplastic lesions and tumours of the liver differs from that of other conjugating enzymes, which often are increased in the altered tissue (Bock *et al.* 1982; Buchmann *et al.* 1985).

SULPHOTRANSFERASES IN CELLS IN CULTURE

In rats, expression of sulphotransferases is sexually dimorphic and strongly focused on the liver. The expression of various sulphotransferases falls dramatically in primary cultures of hepatocytes from male and female rats. This has been demonstrated on the RNA level in particular for ST1C1, EST (ST1E2), ST-20/21, ST-40/41 (STa) and ST-60 (Liu *et al.* 1996). SULT1A1 (AST IV), a form that shows only minor sexual dimorphism, was less sensitive to this down-regulation, and its decrease could be reversed by the addition of dexamethasone to the culture medium. It is probable that this form is responsible for the residual phenol sulphotransferase activities observed in cultured hepatocytes (Grant and Hawksworth 1986; Kane *et al.* 1991; Utesch and Oesch 1992).

In humans, expression of sulphotransferases is sex-independent in general, and several forms display high expression in certain extrahepatic tissues. Preliminary findings suggest that the tissue-dependent expression of sulphotransferases is maintained, at least partially, in primary cultures and cell lines produced from human

tissues (Rearick *et al.* 1987a; Johnson *et al.* 1992; Falany and Falany 1996b; Dooley *et al.* 2000).

In order to achieve maximal sulphonation activity in cells in culture, a relatively high concentration of inorganic sulphate in the medium is required (1 to 3 mM, depending on the cells used) (Schwarz and Schwenk 1984; Chen and Schwarz 1985; van de Poll *et al.* 1990).

Inhibitors and activators of sulphotransferases

SULPHOTRANSFERASE INHIBITORS AS DIAGNOSTIC PROBES—GENERAL ASPECTS

Specific inhibitors for an enzyme can be used to demonstrate its involvement in the metabolism or the activation/inactivation of a xenobiotic. In subcellular systems, the addition or omission of the cofactor PAPS can globally probe the role of sulphotransferases. Selective inhibitors for certain sulphotransferase forms (e.g. DCNP, PCP and DHEA) are occasionally used to specify the involved form(s). This specification is only possible to a limited extent for two reasons. (a) These inhibitors interact with the substrate-binding site; in analogy to their promiscuity for the substrates, different sulphotransferase forms show a broad overlap in their interaction with inhibitors, as illustrated in Table 10.3 for various human sulphotransferases and DCNP; (b) the knowledge about the inhibition of different sulphotransferase forms is insufficient; for example, PCP has only been tested with one human sulphotransferase and two rat sulphotransferases individually.

The examples presented in Tables 10.4 and 10.5 demonstrate that the sulphotransferase inhibitors PCP and DHEA can dramatically influence the metabolic fate and the pharmaco-toxicological effects of many xenobiotics in rodents. Strong modulating effects in animal experiments have also been observed with DCNP, e.g. with regard to the sulphoconjugation of harmol (Mulder and Scholtens 1977) and the activation of nitrotoluenes (Kedderis *et al.* 1984; Rickert *et al.* 1984), β-hydroxylated nitrosamines (Sterzel and Eisenbrand 1986; Kroeger-Koepke *et al.* 1992), 2-nitropropane (Sodum *et al.* 1994), *N*-hydroxy-4′-fluoro-4-acetylaminobiphenyl (van de Poll *et al.* 1989), tamoxifen (Randerath *et al.* 1994a) and safrole (Randerath *et al.* 1994a).

Table 10.3 Inhibition of individual human SULT forms by 2,6-dichloro-4-nitrophenol (DCNP)[a]

Enzyme form[b]	Substrate	IC_{50}, μM	References
SULT1A1*1	4-Nitrophenol, 4 μM	1.44	Raftogianis *et al.* (1999)
SULT1A2*1	4-Nitrophenol, 100 μM	6.94	Raftogianis *et al.* (1999)
SULT1A3	4-Nitrophenol, 3000 μM	86.9	Raftogianis *et al.* (1999)
SULT1B1 (ST1B2)	T_3, 60 μM	400	Fujita *et al.* (1999)
SULT1E1	T_3, 75 μM	30	Li and Anderson (1999)
SULT2A1 (DHEA ST)	DHEA, 5 μM	40	Aksoy *et al.* (1994)

[a] Since DCNP is a competitive inhibitor with respect to the sulpho acceptor, IC_{50} depends on the substrate concentration; the substrates were used at concentrations that lead to near-maximal activity. No data are available about the influence of DCNP on other human SULTs (1C1, 1C2, 2B1a, 2B1b, 4A1).

[b] The name used in the reference cited is given in parentheses if it is different from the systematic name.

