

Mammalian sulfoconjugate metabolism

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Abstract. Sulfoconjugates occur ubiquitously as sulfopolysaccharides, sulfolipids and sulfoproteins. A variety of sulfotransferases catalyze the sulfation process with 3'-phosphoadenosine 5'-phosphosulfate as the sulfate donor. Sulfatases that catalyze the desulfation of different sulfoconjugates are known to be deficient in a number of genetic storage disorders.

Keywords. Sulfoconjugates; sulfotransferases; sulfatases; genetic disorders.

Introduction

Numerous reviews of this subject have been published dating as far back as 1945 (Lugg, 1945). In recent years, there has been wide-spread interest in the study of sulfoconjugates and their metabolism (for selected reviews, see Gregory and Robbins, 1960; Roy, 1960a; Balasubramanian and Bachhawat, 1970; Dodgson and Rose, 1970, 1975; Farooqui, 1980, 1981). This article deals with the more recent aspects.

The importance of sulfur containing compounds stems from the fact that they participate in virtually all living processes. It is present in organisms as constituents of proteins, coenzymes and as major cellular metabolites like glutathione. Although in some cases, sulfate esters may serve no physiological role other than sulfur storage *e.g.* choline O-sulfate), the sulfated glycosaminoglycans of connective tissue or the sulfatides of the nervous system and most cell membranes are excellent examples of their structural function. Protein sulfation of tyrosine residues has been found to occur in most cell types in culture and in many tissues (Huttner, 1982). The recent discovery of a large number of secreted sulfoproteins in plasma (Hille *et al.*, 1984) and neuronal axons (Stone *et al.*, 1984) and the finding that sulfoproteins meet critical requirements for consideration as secretable fast-transported proteins has generated tremendous interest in the study of sulfate metabolism and related enzymes. Further, the drastic reduction in tyrosine O-sulfated proteins in rat fibroblasts in culture infected with Rous sarcoma virus (Liu and Lipman, 1984) in contrast to the increase of tyrosine O-phosphate in a number of malignantly transformed cells are indicative of a transformation mediated change in sulfoprotein level. The sulfated enkephalins present in brain (Unsworth *et al.*, 1982) may act as an inactive pool of enkephalins while the sulfation of neural cell adhesion molecule may have a role in its neurone-neurone and nerve-muscle adhesions (Sorkin *et al.*, 1984). Sulfoconjugation may result in the masking of the physiological activity of certain compounds (*e.g.* steroid hormones, vitamin D, etc.) or serve as a detoxification mechanism (sulfoconjugation of phenols). On the other hand, the presence of cholesterol sulfate in the plasma membrane of spermatozoa may help in sperm capa-

Abbreviations used: PAPS, 3'-Phosphoadenosine 5'-phosphosulfate; *Mr*, molecular weight; MLD, metachromatic leukodystrophy.

citation and fertilization (Langlais *et al.*, 1981), while the sulfation of hexosamines present in lutropin (Parsons and Pierci, 1980) may have a protective effect as it resists enzymatic deglycosylation much like the placental gonadotropins which are protected by peripheral sialic acids.

The discovery of human genetic disorders of sulfate metabolism has led to a major boom in the research on the enzymes that are involved in the synthesis (sulfotransferases) and degradation (Sulfo-hydrolases) of sulfated compounds.

sulfotransferases

Sulfotransferases catalyze the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate (active sulfate or PAPS) to a suitable acceptor containing a phenolic hydroxyl group. There are a variety of sulfotransferases present in animal system. The most widespread among them are arylsulfotransferases present in the soluble fraction of cell preparations where the enzymatic system responsible for the biosynthesis of PAPS exists. Of the arylsulfotransferases, phenolsulfotransferase, steroid sulfotransferase and tyrosylprotein sulfotransferase have been well studied in mammalian system.

