The importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation

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3′-Phosphoadenosine 5′-phosphosulfate (PAPS) is the obligate cosubstrate and source of the sulfonate group in the sulfonation reactions catalyzed by both the cytosolic and membrane-associated sulfotransferases. Sulfation is a high-affinity, low-capacity conjugation system in xenobiotic metabolism partly because the levels of sulfation activity may be limited by PAPS availability. Thus, the rate of PAPS synthesis may be an important factor in determining sulfation activity in tissues. A leveling out of the sulfation of drugs and xenobiotics may occur due to the limiting rate of PAPS synthesis, which may greatly affect their metabolism and biologic properties. PAPS is synthesized from ATP and inorganic sulfate in a two-step reaction. Analyzing the regulation of the enzymes and cofactor levels involved in the synthesis of PAPS is important to our understanding of sulfation activities in cells and tissues.

—Charles N. Falany, Coordinating Editor

ABSTRACT **Sulfation is the transfer of a sulfate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a substrate that is catalyzed by a family of sulfotransferase enzymes. Many different endogenous and xenobiotic molecules are substrates for the sulfotransferases; sulfation affects many different physiological processes, including: *1)* deactivation and bioactivation of xenobiotics, *2)* inactivation of hormones and catecholamines, *3)* structure and function of macromolecules, and *4)* elimination of end products of catabolism. PAPS is the obligate cosubstrate that is synthesized in tissues to make available an "activated form" of sulfate for the sulfation reaction. PAPS participation in the reaction is dependent on its availability, which in turn is dependent on its synthesis, degradation, and ultimately its utilization in the sulfation reaction itself. PAPS synthesis is dependent on the availability of sulfate and on the activity of the two enzymes of its synthesis, ATP-sulfurylase and APS-kinase. Although the kinetic properties of these two enzymes are well described, their in vivo regulation is not fully understood. Sulfation is a high-affinity, low-capacity enzymatic process in which the entire liver content of PAPS can be consumed in less than 2 min. ATP-sulfurylase and APS-kinase can rapidly synthesize additional PAPS. The low capacity of sulfation in rats is due to the limited availability of sulfate, whereas in mice the sulfotransferases appear to limit sulfation capacity. Sulfation rates are not readily enhanced, but they can be decreased. 2,6-Dichloro-4-nitrophenol inhibits phenol-sulfotransferases, but not hydroxysteroid-sulfotransferases. However, the sulfation of phenols and hydroxysteroids can be decreased by factors that decrease sulfate availability such as a low-sulfate diet, other xenobiotics that are sulfated, and molybdate, which inhibits sulfate intestinal absorption, renal reabsorption, and sulfate incorporation into PAPS.—Klaassen, C. D., Boles, J. W. The importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J.* 11, 404–418 (1997)**

Key Words: acetaminophen · sulfate · ATP-sulfurylase · APS-kinase · glutathione

SINCE THE DISCOVERY OF SULFOCONJUGATES in 1875 by Baumann, sulfation has been considered a pathway that precedes the excretion of chemicals. Indeed, chemicals are readily excreted when sulfoconjugated because they are more water-soluble than the parent chemical. However, for some substrates sulfation can be an activation pathway. For instance, the sulfoconjugate of minoxidil is more active in producing desired hypotensive effects than is the parent chemical (1). Also, the sulfate conjugate of *N*-hydroxyl-2-acetylaminofluorine (*N*-OH-2-AAF) adducts to more protein and nucleic acids than does the parent compound, and is the major carcinogenic metabolite (2). Sulfation also plays a role in *1)* the inactivation of thyroid hormones, catecholamines, and steroid hormones, *2)* the structure of sulfolipids, glycosamino-

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glycans, and glycoproteins, *3)* the elimination of bile acids, and *4)* the post-translational modification of tyrosine-containing secretory proteins.