Table 10.4 Modulation of the metabolism and/or pharmaco-toxicological effects of xenobiotics in animals by the sulphotransferase inhibitor PCP (examples)[a]

Agent	Animal model	Modulation of the fate/effect of the xenobiotic
Harmol	Male rat	Decrease in the biliary and urinary excretion of harmol sulphate (by up to 71%); increased excretion of harmol glucuronide (by up to 120%) (Meerman *et al.* 1983)
1-Naphthol	Male rat	Decreased urinary excretion of 1-naphthyl sulphate (by up to 48%); mitigated effect at high doses of 1-naphthol (that may overcome the competitive inhibition by PCP) (Boles and Klaassen 1999)
Toluene, benzyl alcohol, xylene and *o*-methylbenzyl alcohol	Male rat	Strong decrease in the urinary excretion of thio compounds (mercapturic acids) (van Doorn *et al.* 1981)
Benzyl acetate	Male rat	Abolition of the excretion of benzyl mercapturic acid (Chidgey *et al.* 1986)
(*R*)-Stiripentol	Male rat	Inhibition of the chiral inversion (Zhang *et al.* 1994)
Minoxidil	Male rat	Ablation of the hypotensive effect (Duanmu *et al.* 2000)
2-Acetylaminofluorene (AAF)	Male rat	Decreased excretion of glutathione conjugates (probably formed from a reactive sulphoconjugate) (by 67–75%); increase in the *N*-*O*-glucuronide (by 21–32%) (Meerman *et al.* 1983) Complete protection against the induction of liver tumours and resulting death (Ringer *et al.* 1988) Decrease in unscheduled DNA synthesis (determined in the isolated hepatocytes after exposure *in vivo*) (Monteith 1992) Protection against the loss of hepatic OH-AAF sulphotransferase activity (Ringer *et al.* 1985)
	Preweaning mouse	Decrease in hepatic DNA adducts (by 90%) and induced hepatomas (by 80–90%) (Lai *et al.* 1985, 1987)
N-Hydroxy-2-aminofluorene (OH-AAF)	Male rat	Decrease in hepatic RNA adducts (by 61%) and acetylated DNA adducts (by 70%); no influence on the level of deacetylated DNA adducts (Meerman *et al.* 1981). Decrease in the biliary excretion of 1- and 3-(glutathion-*S*-yl)-*N*-acetyl-2-aminofluorene (probably formed from the sulphoconjugate) (by 50%) (Meerman *et al.* 1982)
	Preweaning mouse	Decrease in hepatic DNA adducts (by 75–91%) and hepatomas (by $\geqslant$ 80%) (Lai *et al.* 1987)
2,6-Dinitrotoluene	Male rat	Decrease in binding to hepatic macromolecules (by 65%) and DNA (by $>$ 96%) (Kedderis *et al.* 1984)

continued overleaf

Table 10.4 (*continued*)

Agent	Animal model	Modulation of the fate/effect of the xenobiotic
Safrole	Male rat	Decrease in hepatic DNA adducts and in various cytogenetic effects in hepatocytes (determined in primary culture after the exposure *in vivo*) (Daimon *et al.* 1998)
	Female mouse	Decrease in hepatic DNA adducts (by 91%) (Randerath *et al.* 1994a)
1′-Hydroxysafrole	Preweaning mouse	Decrease in hepatic DNA, RNA and protein adducts (by 50–85%) and hepatomas (by ⩾ 90% (Boberg *et al.* 1983)
	Partially hepatectomised rat	Abolition of the acute lethality and the initiation of (phenobarbital-promoted) enzyme-altered foci in the liver; decrease in hepatic DNA, RNA and protein adducts (by 74–89%); nearly complete abolition of the promotion of diethylnitrosamine-initiated enzyme-altered foci and the formation of neoplasms in the liver (Boberg *et al.* 1987)
Diethylstilboestrol	Female mouse	Decrease in hepatic DNA adducts (by 33–61%) (Moorthy *et al.* 1995)
Tamoxifen	Female mouse	Up to 7-fold intensification of the formation of total hepatic DNA adducts; 11-fold increase in polar adducts but 6-fold suppression of unpolar adducts in the liver; also changes in the pattern of DNA adduct in kidney but not in lung (Randerath *et al.* 1994a,b)
4-Hydroxytamoxifen	Female mouse	4-fold increase in hepatic DNA adducts (Randerath *et al.* 1994a)

[a] PCP also strongly decreased the formation of hepatic macromolecular adducts and/or the induction of tumours by 4-aminoazobenzene, *N*-methyl-4-aminoazobenzene, *N,N*-dimethyl-4-aminoazobenzene and 2-methyl-4-aminoazobenzene (Delclos *et al.* 1984), *N*-hydroxy-4′-fluoro-4-acetylaminobiphenyl (van de Poll *et al.* 1989), *N*-hydroxy-4-acetylaminobiphenyl (Lai *et al.* 1987), 2-nitrotoluene (Rickert *et al.* 1984) and 1′-hydroxy-2′,3′-dehydroestragole (Fennell *et al.* 1985).