Phenolsulfotransferases

The presence of phenolsulfotransferases has been demonstrated in brain, liver, kidney, adrenals, jejunum (Bostrom and Wengle, 1967) and platelets (Hart *et al.*, 1979) of man. There are two types of phenolsulfotransferases designated the P-form which sulfates exogenous phenolic compounds and the M-form that sulfates endogenous phenolic substances such as monoamines (Rein *et al.*, 1982). Both forms were present in human brain and platelets (Young *et al.*, 1985). The P-form of the enzyme may have a role to play in neutralizing dietary phenolic compounds as patients with dietary migraine had significantly lower levels of platelet phenol-sulfotransferase activity than either migrainous patients without a history of dietary provocation or normal controls. The P-form of the enzyme was more severely involved than the M-form (Littlewood *et al.*, 1982). It is suggested that the primary function of both forms of phenolsulfotransferase in brain may not be connected with monoamine metabolism, but may primarily be to protect the brain from low circulating levels of potentially noxious dietary phenols (Rein *et al.*, 1984). Recently Whitemore *et al.* (1985) purified the M-form of phenolsulfotransferase from human brain to about 20,000 fold. The enzyme had a molecular weight (M_r) of 250,000 by gel filtration and it had no phenolsulfating activity. 4-Methoxytyramine was the most preferred substrate. A similar enzyme purified to 272-fold from human brain cortex (Yu *et al.*, 1985) had a M_r of 62,000 and high affinity towards dopamine and m-tyramine. It was moderately active towards noradrenalin and *p*-tyramine while serotonin was a poor substrate.

Steroid sulfotransferases

This group of enzymes catalyze the sulfation of alcoholic hydroxyl groups. Several of them are known that sulfate estrone, estradiol, testosterone, androstenediol, dehy-

droepiandrosterone (Marcus *et al.*, 1980), pregnenolone (Singer *et al.*, 1980) and cholesterol (Lin and Horowitz, 1980). Recently, Tseng *et al.* (1985) have partially purified a human placental estrogen sulfotransferase. It had a pI of 5.8 and M_r of 68,000 on sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Even though the enzyme could sulfate estrone, estriol and dehydroepiandrosterone, estradiol was the best substrate. The effect of androgens and estrogens on rat hepatic bile acid sulfotransferase has been investigated by Kirkpatrick *et al.* (1985). The former had a suppressing effect while estrogens stimulated the sulfotransferase. Consequently, female rats had a 3-fold higher activity than males (Chen and Thaler, 1984). Similar effects were observed on a sulfotransferase described in rabbit uterine endometrium (Munakata *et al.*, 1985). Recently, Chen and Segel (1985) purified a bile salt sulfotransferase from human liver to about 760-fold and showed it to be an -SH enzyme with a pI of 5.2 and M_r of 67,000. The sulfotransferase had no activity towards estrone, testosterone or phenol.

Tyrosylprotein sulfotransferase

Even though the presence of tyrosylprotein sulfotransferase had been demonstrated as early as 1966 (Banerjee and Roy, 1966), the real interest was awakened only recently when Huttner (1982) demonstrated the protein sulfation on tyrosine residues of virtually all cell lines and tissues. This was followed up by a large number of investigators unraveling the secretory nature of most sulfoproteins. A tyrosylprotein sulfotransferase has been described by Lee and Huttner (1985) in the Golgi membrane fraction of a bovine adrenal medulla homogenate. The enzyme could sulfate tyrosine residues of tubulin in the presence of a nonionic detergent. A polymer, (glutamyl, alanyl, tyrosinyl) served as a high affinity substrate. A similar enzyme has been described by Vargas *et al.* (1985) in a crude microsomal fraction from rat cerebral cortex. Various cholecystokinin fragments or derivatives could act as sulfate acceptors. Peptides with an N-terminal tyrosine residue (*e.g.* enkephalins or CCK-7) were not sulfated. The importance of these sulfoproteins are yet to be worked out. Other than as secretory proteins, they may be involved in the overall function of the protein in an identical manner as phosphoproteins.

Sulfotransferases such as choline sulfotransferases are involved in the sulfation of choline, dimethylaminoethanol and dimethylethylaminoethanol (Fitzgerald, 1973; Kaji and Gregory, 1959; Nissen and Benson, 1961). There are sulfotransferases that sulfate aromatic amines such as aniline or 2-naphthylamine (Roy, 1960b). In rat hepatocytes sulfation of the phenolic hydroxyl group of iodothyronines facilitate the deiodination of these compounds (Otten *et al.*, 1983). Sulfolipid sulfotransferases are present in the brain (Mckhann *et al.*, 1965; Balasubramanian and Bachhawat, 1965; Cumar *et al.*, 1968), kidney (Fleischer and Smigel, 1978) and testes where it is involved in spermatogenesis (Lingwood, 1985). The rat kidney galactocerebroside sulfotransferase has been purified to apparent homogeneity. It was hydrophobic in nature with a M_r of 64,000 and pI of 5.1 with essential arginine residues at the active site (Tennekoon *et al.*, 1985).