THE SULFOCONJUGATION REACTION

Sulfation is the conjugation of a substrate with a sulfuryl group ($-SO_3^-$). This reaction occurs in the cytosol or is associated with the membranes of the Golgi apparatus of the cell. The cosubstrate 3′-phosphoadenosine 5′-phosphosulfate (PAPS)[2] donates its sulfuryl group ($-SO_3^-$) to a substrate (R-OH). The products are 3′-phosphoadenosine 5′-phosphate (PAP) and the sulfoconjugate ($R\text{-}OSO_3^-$).

$$PAPS + R\text{-}OH \xrightarrow{\text{sulfotransferase}} PAP + R\text{-}OSO_3^-$$

The sulfation reaction is catalyzed by a family of enzymes, the sulfotransferases (ST), whose individual family members were originally classified on the basis of their affinity and activity for substrates. However, individual sulfotransferases catalyze the transfer of the sulfuryl group from PAPS to different classes of substrates, hence their substrate specificities overlap. Many different chemicals are sulfotransferase substrates. Their sulfation is dependent on the presence of a functional group, such as a hydroxy group, which acts as a sulfuryl group acceptor. In many cases, the functional group of a chemical is exposed or added as a consequence of phase I biotransformation. The most common functional group that accepts the sulfuryl group is the hydroxyl group present in phenols, alcohols, and hydroxyl amines, which form sulfate esters. Also, the unprotonated form of amines can serve as an acceptor of sulfuryl groups to form sulfamates ($RNHSO_3H$).

Sulfation in higher organisms has a strict requirement for PAPS. This has been demonstrated by the following: *1)* sulfation does not proceed in the absence of PAPS in vitro or under conditions that limit PAPS synthesis, *2)* the putative binding site for PAPS on the sulfotransferases is conserved between different classes of sulfotransferases within different tissues and between different species (3, 4), and *3)* structural analogs of PAPS bind to and inhibit sulfotransferases (5).

PAPS is the "activated" form of sulfate and is synthesized from endogenous sulfate. Serum sulfate equilibrates rapidly with PAPS in vivo, which is then available for sulfation (6). Therefore, in vivo sulfation of many chemicals has been shown to be dependent on endogenous sulfate concentrations (7, 8).

[2] Abbreviations: AA, acetaminophen; APS, adenosine 5′-phosphosulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PPi, pyrophosphate.

AVAILABILITY OF INORGANIC SULFATE

Inorganic sulfate is essential for PAPS synthesis, and several mechanisms maintain inorganic sulfate homeostasis. Sulfate pools are replenished by the sulfoxidation of sulfur-containing amino acids (cysteine and methionine) yielding inorganic sulfate, transport-mediated intestinal absorption of inorganic sulfate, transport-mediated renal reabsorption of inorganic sulfate, degradation of sulfate-containing macromolecules, and the activity of sulfatases.

Intestinal absorption of sulfate

Tissue concentrations of sulfate are also dependent on inorganic sulfate supplied in the diet (9). Food and water are both sources of sulfate, with tap water contributing about 10% of the total dietary source (10). Intestines, which absorb sulfate rapidly and nearly completely (11), contain an electroneutral sodium-sulfate cotransport system (12). In addition, both L- and D-cysteine can be converted to inorganic sulfate (8).

Renal reabsorption of sulfate

An important factor in the homeostasis of sulfate is its renal reabsorption (13). The primary mechanism for renal reabsorption of sulfate across the brush-border membrane of proximal tubular cells is an electroneutral, sodium-dependent, capacity-limited transporter (12), which has been characterized by expression cloning (14). Also, an anion exchange has been identified in the rat renal cortical brush-border membrane but is believed to play only a minor role in sulfate reabsorption (15).

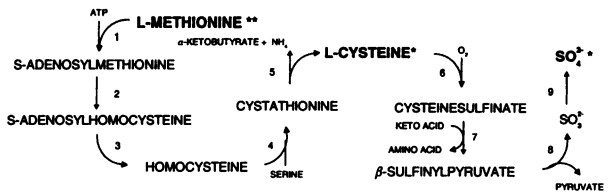
Renal reabsorption of sulfate is regulated by sulfate load. When sulfate delivery to the kidneys is increased in a stepwise manner by sulfate infusion in the rat, a maximum renal reabsorption of sulfate is achieved (T_m). When sodium sulfate infusion continues over a period of time, the T_m for sulfate decreases, implying that the sulfate transporter can be down-regulated by sulfate (16). Conversely, diets low in inorganic sulfate, cysteine, and methionine result in a decrease in the urinary excretion of inorganic sulfate in humans (17) and rats (18), because the renal fractional reabsorption of inorganic sulfate increases to maintain serum inorganic sulfate levels (19). The T_m for sodium-sulfate cotransport is increased in renal brush border and basolateral membrane vesicles from rats fed diets low in sulfate, indicating up-regulation of the sodium-sulfate cotransporter (19). Renal sulfate reabsorption is key to the rapid fine-tuning of sulfate homeostasis.