Table 10.5 Examples of modulation of the metabolism and/or pharmaco-toxicological effects of xenobiotics in animals by the sulphotransferase inhibitor DHEA

Agent	Animal model	Modulation of the fate/effect of the xenobiotic
Atropine	Guinea pig	Inhibition of dehydration (by 57%) (Wada *et al.* 1994)
6-Hydroxmethyl-benzo[*a*]pyrene	Preweaning female rat	Prevention of the formation of hepatic DNA adducts (Surh *et al.* 1989)
7-Hydroxmethyl-12-methylbenz[*a*]-anthracene	Preweaning female rat	Prevention of the formation of hepatic DNA adducts (Surh *et al.* 1987)
2-Nitropropane	Male rat	Marked decrease in various RNA and DNA modifications, e.g. 8-NH_2-dGua (by 92%) (Sodum *et al.* 1994)

2,6-DICHLORO-4-NITROPHENOL (DCNP) AND PENTACHLOROPHENOL (PCP)

Various phenols with electron-withdrawing substituents, such as Cl and NO_2, are poor substrates but potent competitive inhibitors of rat PSTs (Mulder and Scholtens 1977; Koster *et al.* 1979). Among them, DCNP and PCP show the highest inhibitory activity *in vivo*. Many other phenols that are inhibitors and competitive substrates *in vitro* did not significantly affect the sulphoconjugation *in vivo* (Koster *et al.* 1979).

In general, PCP is a more potent inhibitor than DCNP. Thus, partially purified rat AST IV was inhibited 97.7% and 51.3% by 1 μM PCP and DCNP, respectively (Sodum *et al.* 1994). Initial observations suggesting a relatively high toxicity of PCP were not confirmed in later studies, when purified batches of PCP were used. Indeed, PCP can be utilised for long-term inhibition of sulphotransferases in animal experiments (Meerman *et al.* 1983).

When PCP or DCNP are used as diagnostic inhibitors, it is important to take into account the very limited knowledge about the interaction of these inhibitors with individual forms of sulphotransferases. Rat AST IV and rSULT1C1 are the only rodent sulphotransferases that appear to have been studied as individual (purified or cDNA-expressed) enzymes with PCP. The inhibition of AST IV by PCP is competitive with regard to the sulpho acceptor ($K_i = 0.2$ μM); it could be defined as a dead-end inhibition, since no PCP sulphate is formed (Duffel and Jakoby 1981). rSULT1C1 (ST1C1) was inhibited completely in the presence of 1 μM PCP (Nagata *et al.* 1993). However, PCP was conjugated by a purified amine sulphotransferase from the guinea pig (Ramaswamy and Jakoby 1987). Likewise PCP, added to a tank with Zebra fishes, was converted to its sulphoconjugate to an extent of 31.4% (Kasokat *et al.* 1987). Sulphonation of PCP has also been observed in the sea urchin (Tjeerdema *et al.* 1994), abalone (Tjeerdema and Crosby 1992) and goldfish (Stehly and Hayton 1988).

Inhibition by DCNP has been studied with several individual human sulphotransferase forms (Table 10.3), but not with any highly purified or cDNA-expressed rodent forms. Inhibition of rat enzymes has been investigated in cytosol preparations of hepatic and extrahepatic tissues (e.g. Mulder and Scholtens 1977; Wong *et al.* 1993). DCNP is an alternate-substrate inhibitor of rat hepatic PST(s) *in vitro* ($K_m = 4.3$ μM) (Seah and Wong 1994).

Nevertheless, no sulphoconjugation of either DCNP or PCP has been observed in the rat *in vivo*. Both compounds can elevate hepatic PAPS levels, possibly via inhibition of the conjugation of endogenous substrates (Dills and Klaassen 1986). Through this mechanism, they may enhance the sulphonation of compounds that are conjugated by sulphotransferases resistant to these inhibitors, as has been shown for DHEA (Boles and Klaassen 1998a).

In subcellular preparations of mouse liver, PCP inhibited OH-AAF sulphotransferase activity ($IC_{50} < 1$ μM) without affecting the *N*-hydroxy-2-aminofluorene acetyltransferase, OH–AAF deacetylase and OH–AAF *N,O*-transacylase ($IC_{50} > 10$ to 100 μM) (Lai *et al.* 1985). Shinohara *et al.* (1986) reported that PCP, but not DCNP, inhibits the *O*-acetylation of various aromatic hydroxylamines in rat and hamster liver cytosols ($IC_{50} = 12$ to 25 μM). Flammang and Kadlubar (1986) found a moderate inhibition ($\sim 40\%$) of rat acetyltransferase and *N,O*-transacylase activities in the presence

of 100 μM PCP. PCP also inhibited murine microsomal epoxide hydrolase ($IC_{50} = 35$ μM) and a glutathione transferase activity ($IC_{50} = 23.5$ μM) *in vitro* (Moorthy *et al.* 1995). Thus, inhibition of non-sulphotransferase enzymes requires substantially higher concentrations of PCP or DCNP than inhibition of certain PSTs. Under conditions that led to inhibition of sulphoconjugation, DCNP did not affect other conjugation reactions in the rat *in vivo* (Koster *et al.* 1979). Of course, only representative reactions could be studied.

Enzyme induction may occur, especially when inhibitors are administered to animals over long periods. Induction of hepatic CYP enzymes has been observed in rats treated for two weeks with PCP (Vizethum and Goerz 1979). Likewise, PCP induced hepatic glucocorticoid sulphotransferase (form STI) in male rats (Singer *et al.* 1984).