Different sulfotransferases are present for the sulfation of glycosaminoglycans resulting in the formation of chondroitin 4- and 6-sulfates, heparan sulfate (Suzuki *et al.*, 1961) and dermatan sulfate (Hasegawa *et al.*, 1961). Balasubramanian and Bachhawat (1964) demonstrated the presence of a mucopolysaccharide sulfotrans-

ferase in rat brain cytosol that could sulfate a variety of substrates. Habuchi and Miyashita (1982) separated chondroitin 4- and 6-sulfotransferase from 14-day old chick embryos by gel filtration and showed that addition of polyamines and basic proteins to the incubation medium had an activating effect on both transferases.

Investigations by various workers (Young, 1973; Richmond *et al.*, 1972, 1973; DeLuca *et al.*, 1973) have localized the glycosaminoglycan sulfotransferases on the Golgi and concluded that sulfating enzymes were located together with polymerizing enzymes in an enzyme complex so that sulfation of the heteropolysaccharide chain proceeded during or immediately following polymerization. Two keratan sulfotransferases have been partially purified from isolated bovine corneal cells and shown to have a M_r each of 220,000 and 140,000, respectively. The preparation did not show any chondroitin sulfotransferase activity and both enzymes acted in a co-operative manner on keratan sulfate fragments, which was the best preferred substrate (Rueter and Kresse, 1984). Evidence for the recognition of the N-acetylgalactosamine residues in the non-reducing terminal regions of oligosaccharides by the transferases was revealed by Delfert and Conrad (1985) while working with microsomal preparations from cultured chick embryo chondrocytes which showed a high rate of sulfation at the reducing ends of shorter oligosaccharides, but decreased with increasing chain length.

On the whole, sulfotransferases are involved in the synthesis of all the biologically occurring sulfoconjugates and thus participate in the overall functioning of the system.

Sulfohydrolases

The class of enzymes that hydrolyze esters of sulfate and related sulfoconjugates are collectively called sulfohydrolases or sulfatases. The role of sulfatases is profound in maintaining the concentrations of various sulfoconjugates in physiological fluids or the turnover of sulfated compounds in tissues, in cell-mediated cytolysis or in the breakdown of dietary sulfate esters. If the recent developments in the study of sulfated cell surface glycosaminoglycans (Wieland, 1982; Dietrich, 1984; Hook *et al.*, 1984) are any indication, sulfohydrolases have a very important role to play in cell growth and cell division.

Sulfatases may be generally classified into alkyl, aryl and carbohydrate sulfatases.

Alkylsulfatases

Sulfatases that hydrolyze the sulfate esters of simple alcohols such as methanol, ethanol, etc. are termed as alkylsulfatases. Although the formation of alkylsulfate esters in mammalian tissues is well known (Vestermark and Bostrom, 1959; Spencer, 1960), the corresponding hydrolases have not been described so far except a choline sulfatase in higher fungi and some bacteria.

Steroid alkylsulfatases such as dehydroepiandrosterone sulfatase, a microsomal enzyme rich in placenta and testes (French and Warren, 1967) are widely known. Sulfatases that hydrolyze sulfate esters of estrone, pregnenolone, testosterone, cholesterol and *p*-nitrophenol have been described in rat brain (Iwamori *et al.*, 1976a,b). Recently, Milewich *et al.* (1984) demonstrated the presence of steroid sulfatase in more than 30 different tissues from mice.

Steroid sulfatase is involved in the mechanism of sperm capacitation and ovum penetration (Langlais *et al.*, 1981). Studies by Ropers and Wieberg (1982) suggested that the steroid sulfatase activity in cultured cells of wood lemming is directly correlated to the number of X-chromosomes and that the sulfatase gene is also X-linked and not subject to inactivation while in mice the steroid sulfatase gene is subject to the normal pattern of X-inactivation (Crocker and Craig, 1983).