Chemicals can have a direct effect on renal tubular sulfate reabsorption. Salicylic acid increases the renal

clearance of inorganic sulfate in vivo (21); a partial explanation is that salicylic acid inhibits inorganic sulfate transport at the basolateral membrane (21). Probenecid, an inhibitor of renal organic anion secretion, inhibits the renal reabsorption of inorganic sulfate as determined by decreases in serum sulfate concentrations, and increases in renal clearance of inorganic sulfate (22).

Amino acids as a source of sulfate

Both L-methionine (L-Met) and L-cysteine (L-Cys) are sulfur-containing amino acids that can be a source of sulfate. These amino acids originate from the diet and the breakdown of protein. Cysteine can be formed from methionine and glutathione catabolism. During their catabolism, L-Met and L-Cys are broken down to their carbon skeletons; sulfite, as noted below, is broken down by a series of trans-sulfurations and transaminations. Sulfite oxidase readily oxidizes sulfite to sulfate (23).



1, L-methionine S-adenosyltransferase; 2, L-homocysteine S-methytransferase; 3, S-adenosyl-L-homocysteine hydrolase; 4, cystathionine β-synthetase; 5, L-cystathionine cysteinelyase; 6, cysteine dioxygenase; 7, cysteine sulfinate transaminase; 8, nonenzymatic; 9, sulfite oxidase.
* Supplied in the diet.
** Essential nutrient in most species.

Cysteine, methionine, and sulfate are not used equally to supply tissues with inorganic sulfate. The infusion of sodium sulfate, cysteine, and methionine increases serum and tissue sulfate concentrations in a time- and dose-dependant manner (**Fig. 1**), with sodium sulfate being the most efficacious, cysteine intermediate, and methionine the least efficacious (25).

L-Cysteine is not only a precursor to sulfate, but also to glutathione. Decreases in cysteine availability can decrease glutathione in addition to sulfate concentrations (25–27). Both isomers (D-+L-) of sulfur-containing amino acids can be used as sulfate precursors. However, D-cysteine, the nonphysiological isomer of cysteine, does not participate in protein or glutathione synthesis but is sulfoxidized to inorganic sulfate, thereby increasing the sulfation of substrates (28). In contrast, *N*-acetyl-L-cysteine increases glutathione and sulfate concentrations, yet *N*-acetyl-D-cysteine does not increase either glutathione or sulfate concentrations. These acetylated forms of cysteine are deacetylated by *N*-acylases before they are available for glutathione synthesis or sulfoxidation to sulfate; these *N*-acylases are stereoselective for *N*-acetyl-L-cysteine (29).

Sulfatase activity as a source of sulfate

Macromolecules that undergo normal degradation within lysosomes can release sulfate by the activity of sulfatases (30). Likewise, the activity of sulfatases on bioactive molecules (steroids and catecholamines) may add sulfate to the sulfate pool.

The activity of sulfatase is regulated in part by negative feedback of its product. Inorganic sulfate and cysteine repress the synthesis of arylsulfatases in bacteria, but methionine has no such effect (31, 32). Human placental sterylsulfatases, which also exhibit arylsulfatase activity, are inhibited by their products, free phenols or steroids and inorganic sulfate. These findings are similar to those of the arylsulfatases of lysosomal origin. Diisothiocyano-dihydrostilbene-2,2′-disulfonic acid (DIDS) and other inhibitors of carrier-mediated anion transport inhibit the activity of this sterylsulfatase (33).

Cellular uptake and utilization of sulfate

Sulfate enters cells by active transport that is inhibited by DIDS (34). The sulfate transporter from the canalicular surface of rat hepatocytes has been cloned; mRNA from kidney, muscle, and brain of rats as well as from the liver of mice has been identified as the possible transcript for the sulfate transporter in the respective tissues (35).