DHEA AND OTHER INHIBITORS OF HSTs

DHEA is an inhibitor (alternate substrate) of the 1-HMP, 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 6-hydroxymethylbenzo[*a*]pyrene sulphotransferase activities in rat hepatic cytosol (Surh *et al.* 1989, 1990, 1991). It has been used in animals to probe the role of HSTs in the metabolism and bioactivation of xenobiotics (Table 10.5).

DHEA-S is a product inhibitor of rat HST 1 ($K_i = 4$ μM) (Lyon and Jakoby 1980) and HST 2 ($K_i = 290$ μM) (Marcus *et al.* 1980). The inhibition is competitive with regard to the substrate DHEA. DHEA-S decreased the formation of ductal carcinomas by *N*-nitroso-bis(2-oxopropyl)amine in Syrian hamsters (Tsutsumi *et al.* 1995). Propylene glycol strongly reduced the DNA alkylation in the liver of rats treated with *N*-nitrosomethyl(2-hydroxyethyl)amine (Kroeger-Koepke *et al.* 1992). It has been postulated that these effects occurred via inhibition of HSTs.

When modulating effects of DHEA and DHEA-S are analysed, it is important to take into account that these compounds not only inhibit sulphotransferases but also exhibit numerous intrinsic activities on the organism and are the metabolic precursors of other steroid hormones. Furthermore, DHEA can diminish the expression of CYP1A1 protein via a stimulation of the degradation of CYP1A1 RNA (Ciolino and Yeh 1999).

MOLYBDATE

Molybdate lowers the serum levels of inorganic sulphate and the availability of PAPS. It is a relatively potent inhibitor of the sulphoconjugation of paracetamol (Oguro *et al.* 1994), harmol (Boles and Klaassen 1998b), 1-naphthol (Boles and Klaassen 1999) and DHEA (Boles and Klaassen 1998a) in the rat *in vivo*. Thus, it appears that sulphonation is decreased regardless of the enzyme form involved; this characteristic is consistent with the postulated mechanism of inhibition. However, the PAPS depletion is tissue-dependent. Molybdate strongly reduced the hepatic PAPS level, but showed negligible effects on the renal level in the rat (Oguro *et al.* 1994). Furthermore, the decrease in the sulphonation was stronger at high doses of the substrates (requiring a high expenditure of PAPS) than at low doses. The opposite was observed with PCP and DCNP (in agreement with the proposed mechanism, competitive inhibition of sulphotransferases).

In the cases described above, molybdate did not inhibit metabolic pathways competing with sulphonation, in particular glucuronidation. In some cases, these pathways were even enhanced, probably because substrate is saved due to decreased sulphonation.

OTHER INHIBITORS

Numerous drugs (Singer *et al.* 1984; Bamforth *et al.* 1992; Harris *et al.* 1998; Vietri *et al.* 2000), food additives (Bamforth *et al.* 1993) and phytochemicals (Gibb *et al.* 1987; Harris and Waring 1996; Glatt 2000b; Otake *et al.* 2000) are potent inhibitors (and, in part, competitive substrates) of sulphotransferases *in vitro*. Various flavonoids inhibit hSULT1A1 with K_i values of < 1 μM (Ghazali and Waring 1999; De Santi *et al.* 2000). Several hydroxylated metabolites of polychlorinated biphenyls showed IC_{50} values of < 1 nM towards human EST; it has been suggested that this EST inhibition may be the cause of the oestrogenic activity of polychlorinated biphenyls observed in rodents (Kester *et al.* 2000). Tetraalkyl (*n*-propyl, *n*-butyl, *n*-pentyl) ammonium salts are potent inhibitors of DHEA and cortisol sulphotransferase activities ($IC_{50} = 15$ to 66 μM), but they do not affect the sulphonation of 2-naphthol in rat liver cytosol preparations (Matsui *et al.* 1995).

With some chiral compounds, one enantiomer is a substrate of a sulphotransferase whereas its antipode is an inhibitor (Rao and Duffel 1991a; Banoglu and Duffel 1997, 1999). Therefore, caution is required when testing racemic compounds as substrates or inhibitors of sulphotransferases.

Various primary alcohols are substrates of sulphotransferases, but can also be oxidised enzymically via aldehydes to carboxylic acids. The hydrated forms of the aldehydes and the carboxylic acids are nucleophiles and thus potential substrates of sulphotransferases. Whereas sulphonation of such compounds has not been observed to date, some aryl aldehydes and aryl carboxylic acids have displayed relatively strong competitive inhibition of rat AST IV (Rao and Duffel 1991b; Duffel and Zheng 1993).

Various aromatic hydroxylamines are substrates of sulphotransferases. During their incubation in enzyme assays, they may be autoxidised to potent irreversible sulphotransferase inhibitors (King and Duffel 1997).

METAL IONS, pH

Interestingly, the medium composition (salts, pH) not only affects the level of activity of sulphotransferases, but also can modify their substrate specificity. The pH effect is trivial for substrates whose protonation is altered in the investigated range, e.g. certain phenols and amines (Ramaswamy and Jakoby 1987). However, the pH optimum of rat AST IV can also vary between 5.5 and 9 for substrates whose protonation is not altered (Sekura and Jakoby 1981; Marshall *et al.* 1997). Some of these substrates were conjugated only within a small pH range that did not overlap with the appropriate pH range for another substrate. Likewise, the addition of $MnCl_2$ to human M-PST (SULT1A3) drastically affected its substrate and stereo selectivity towards tyrosine derivatives (Sakakibara *et al.* 1997). For example, the activity towards D-*p*-tyrosine

was stimulated 130-fold in the presence of 10 mM $MnCl_2$, whereas the activity towards L-dopa was decreased by a factor of 3.7 under the same conditions.