The insoluble nature of steroid sulfatase had hampered attempts at purification until Noel *et al.* (1983) and Burns (1983) purified the enzyme 100- and 300-fold, respectively from human placental microsomes. The preparation obtained by Noel *et al.* (1983) was heterogeneous in nature and it could hydrolyze sulfate esters of cholesterol, dehydroepiandrosterone and *p*-nitrophenol while the sulfatase purified by Burns was homogeneous and hydrolyzed dehydroepiandrosterone sulfate and *p*-nitrophenylsulfate. Both preparations exhibited a M_r range of 72-74,000 daltons.

There is evidence to suggest that different sulfatasases are responsible for the hydrolysis of the sulfate esters of estrone, dehydroepiandrosterone, cholesterol and *p*-nitrophenol (Eto *et al.*, 1974; Mathew and Balasubramanian, 1982; Milewich and Garcia, 1985; Prost *et al.*, 1985). Further Simard *et al.* (1985) have separated steroid sulfatase and arylsulfatase C (4-methylumbelliferyl or *p*-nitrophenylsulfatase) by cellogel electrophoresis and demonstrated that they are immunologically different. It is possible that steroid sulfatase could hydrolyze the artificial substrates of arylsulfatase C and not *vice versa* (Horwitz *et al.*, 1986; Simard *et al.*, 1985). Steroid sulfatase is stimulated by phosphatidylcholine (McNaught and France, 1980). The significantly lower level of this lipid in steroid sulfatase deficient placenta (McKee and France, 1983) suggest that phosphatidylcholine may have a role to play in Steroid sulfatase activity.

Arylsulfatasases

Arylsulfatasases are the most widely distributed of all the sulfohydrolases. The arylsulfatase present in natural killer cells are involved in the lysis of the target cell membrane (Zucker-Franklin *et al.*, 1983). They are classified into arylsulfatase A, B and C. The first two are soluble lysosomal enzymes and they are inhibited by phosphate and sulfate while arylsulfatase C is an insoluble microsomal enzyme which is not affected by phosphate or sulfate. Many of the arylsulfatasases have been purified and identified (table 1).

Arylsulfatase A: Arylsulfatase A is perhaps the best studied of all the arylsulfatasases. It has been purified from a variety of sources by a large number of workers (Hess, 1983; Waheed and Van Etten, 1985a; Laidler *et al.*, 1985; Nakamura *et al.*, 1984). Sulfatase A is an acidic glycoprotein (Balasubramanian and Bachhawat, 1975) with a pI of 4.6-4.8 (Eto *et al.*, 1974). It has a monomeric M_r of 102,000-107,000 and a carbohydrate content of 15-25%. The enzyme is rich in proline, aspartic and glutamic acids and hydrolyzes sulfate esters such as cerebroside 3-sulfate, seminolipid, psychosine sulfate, ascorbic acid 2-sulfate and tyrosine O-sulfate (Farooqui, 1980).

The arylsulfatase A purified from human liver (James and Austin, 1979) contained two subunits of M_r 69,000 and 57,000 which on further studies proved similar suggesting that the smaller subunits might have been derived from the larger one. However, an arylsulfatase A purified from human urine (Laidler *et al.*, 1985)

Table 1. Properties of arylsulfatases.

	Arylsulfatase A	Arylsulfatase B	Arylsulfatase C
Localization	Lysosomal	Lysosomal	Microsomal
Nature	Glycoprotein	Glycoprotein	Glycoprotein
Isoelectric point	3.8	8.0	8.1
Molecular weight	100,000	38,000	280,000
Sedimentation coefficient	6.5	4.5	4.85
Kinetics	Abnormal	Normal	Normal
pH optimum	Acidic	Acidic	Alkaline
Effect of sulfate	Competitive inhibition	Non-competitive inhibition	No effect
Effect of cyanide	No effect	No effect	Inhibition
Effect of silver	Marked inhibition	Slight stimulation	Inhibition
Synthetic substrates	Nitrocatechol sulfate Methylumbelliferyl sulfate Cerebroside 3-sulfate Tyrosine O-sulfate Ascorbic acid 2-sulfate Lactosyl sulfate	Nitrocatechol sulfate Methylumbelliferyl sulfate UDP-N-acetylgalactosamine 4-sulfate	<i>p</i> -nitrophenyl sulfate <i>p</i> -acetylphenyl sulfate Steroid sulfates?
Natural substrates	Sulfogalactosyl sphingosine Sulfogalactosyl glycerolipid		

exhibited peptide subunits of 63,500 and 54,500 daltons. The subunits were immunologically similar and their nonidentity is attributed to differing extents of glycosylation (Waheed *et al.*, 1983). The enzyme was both phosphorylated and sulfated (Waheed and Van Etten, 1985b) and had essential arginine residues (James, 1979). It exhibits abnormal kinetics and the activity is linear only upto 10–15 min due to a substrate induced inactivation of the enzyme, which is accompanied by the binding of a sulfate ion from the substrate (Prosser and Roy, 1980; Waheed and Van Etten, 1980) followed by reactivation by sulfate in the presence of the substrate. Roy (1985) described arylsulfatase A as a hysteretic enzyme and proposed a progress curve for the reaction catalyzed by the enzyme.