For most isolated cell cultures, extracellular inorganic sulfate is the predominate source of sulfate. However, not all tissue types use inorganic sulfate as the major source of sulfate. Intestinal cells and kidney cells use inorganic sulfate most effectively, as measured by the sulfation of 7-hydroxycoumarin in vitro in isolated cells. But kidney cells also use cysteine, *N*-acetylcysteine, and glutathione as sulfate sources, whereas in lung cells, cysteine is the most efficient source of sulfate (36). The source of sulfate in human lung fibroblast cell culture is predominately inorganic sulfate. However, at less than physiological extracellular concentrations of sulfate, cysteine is a major contributor to the intracellular sulfate pool in fibroblast cells (37). The sulfation of proteoglycans in glomeruli and mesangial isolated cells differs from other cell cultures in that their sulfation is independent of media content of inorganic sulfate and is, instead, dependent on methionine, which is subsequently converted to sulfate (38).

Altered sulfate homeostasis

A number of physiological states alter sulfate homeostasis. For example, age, time of the day, gestational period, and disease states all have an effect on sulfate

concentrations in body fluids, which may affect sulfation.

Sulfate homeostasis changes with the stage of development of an organism. For example, serum sulfate concentrations in humans decrease from 470 μM at day 1 (at birth) to 330 μM at 36 months of age and thereafter (39). Cerebrospinal (CSF) fluid concentrations of inorganic sulfate are lower than serum concentrations, and decrease between birth and 3 years of age. However, the ratio of sulfate in CSF to serum is less than that predicted by the Gibbs-Donnan equilibrium, which suggests that sulfate is transported out of the CSF (40).

Urinary excretion rates of sulfate increase in aged rats during periods of sulfate depletion due to decreased tubular reabsorption of inorganic sulfate (41). Normally, aged rats and guinea pigs have decreased urinary excretion of inorganic sulfate compared with younger animals. This decrease in urinary excretion of inorganic sulfate in aged guinea pigs is a result of decreased capacity of the renal tubular transport of sulfate (42), which in turn is most likely due to lower density of sodium-sulfate cotransporters in aged guinea pigs (43). Aged rats administered acetaminophen (300 mg/kg) to deplete sulfate levels, followed by sulfate administration (sodium sulfate, 2 mmol/kg), show lower reabsorption rates of inorganic sulfate than do younger rats.

Sulfate concentrations in maternal and fetal compartments change during gestation, indicating altered demands for sulfoconjugation during development. In fact, inorganic sulfate concentrations increase in human amniotic fluid during the third trimester (44). Serum sulfate and renal reabsorption of inorganic sulfate also increase in third-trimester mothers, which makes more sulfate available for the fetus (45, 46). Fetal serum inorganic sulfate is higher than maternal serum inorganic sulfate; however, the mechanism for this gradient is unknown (47).

Other physiological factors also alter urinary excretion of inorganic sulfate, which may affect inorganic sulfate availability. Menopause results in lower serum sulfate concentrations that may be a result of decreased renal sulfate reabsorption (48). Blood pH affects renal reabsorption of inorganic sulfate; a blood pH of 7.49 increases, whereas a blood pH of 7.29 decreases, renal excretion of sulfate (49).

Some diseases appear to affect sulfate homeostasis, which may affect the ability to sulfoconjugate. Patients with rheumatoid arthritis have depressed concentrations of inorganic sulfate in serum and synovial fluid, and raised serum concentrations of cysteine, indicating a deficiency in the enzymatic degradation of cysteine to sulfate, notably cysteine dioxygenase (50). Decreased concentrations of inorganic sulfate in the sweat of cystic fibrosis patients may be due to defects in anion permeability in the ductual epithelium of the sweat gland (51).