SUICIDE SUBSTRATES

Incubation of purified rat AST IV (probably containing rSULT1C1) with [^{14}C]-OH-AAF and PAPS led to the radiolabelling of the enzyme (primarily at cysteine residues) and to a concurrent loss of OH-AAF sulphotransferase activity (Ringer *et al.* 1992). Omission of PAPS, addition of an enzyme inhibitor (PCP) and the presence of high levels of an exogenous nucleophile (methionine) abolished these effects, suggesting that they were caused by the product sulphooxy-AAF and that this product was released from the active centre before adduction. Therefore, this self-inactivation may be favoured when using purified enzyme. However, it may also be involved in the decrease in AST IV protein and enzyme activity observed in AAF-treated rats.

The PAPS-dependent inhibition by OH-AAF has to be distinguised from the PAPS-independent, autoxidation-mediated inhibition observed with its deacetylated congener, *N*-hydroxy-2-aminofluorene.

SULPHOTRANSFERASE INHIBITION BY DRUGS IN THE HUMAN

Data on drug–drug interactions in the human via inhibition of sulphotransferases are scarce. If paracetamol and salicylamide, two substrates of sulphotransferases, were administered together to human subjects, the sulphonation rate of each substrate was lower than when the compounds were administered individually (Levy and Yamada 1971). Dapsone significantly decreased the sulphonation of paracetamol in a clinical study; lamivudine had a similar effect in the cross-sectional part, but not in the longitudinal part, of the study (O'Neil *et al.* 1999).

Strain differences in animals and genetic polymorphisms in the human

STRAIN DIFFERENCES

Only scarce information is available about strain-dependent differences of sulphotransferases in laboratory animals. King and Olive (1975) reported that Fischer 344 rats express much higher hepatic OH-AAF sulphotransferase activity than Sprague–Dawley rats. In particular, Sprague–Dawley females were virtually deficient of this activity, whereas Fischer 344 females showed activities that were even higher than those in Sprague–Dawley males. Maus *et al.* (1982) determined the basal and dexamethasone-induced levels of PST activity (substrate 3-methoxy-4-hydroxyphenylglycol) in liver, kidney and brain of various rat strains; they did not find any major differences between the ten strains investigated (including Sprague–Dawley and Fischer 344). ACI rats exhibited lower hepatic T_3 sulphotransferase activity than Sprague–Dawley rats (Gong *et al.* 1992).

The AKR mouse showed higher *N*-sulphotransferase activities towards several substrates than three other strains (BALB/c, C57/6 and DBA/2) (Shiraga *et al.* 1995).

INTERINDIVIDUAL DIFFERENCES AND GENETIC POLYMORPHISMS IN HUMANS

STUDIES IN VIVO

Paracetamol, an analgesic, is chiefly excreted as sulpho and glucuronic acid conjugates in the urine. In several studies, the ratio of these conjugates in 8- or 24-hour urine showed an approximately 6-fold variation among healthy adult subjects, e.g. 0.25 to 1.41 (Critchley *et al.* 1986), 0.22 to 1.42 (Lee *et al.* 1992) and 0.26 to 1.33 (Esteban *et al.* 1996). It has not yet been elucidated to which extent this variation is due to differences in the levels and properties of sulphotransferases, UDP-glucuronosyltransferases or other factors (e.g. cofactor supply), and whether genetic or environmental factors are more important. On the one hand, the genetic contribution to the total interindividual variation appeared to be low in a study performed in mono- and di-zygotic twins (Nash *et al.* 1984). On the other hand, Chinese excreted a significantly higher percentage of the sulphoconjugate (35.9%) than Indians (28.9%) ; since both populations, university students in Singapore, had a similar life-style, the role of genetic factors is probable (Lee *et al.* 1992). Methyldopa (Campbell *et al.* 1985), salicylamide (Levy and Yamada 1971; Bonham Carter *et al.* 1983), ritodrine (Brashear *et al.* 1988) and diflunisal (Herman *et al.* 1994) are other drugs that have been used to study individual differences in the rate of excretion sulphoconjugates in the urine.

Phenotyping in tissue samples

The level of sulphotransferase activity and immunoreactive protein substantially varies among tissue samples obtained from different subjects (examples in Table 10.6). In liver, gut and lung samples from adult subjects, 4- to 12-fold variations are typical for sulphotransferase activities towards various substrates. Much larger variations have been observed in platelets, which express SULT1A1 and 1A3. Due to their accessibility, platelets have been used extensively for studying intra- and inter-individual variations of sulphotransferases (examples in Tables 10.6 and 10.8).