An atypical arylsulfatase purified from chicken brain (Farooqui and Bachhawat, 1972) hydrolyzed cerebroside 3-sulfate, but had properties of both arylsulfatase A and B. The possibility of a single enzyme hydrolyzing the substrates of both arylsulfatase A and B cannot be ruled out as only a single arylsulfatase was present in chicken brain (Mathew and Balasubramanian, 1984).

Das and Bishayee (1980) separated sheep brain arylsulfatase A into high and low-uptake forms. The former was susceptible to alkaline phosphatase indicating the presence of phosphate residues. The enzyme purified from human liver exhibited molecular heterogeneity (Sarafian *et al.*, 1984). Sialic acid residues were partly responsible as neuraminidase treatment could bring down the heteromer bands to only 3. Similar studies in lung tumors (Nakamura *et al.*, 1984) and bladder cancer patients (Mitsuhashi *et al.*, 1984) suggest an increased level of microheterogeneity of arylsulfatase A in transformed tissues. Recently, (Waheed and Van Etten, 1985b) demonstrated the variations in carbohydrate content of arylsulfatase A in normal and carcinoma cell lines and concluded that the carbohydrate free enzymes were essentially similar with a M_r of 59,000 daltons and attributed this to an elevated level of glucosyltransferase.

Arylsulfatase B: Arylsulfatase B is similar to arylsulfatase A in being a soluble lysosomal glycoprotein exhibiting molecular heterogeneity (J. Mathew and A. S. Balasubramanian, unpublished results). It is cationic in nature and has been purified from a variety of sources (Agogbua and Wynn, 1976; Farooqui and Roy, 1976; McGovern *et al.*, 1982; Weller and Austen, 1983). Multiple forms of arylsulfatase B has been reported from ox tissues (Bleszynski *et al.*, 1969), human placenta (Gniot-Szulzycka, 1972), human brain and liver (Harzer *et al.*, 1973) which can be separated by chromatographic and electrophoretic methods. The nature and significance of these multiple forms are not clear at present even though sialic acid (Harris *et al.*, 1982), phosphate residues (Uehara *et al.*, 1983) and varying carbohydrate content have been implicated (Farooqui and Roy, 1976).

Sulfatase B from ox liver and brain is shown to be rich in proline and basic aminoacids. The enzyme has large amounts of tyrosine and an essential histione residue in its active site. The ability of arylsulfatase B to hydrolyze UDP-N-acetylgalactosamine 4-sulfate was first demonstrated by Fluharty *et al.* (1975) and later confirmed by using homogeneous preparations of arylsulfatase B (Farooqui, 1976). The enzyme could also hydrolyze glucosamine 4,6-disulfate, but not glucosamine 6-sulfate.

A minor anionic form of arylsulfatase B present in significant amounts only in brain, but not in other tissues like liver, kidney, testes and placenta was isolated by

Stevens *et al.* (1977). The anionic nature of the enzyme is due to its phosphoprotein nature (J. Mathew and A. S. Balasubramanian, unpublished results). The anionic B purified from monkey brain had a M_r of 30,000 and a phosphate content of about 7.0 mol of inorganic phosphorus per mol of protein (Lakshmi and Balasubramanian, 1984).

Arylsulfatase B is located on chromosome 5 as compared to chromosome 22 of arylsulfatase A and the fact that each enzyme is expressed independently provides evidence against any common structural feature between the two enzymes (DeLuca *et al.*, 1979).