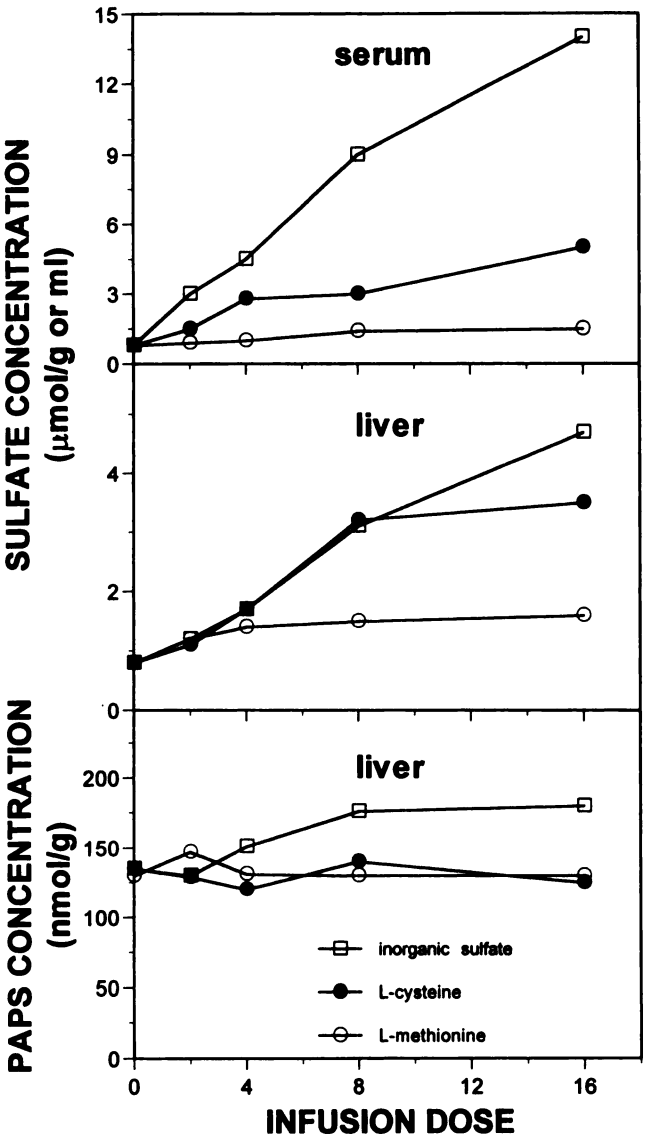


Figure 1. Effects of various dosages of sodium sulfate, cysteine, and methionine on serum sulfate, hepatic sulfate and hepatic 3′-phosphoadenosine 5′-phosphosulfate (PAPS) concentrations. Serum sulfate (top panel), hepatic sulfate (middle panel), and hepatic (PAPS) (lower panel) concentrations were determined at the end of a 2 h infusion of 2–16 $mmol \cdot kg^{-1} \cdot 2\ h^{-1}$ of sodium sulfate, cysteine, or methionine to rats Data previously published (24).

Although altered sulfate homeostasis occurs in some physiological or diseased states, the effect of such changes on sulfoconjugation is not known. However, the oversulfation of high- molecular weight glycoconjugates (HMG) of airway epithelial cells in cystic fibrosis patients is not due to elevated levels of sulfate or enhanced sulfate transport, but more likely results from increased synthesis of PAPS or increased sulfotransferase activity (52). Conversely, in two human osteochondrodysplasic syndromes—diastrophic dysplasia and type IB achondrogenesis—the cartilage matrix is deficient in sulfated proteoglycans and has

low adenosine'-phosphosulfate (APS) and PAPS concentrations. The undersulfation of glycosaminoglycans on the proteoglycan molecules in these syndromes is believed to result from deficient sulfate transport as a consequence of mutations in the gene coding for the sulfate transporter (53, 54).

The role of sulfate in PAPS synthesis

Limiting sulfate availability usually does not decrease steady-state concentrations of PAPS when sulfation is minimal. However, limiting sulfate availability decreases the ability to synthesize PAPS during the sulfation of high doses of exogenous substrates (10). Therefore, the ability to synthesize PAPS from sulfate is a more important factor in sulfation capacity than is PAPS steady-state concentrations.

Consumption of tissue sulfate can limit PAPS synthesis, but increased tissue sulfate does not increase tissue concentrations of PAPS at steady state. For example, infusion of sodium sulfate, cysteine, and methionine increases serum and tissue (liver, kidney, and brain) sulfate concentrations in a dose- and time-dependent manner (24). However, tissue PAPS levels are not increased markedly (Fig. 1). PAPS concentrations in the kidney and brain are not influenced by increased tissue sulfate concentrations; only in the animals infused with high doses of sodium sulfate were hepatic PAPS concentrations increased, and then by only 30–35%. Therefore, it appears that steady-state PAPS concentrations in various tissues cannot be increased by increasing tissue sulfate concentrations.