Platelet SULT1A1 activity (usually measured with 4-nitrophenol as a characteristic substrate) correlates with the activity in other tissues, e.g. brain, intestine and liver (reviewed by Weinshilboum 1990). It also correlates with the platelet level of immunoreactive SULT1A1 protein (Jones *et al.* 1993). Furthermore, high activity in platelets is associated with the high-thermostability phenotype and the *SULT1A1*1* genotype (see below), although this factor cannot explain all variations of the platelet SULT1A1 activity (Raftogianis *et al.* 1999).

The frequency distribution of platelet SULT1A3 activity (usually measured with dopamine as the diagnostic substrate) is normal in newborns but more complex in adults (Pacifici and Marchi 1993). No significant correlation was observed between the level of SULT1A3 activity in platelets and that in liver, small-intestinal mucosa and brain (reviewed by Weinshilboum 1990).

DHEA sulphotransferase activity in liver and duodenum is primarily mediated by SULT2A1. The frequency distribution of this activity in hepatic samples from 94 different subjects was bimodal with approximately 25% of the subjects included in the high-activity group (Aksoy *et al.* 1993). A bimodal distribution of the level of

Table 10.6 Variation of sulphotransferases activity in tissue samples from different subjects[a]

Substrate	Tissue	Number of subjects	Variation factor[b]	References
4-Nitrophenol	Platelets	905	55	Raftogianis *et al.* (1999)
4-Nitrophenol	Platelets	100	>100	Pacifici and Marchi (1993)
4-Nitrophenol	Liver	100	10	Pacifici *et al.* (1997c)
4-Nitrophenol	Lung	96	12	Pacifici *et al.* (1996)
4-Nitrophenol	Jejunum	64	11	Sundaram *et al.* (1989)
4-Nitrophenol	Duodenum	100	8	Pacifici *et al.* (1997c)
4-Nitrophenol	Colon	56	16	Pacifici *et al.* (1997b)
2-Naphthol	Platelets	174	>100	Frame *et al.* (1997)
2-Naphthol	Liver	42	4.8	Pacifici *et al.* (1988)
Dopamine	Platelets	100	>100	Pacifici and Marchi (1993)
Dopamine	Jejunum	64	11	Sundaram *et al.* (1989)
Dopamine	Lung	96	7	Pacifici *et al.* (1996)
DHEA	Liver	94	4.6	Aksoy *et al.* (1993)
Ritodrine	Liver	100	5.4	Pacifici *et al.* (1998)
Ritodrine	Duodenum	100	8	Pacifici *et al.* (1998)
(−)-Salbutamol	Liver	100	4.6	Pacifici *et al.* (1997c)
(−)-Salbutamol	Lung	96	6	Pacifici *et al.* (1996)
(−)-Salbutamol	Duodenum	100	5.6	Pacifici *et al.* (1997c)
Desipramine	Platelets	105	64	Romiti *et al.* (1992)
Desipramine	Liver	118	27	Romiti *et al.* (1992)
Minoxidil	Platelets	100	>100	Pacifici *et al.* (1993a)
Minoxidil	Liver	118	10	Pacifici *et al.* (1993a)

[a] Adult subjects.
[b] Ratio of highest to lowest activity observed (sometimes estimated from figures in the cited article).

immunoreactive SULT2A1 protein was also observed in jejunal tissue samples; however, in this case only 13% of the subjects were in the low SULT2A1 level group (Her *et al.* 1996).

Genetic polymorphisms

Single nucleotide polymorphisms (SNPs) have been detected in each of the ten human *SULT* genes known (reviewed by Glatt and Meinl 2001). The only other genetic polymorphisms reported involve single-base deletions in introns of *SULT1A* and *SULT4A1*. Most of the SNPs observed in *SULTs* do not affect the amino acid sequence, nor are any influences on the expression known. I present here only those polymorphisms that affect the amino acid sequence and have been investigated with regard to their frequency and/or functional aspects.

Raftogianis *et al.* (1997, 1999) have detected 15 different *SULT1A1* alleles, which encode four different amino acid sequences. The frequencies of these alloenzymes in 150 random blood donors are shown in Table 10.7. Two further amino acid sequence variants of SULT1A1 have been reported by other groups (Table 10.7). Subjects with the *SULT1A1*2/*2* genotype showed a 7.7-fold lower platelet enzyme activity than subjects with the *SULT1A1*1/*1* genotype (Raftogianis *et al.* 1997). In the liver,

Table 10.7 Genetic polymorphisms of human *SULT1A1 and 1A2* involving amino acid substitutions

SULT form	Alloenzyme[a]	Amino acid substitution	Allele frequency (Caucasians)[b]
1A1	*1	(reference sequence)	0.674
	*2	Arg213His	0.313
	*3	Met223Val	0.010
	*4	Arg37Gly	0.003
	*V	Ala147Thr, Glu181Gly, Arg213His	0
	*VI	Pro90Leu, Val243Ala	0
1A2	*1	(reference sequence)	0.508
	*2	Ile7Thr, Asn235Thr	0.287
	*3	Pro19Leu	0.180
	*4	Ile7Thr, Arg184Cys, Asn235Thr	0.008
	*5	Ile7Thr	0.008
	*6	Asn235Thr	0.008