Cancer associated modification of arylsulfatase B has been reported in lung tumors (Gasa *et al.*, 1981) which is speculated to be due to transformation mediated elevation of sialyltransferase and protein kinase (Niinobe *et al.*, 1979; Kottgen *et al.*, 1978) resulting in the phosphorylation of arylsulfatase B both on its protein and carbohydrate moiety (Gasa and Makita, 1983). Human brain anionic B was taken up rapidly by multiple sulfatase deficient fibroblasts whereas arylsulfatase B was practically not taken up into the cells. Alkaline phosphatase treatment could abolish this high-uptake activity without diminishing its catalytic activity (Kureha and Eto, 1983).

Steckel *et al.* (1983) showed that arylsulfatase B synthesized and secreted as a 64,000 M_r precursor was processed to a 62,000 dalton intermediate and mature forms of M_r 47,000, 40,000 and 31,000 daltons. The processing of 62,000 M_r intermediate to the pair with M_r 40,000 and 31,000 depended on cysteine proteinases (Steckel *et al.*, 1985).

Arylsulfatase C: The presence of arylsulfatase C was first demonstrated by Dodgson *et al.* (1954) in rat liver microsomes and later in various human tissues (Dodgson *et al.*, 1956). The enzyme is located in the endoplasmic reticulum and the nuclear outer membrane (Zemelman *et al.*, 1985). The numerous similarities observed between mammalian arylsulfatase C and steroid sulfatase had led to the hypothesis that a single enzyme is responsible for both activities (Dolly *et al.*, 1972; Balasubramanian, 1976; Iwamori *et al.*, 1976b). The chaotropically solubilized arylsulfatase C from monkey brain has been shown to require a dialyzable activator which had no effect on the estrone sulfatase activity from the same source (Lakshmi and Balasubramanian, 1979). This led to the possibility of separate enzymes responsible for the hydrolysis of *p*-nitrophenylsulfate and estrone sulfate. Making use of the hydrophobic nature of arylsulfatase C, it could be partially separated from estrone sulfatase by hydrophobic interaction chromatography (Mathew and Balasubramanian, 1982).

Persistent attempts by workers to purify arylsulfatase C were unsuccessful until Moriyasu *et al.* (1982) obtained a homogeneous preparation from rat liver microsomes. The native enzyme had a M_r of 280,000 by gel filtration while the monomeric M_r was 72,000. It was a glycoprotein with a pI of 8.1. The carbohydrate part of the enzyme was rich in mannose and N-acetylglucosamine. It is not known whether steroid sulfatase activity is associated with this preparation.

The physiological substrate of arylsulfatase C is not known. The enzyme purified from placenta (Noel *et al.*, 1983; Burns, 1983) has steroid sulfatase activity. Arylsulfatase C purified from human skin fibroblasts (Simard *et al.*, 1985) exhibited two immunologically different bands on cellophore electrophoresis. Both forms hydrolyzed

the artificial substrate 4-methylumbelliferyl sulfate, while only the slow form showed any steroid sulfatase activity. Hence it is possible that the fast form is the 'true' arylsulfatase C while the slow form is steroid sulfatase.

Carbohydrate sulfatases

A large number of carbohydrate sulfate esters such as lactose 6-sulfate, sulfated glycosaminoglycans, ascorbic acid 2-sulfate, N-acetylglucosamine 6-sulfate, cellulose polysulfate, etc. are known at present and the sulfatases that hydrolyze the sulfate part of these compounds are collectively referred to as glycosulfatases or carbohydrate sulfatases. The enzyme is present in mollusks, bacteria and molds (Dodgson and Rose, 1975). Habuchi *et al.* (1979) have identified 5 glycosaminoglycan sulfatases from rat skin. They are N-acetylglucosamine and N-acetylgalactosamine 6-sulfate sulfatase, N-acetylgalactosamine 4-sulfate sulfatase, heparan sulfate N-sulfatase and iduronate sulfatase. The deficiency of any one of these may give rise to a specific storage disorder. Iduronate sulfatase has been purified from human liver to 80-fold and shown to be of heterogeneous nature. It had a pH optimum of 4.0 and was free of other sulfatases (Yutaka *et al.*, 1982).

Recently, Benitez and Halver (1982) purified an ascorbic acid 2-sulfatase from rainbow trout. It had a M_r of 117,500 and a pH optimum of 6.0 and was structurally identical to human arylsulfatase A. Placental N-acetylgalactosamine 6-sulfate sulfatase has been purified by Glossl *et al.* (1979) and shown it to be a glycoprotein of M_r 100,000 by gel filtration. It had a monomeric M_r of 78,000. A similar enzyme, N-acetylglucosamine 6-sulfate sulfatase has been purified from human urine and shown to be a glycoprotein of M_r 97,000 with a pH optimum of 5.5. It is also active towards glucose 6-sulfate (Basner *et al.*, 1979a). On the other hand, heparan sulfate N-sulfatase, a lysosomal enzyme purified from human urine had a pH optimum of 4.5, pI of 4.7 and a M_r of 120,000 (Friedman and Arsenis, 1972, 1974).

Genetic disorders of sulfate metabolism

The genetic disorders of sulfate metabolism are characterized by the abnormal accumulation and excretion of different metabolites corresponding to the type of defect (table 2). The accumulation of these compounds have been demonstrated in liver, kidney and brain. Mental retardation is invariably associated with many of these disorders.

Metachromatic leukodystrophy or arylsulfatase A deficiency

The physiological substrate of arylsulfatase A, cerebroside 3-sulfate accumulates in brain, peripheral nerve, kidney and other visceral organs of the patient as a result of this genetic disorder. It is an autosomal recessive disorder classified into late infantile, juvenile and adult disorders. The first demonstration of arylsulfatase A deficiency in metachromatic leukodystrophy (MLD) came from the work of Austin *et al.* (1963). Diagnosis is carried out by measuring the urinary excretion of cerebroside 3-sulfate, presence of metachromatic deposits in sural nerve and by the deficiency of arylsulfatase A in urine, leukocytes or skin fibroblasts (Brady, 1978). Several studies

Table 2. Storage diseases associated with the deficiency of sulfatases.

Disease	Accumulated product	Enzyme deficient
Metachromatic leukodystrophy	Cerebroside 3-sulfate	Arylsulfatase A (cerebroside 3-sulfatase)
Maroteaux-Lamy syndrome	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
Hunter's syndrome	Dermatan sulfate	Iduronate sulfatase
Sanfilipo syndrome A	Heparan sulfate	Heparan N-sulfatase
Morquio's syndrome	Heparan sulfate	N-acetylgalactosamine 6-sulfatase
Placental sulfatase deficiency	Keratan sulfate	Steroid sulfatase
Multiple sulfatase deficiency	Steroid sulfate	Arylsulfatase A, B and C, steroid sulfatase, heparan N-sulfatase, iduronate 2-sulfate sulfatase, N-acetylglucosamine 6-sulfate sulfatase, N-acetylgalactosamine 6-sulfate sulfatase
I-Cell disease and pseudo-Hurler polydystrophy	Cerebroside 3-sulfate Steroid sulfates Mucopolysaccharides Mucopolysaccharides and glycolipids	Almost all lysosomal enzymes deficient in fibroblasts; enzymes present extracellularly

have shown that MLD patients have a mutated sulfatase A which is immunologically cross-reactive with monospecific antibody, but had very little enzyme activity (Neuwelt *et al.*, 1973; Shapira and Nadler, 1975).

There is marked demyelination in the nervous system of patients suffering from MLD. Recent studies have demonstrated pseudoarylsulfatase A deficiency in fibroblasts from healthy individuals (Chang and Davidson, 1983). The enzyme was similar to normal arylsulfatase A except for a slight differences in pI value. Low arylsulfatase A activity has been reported in leukocytes and skin fibroblasts of healthy members of families having an MLD patient (Dubois *et al.*, 1977; Butterworth *et al.*, 1978). In the late infantile form of MLD, sulfatase A is deficient in brain, liver and spleen (Yamaguchi *et al.*, 1975).

Maroteaux-Lamy syndrome (arylsulfatase B deficiency)

Arylsulfatase B deficiency is characterized by severe skeletal deformities, gross corneal opacity, hepatosplenomegaly, growth retardation and increased urinary excretion of dermatan sulfate as N-acetylgalactosamine 4-sulfate residues of dermatan sulfate are the physiological substrate of the enzyme. Recent studies (Gasper *et al.*, 1984) have indicated that allogenic bone marrow transplant from a histocompatible donor could reverse an advanced state of arylsulfatase B deficiency in a two year old Siamese cat as evidenced by a normalization of urinary glycosaminoglycan excretion, leukocyte arylsulfatase B activity and sustained improvement in clinical symptoms. Hence it would be only a question of time before man can be treated in a similar manner.