[a] The Arabic numerals of alleles/alloenzymes have been introduced by Raftogianis *et al.* (1997, 1999). Roman numerals are working designations for other variants; since they are only known from individual sequences (Jones *et al.* 1995a; Hwang *et al.* 1995), cloning/sequencing errors cannot be ruled out completely.
[b] Frequencies determined in US Caucasians, 150 random blood donors (1A1) or 61 liver samples (1A2) (Raftogianis *et al.* 1999). The frequencies of the 1A1 Arg213His and 1A2 Asn235Thr polymorphisms have also been determined in various other populations (reviewed by Glatt *et al.* 2001).

however, *SULT1A1*2* was not consistently associated with a low level of enzyme activity (Raftogianis *et al.* 1999), suggesting a tissue-dependent modulation of the SULT1A1 activity by the genotype and/or a contribution of other sulphotransferase forms to the investigated activity in the liver. Differences in enzyme kinetic parameters have been observed between cDNA-expressed SULT1A1 alloenzymes (Raftogianis *et al.* 1999). With all promutagens studied (1-HMP, *N*-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, OH-AAF, 2-nitropropane), SULT1A1*His^{213} (*2 and *V) alloenzymes were less active than the wild-type form (*1) (Glatt *et al.* 2001).

A total of 13 different *SULT1A2* alleles, encoding six alloenzymes, have been reported (Ozawa *et al.* 1995b; Zhu *et al.* 1996; Raftogianis *et al.* 1999). The two most common alloenzymes differ by two amino acid exchanges (Ile7Thr and Asn235Thr). In particular, the exchange in codon 235 appears to be functionally important; it strongly decreases the affinity for the standard substrate, 4-nitrophenol (Brix *et al.* 1998; Raftogianis *et al.* 1999; Meinl *et al.* 2001a). Expression of SULT1A2*1 and *5 (having Asn in position 235) in *S. typhimurium* led to a stronger activation of the promutagens OH-AAF, 2-hydroxylamino-5-phenylpyridine and 1-HMP than expression of SULT1A2*2 and *6 (Thr in position 235) (Meinl *et al.* 2001a).

The *SULT1A2* alleles are in a linkage disequilibrium with the alleles of the neighbouring *SULT1A1* gene (Raftogianis *et al.* 1999; Engelke *et al.* 2000a). Usually alleles that encode the high-activity enzyme variants (SULT1A1*Arg^{213} and 1A2*Asn^{235}) are associated with each other; accordingly, the alleles encoding the low-activity enzyme variants (SULT1A1*His^{213} and 1A2*Thr^{235}) form another common haplotype.

Several rare nucleotide exchanges in the *SULT2A1* gene (some leading to amino acid exchanges) were detected (Table 10.1), but could not be related to the levels of

enzyme activity or immunoreactive protein in hepatic samples (Wood *et al.* 1996). Mutant proteins (Thr90Ser and/or Leu159Val) expressed in COS-1 cells did not differ in their K_m values for PAPS and DHEA from the wild-type protein.

For SULT1B1, two different cDNA sequences have been published, but not yet verified; they would imply an amino acid exchange, Glu186Gly (Table 10.1). Although the pattern of platelet SULT1A3 activity in families suggests that genetic polymorphisms affect this enzyme (Price *et al.* 1988), these have not yet been elucidated. Since the identical cDNA sequence was invariably isolated from human tissues in several laboratories, it is probable that critical polymorphism(s) are located outside the coding region or even outside the *SULT1A3* gene.

SULT genotypes and phenotypes in different ethnic groups

The percentage of sulphoconjugate among the urinary metabolites of paracetamol is higher in Chinese than in Indians (Lee *et al.* 1992). The mean platelet sulphotransferase activities towards phenol, 4-nitrophenol and 2-naphthol (which are mediated by SULT1A1) are nearly 2-fold higher in African-Americans than in Caucasians, whereas the activities towards dopamine (mediated by SULT1A3) are similar in both ethnic groups (Anderson and Jackson 1984; Frame *et al.* 1997). Significant differences in platelet SULT1A1 as well as 1A3 activities were observed between Finns and Italians (Brittelli *et al.* 1999). A pronounced sexual dimorphism for the SULT1A1 activity was detected in Finns but not in Italians. The finding is unusual, since sexual dimorphisms of sulphotransferases have been detected only very rarely in the human. It is likely that a special life-style, rather than genetic factors, was involved in the aberrant platelet SULT1A1 activity in Finnish males. A role for non-genetic influences is also demonstrated by a study conducted in Italy: platelet SULT1A1 and 1A3 activities showed seasonal rhythms; they were ~ 4-fold higher in summer than in winter (Marazziti *et al.* 1995).

Lower frequencies of $SULT1A1^{*}His^{213}$ alleles have been observed in Chinese (0.110) and Japanese (0.168) than in Nigerians (0.269) and various Caucasian populations (0.311 to 0.365); $SULT1A1^{*}Val^{223}$ (allele $^{*}3$) occurs in Caucasians in the USA but has not been found in Caucasians in Germany nor in Chinese; it is rather common in African Americans (allele frequency 0.229) (reviewed by Glatt *et al.* 2001).