Hunter's syndrome (iduronate sulfate sulfatase deficiency)

It is a sex-linked recessive trait characterized by abnormal accumulation of heparan sulfate, dermatan sulfate and by the deficiency of L-iduronate sulfate sulfatase. It is known to occur only in hemizygous males, while the females who are heterozygous are probably protected from the disorder by an enzyme transfer from normal to abnormal cells. The syndrome can be diagnosed by assaying iduronate sulfate sulfatase activity in serum, lymphocytes, fibroblasts and hair roots (Liebaers and Neufeld, 1976; Migeon *et al.*, 1977; Yutaka *et al.*, 1978).

Morquio's syndrome

This syndrome is characterized by abnormal accumulation and excretion of keratan sulfate and chondroitin 6-sulfate and distinguishable from others by such characteristic clinical features as disproportionate dwarfism, spondiloepiphyseal dysplasia, dental anomalies, corneal clouding but normal intellect. Patients are deficient in N-acetylgalactosamine 6-sulfate sulfatase (Singh *et al.*, 1976) and galactose 6-sulfate sulfatase (Yutaka *et al.*, 1982).

Sanfilippo 's syndrome type A (heparan sulfate N-sulfatase deficiency)

Patients with this syndrome suffer from severe neurological symptoms but mild

physical alterations. It can be diagnosed by assaying heparan sulfate N-sulfatase in fibroblasts and peripheral leukocytes (Schmidt *et al.*, 1977).

Placental steroid sulfatase deficiency

Steroid sulfatase and arylsulfatase C (*p*-nitrophenylsulfatase) are absent in the placenta of pregnant women having this deficiency syndrome. It is characterized by low urinary estrogen excretion compared to normal and pregnant women. This disorder can be easily distinguished from other sulfatase deficiencies both by being sex-linked and causing pregnancy complications (Oakey, 1978). The babies are usually males and develop an ichthyotic skin condition probably caused by excessive accumulation of cholesterol sulfate in the pathological scale (Williams and Elias, 1981) in the first year. Corneal dystrophy has been described in patients with X-linked ichthyosis and some female carriers (Sever *et al.*, 1968).

Recent studies have shown that steroid sulfatase deficient placental microsomes have a significantly lower percentage of phosphatidylcholine as compared to normal placental microsomes (McKee and France, 1983). This finding is important especially since phosphatidylcholine can stimulate steroid sulfatase (McNaught and France, 1980). The disorder can be diagnosed by subjecting the patients to dehydroepiandrosterone and dehydroepiandrosterone sulfate loading tests (Braunstein *et al.*, 1976) or by measuring the cholesterol sulfate content in the pathological scales (William and Elias, 1981).

Multiple sulfatase deficiency

It is an autosomal recessive trait characterized by a profound deficiency of arylsulfatase C along with arylsulfatase A, B and steroid sulfatase resulting in the accumulation of cerebroside 3-sulfate, steroid sulfates and mucopolysaccharides (Eto *et al.*, 1974; Murphy *et al.*, 1971; Austin, 1973). The suggestion that the deficient arylsulfatases must be having a common subunit controlled by a single gene (Moser *et al.*, 1972) has been ruled out by the fact that arylsulfatase A and B are assigned to different chromosomes (DeLuca *et al.*, 1979; Hors-Cayla *et al.*, 1979). Nine different sulfatases (arylsulfatase A, B and C, cholesterol sulfatase, dehydroepiandrosterone sulfatase, iduronate 2-sulfate sulfatase, N-acetylgalactosamine 6-sulfatase, N-acetylglucosamine 6-sulfate sulfatase and heparan sulfate N-sulfatase) are shown to be deficient in this syndrome (Banser *et al.*, 1979b). Studies by Chang *et al.* (1983) using cultured fibroblasts from 7 different patients have suggested typing of the patients into 3 groups: (i) deficient in A, B and C; (ii) deficient in A and C with half or near normal levels of B and (iii) same as in (ii), but arylsulfatase A activity bands are detectable.

Recent studies using fibroblasts from multiple sulfatase deficient patients demonstrate a decreased stability of arylsulfatase A and the 47,000 dalton form of arylsulfatase B (Steckel *et al.*, 1985) and steroid sulfatase (Horwitz *et al.*, 1986). The root cause of this disorder is still not known. They are probably secondary manifestations of a yet unknown primary genetic defect.

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