Associations between disease, age and SULT genotype or phenotype

In several studies, a decrease in platelet sulphotransferase (usually SULT1A1) activity was observed in patients suffering from migraine (Table 10.8). Aberrant platelet sulphotransferase activities were also detected in patients with certain psychiatric disorders and in colon tumour patients. Genotyping of *SULTs* is rather new and therefore has only been used in very few epidemiological studies (Table 10.8); associations between *SULT* genotype and longevity, obesity and certain cancer types have been reported, but require corroboration in prospective studies.

Table 10.8 SULT1A1 phenotype and genotype: association with diseases and other health-related parameters

Investigated disease/factor	Test parameter	Observation
Migraine	Sulphotransferase activities in platelets	Decreased 1A1 activity but normal 1A3 activity (Davis *et al.* 1987; Marazziti *et al.* 1996; Alam *et al.* 1997), especially in patients with diet-induced migraine (Littlewood *et al.* 1982) Divergent result: normal 1A1 activity but decreased 1A3 activity (Jones *et al.* 1995b)
	Urinary paracetamol metabolites, ratio of sulphoconjugate to glucuronide	Not significantly different from controls (Alam *et al.* 1997)
Psychiatric disorders	Sulphotransferase activities in platelets	1A1 and 1A3 activities elevated in patients with obsessive-compulsive disorder and manic patients; normal in dysthymic patients, bipolar depressives and patients with panic disorder; decreased in unipolar depressives (Marazziti *et al.* 1996)
Hyertension (in males)	SULT1A1 Arg213His and SULT1A2 Asn235Thr polymorphisms	Frequencies not significantly different from controls (Engelke *et al.* 2000b)
Obesity (in males)	SULT1A1 Arg213His and SULT1A2 Asn235Thr polymorphisms	Increased frequencies of 1A1*His^{213} and 1A2*Thr^{235} alleles (Engelke *et al.* 2000b)
Longevity	SULT1A1 Arg213His polymorphism	Decrease in the frequency of the 1A1*His^{213} allele with increasing age (Coughtrie *et al.* 1999)
Colon cancer	2-Naphthol (SULT1A1) sulphotransferase activity in platelets	Increased frequency of the slow-activity phenotype (57% versus 40% in the controls) (Frame *et al.* 1997)
Prostate cancer	SULT1A1 Arg213His polymorphism	Frequencies not significantly different from controls (Steiner *et al.* 2000)
Breast cancer	SULT1A1 Arg213His polymorphism	Frequencies not significantly different from controls; however, association of the 1A1*Arg^{213} allele(s) with early onset of breast tumour and with the likelihood of having other tumours in addition to breast cancer (Seth *et al.* 2000)

Competition of sulphotransferases with other enzymes

Nearly every substrate of a sulphotransferase is also metabolised by UDP-glucuronosyltransferases. The sulphotransferases show lower apparent K_m values for many phenolic substrates than the UDP-glucuronosyltransferases (Mulder and Meerman 1978). Besides, the supply of the cofactor PAPS becomes limiting more readily than that of UDP-glucuronic acid. Therefore, it is not surprising that a decrease in the ratio of sulphonated metabolites to glucuronidated metabolites in urine and/or bile was frequently observed when the dose of the xenobiotic was increased, e.g. for phenol,

harmol, salicylamide, 4-nitrophenol, 1-naphthol and paracetamol in the rat (Mulder and Meerman 1978; Weitering *et al.* 1979; Hjelle *et al.* 1985; Kane *et al.* 1991; Kim *et al.* 1995) or perfused rat liver (Minck *et al.* 1973; Mulder *et al.* 1975); for paracetamol (Liu and Klaassen 1996c), *o*-phenylphenol (Bartels *et al.* 1998) and phenol (Kenyon *et al.* 1995) in the mouse; for 4-nitrophenol in the perfused foetal sheep liver (Ring *et al.* 1996); for phenol and 1-naphthol in various non-human primates (Mehta *et al.* 1978) and for paracetamol in the human (Clements *et al.* 1984). However, other dosage effects have also been observed, although much more rarely. The proportion of xamoterol excreted as sulphoconjugate remained constant over a 100-fold dose range in the dog (Groen *et al.* 1988). When the dose of diflusinal administered to humans was increased, the recovery of urinary diflunisal sulphate was increased (Loewen *et al.* 1988). In an *in-situ* intestinal loop preparation of the rat, glucuronidation rather than sulphonation became saturated at relatively low doses of various phenols, which were administered into the gut lumen (Goon and Klaassen 1990, 1991).

The trend to develop highly potent drugs that require a very low dosage may lead to an increase in the importance of the sulphonation pathway in pharmacology. Also in environmental toxicology, which usually deals with low exposure levels, the sulphonation pathway may be more important than in long-term carcinogenicity studies in animals, for example, where high doses are used. This discrepancy between human-relevant and animal-experimental situation is problematic because sulphonation involves a much higher risk of the formation of a reactive metabolite than does glucuronidation.

Competition between sulphotransferases and alcohol dehydrogenases is toxicologically significant with benzylic alcohols (L. Ma and H. R. Glatt, unpublished results). *N*-Acetylation and *N*-sulphonation are detoxification pathways for various aromatic amines, whereas *O*-acetylation and *O*-sulphonation of the corresponding hydroxylamines represent alternative activation pathways.

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