PAPS AVAILABILITY

The availability of PAPS for sulfation in vivo is dependent on its synthesis, transport (with regard to the sulfation of macromolecules), degradation, and utilization. PAPS tissue concentrations are low (4–80 nmol/g tissue) (**Table 1**) (55–57) compared with tissue concentrations of uridine 5'-diphosphoglucuronic acid (UDP-GA) (200 nmol/g of liver) required for glucuronidation or with glutathione (GSH) (5000 mmol/g of liver) required for glutathione conjugation. PAPS concentrations differ between tissues; the liver is consistently the tissue with the highest PAPS concentration. Also, there are species differences in PAPS tissue concentrations, with rats having one of the highest concentrations.

PAPS tissue concentrations are low compared with the amount of PAPS required to sustain high rates of sulfation, indicating high rates of PAPS biosynthesis. For example, hepatic PAPS concentration in the rat is 30–70 nmol/g of liver, yet the rate of sulfation can be as high as 100 nmol/min/g liver (58). Thus, the entire hepatic PAPS pool can be utilized in less than 1 min. Therefore, rapid biosynthesis of PAPS is required for sulfation.

PAPS synthesis

PAPS is synthesized rapidly in a two-step, coupled reaction. The first step combines ATP and inorganic sulfate (SO_4^{2-}) to form APS and pyrophosphate (PPi). The reaction is catalyzed by ATP-sulfurylase (EC 2.7.7.4) in the presence of Mg^{2+}.

$$ATP + SO_4^{2-} \overset{\text{ATP-sulfurylase}}{\leftrightarrows} APS + PPi$$

TABLE 1. *3'-Phosphoadenosine 5'-phosphosulfate (PAPS) concentration in various tissues from different species*[a]

	PAPS (nmol/g of tissue)							
	Rat		Mouse		Rabbit	Dog		
Tissue	Male	Female	Male	Female	Female	Male	Female	Human
Liver	76.8	67.9	29.4	32.7	32.7	17.3	16.1	22.6[b]
Kidney:								
whole	15.2	16.1	12.1	20.0	—	—	—	4.8[b]
cortex	—	—	—	—	12.3	9.9	9.8	—
medulla	—	—	—	—	7.8	7.5	6.5	—
Lung	8.8	8.2	13.9	12.1	10.4	6.9	7.5	4.3[b]
Intestine:								
whole	8.5	9.6	13.3	6.7	12.1	8.0	7.2	—
ileum	—	—	—	—	—	—	—	12.8[b]
Ascending colon	—	—	—	—	—	—	—	8.1[b]
Descending colon	—	—	—	—	—	—	—	7.5[b]
Sigmoid colon	—	—	—	—	—	—	—	6.2[b]
Brain	7.1	7.2	6.7	—	—	—	—	—
Placenta	—	—	—	—	—	—	—	3.6[c]
Fetal liver	—	—	—	—	—	—	—	10.1[c]

[a] Unless otherwise noted, all values are from ref 55. [b] From ref 56. [c] From ref 57.

The subsequent step combines the APS formed in the first step with additional ATP to form PAPS and ADP. This reaction is catalyzed by APS-kinase (EC 2.7.1.25) in the presence of Mg^{2+}.

$$ATP + APS \overset{\text{APS-kinase}}{\leftrightharpoons} PAPS + ADP$$

ATP-sulfurylase

The deduced amino acid sequence of cDNAs cloned from the ATP-sulfurylase gene of plants (59, 60), bacteria (61), fungus (62), and yeast (63) show two conserved areas, high in basic residues, that are thought to be the binding sites for MgATP and sulfate (62). It is believed that MgATP and sulfate add to these binding sites in a random order, but product release is ordered because MgPPi is released before APS. This mechanism is supported by initial velocity, product inhibition, dead-end inhibition, and alternative substrate kinetics (64, 65). Little is known concerning the regulation of ATP-sulfurylase in higher organisms. However, in yeast, ATP-sulfurylase appears to be regulated at the level of transcription because ATP-sulfurylase is implicated in the biosynthesis of methionine in yeast, and methionine in the media of yeast represses both ATP-sulfurylase activity and mRNA concentrations (63).

Only ATP is effective as a nucleoside triphosphate substrate for ATP-sulfurylase. Although ATP-sulfurylase has some activity toward dATP and CTP, it is possible that this activity is a consequence of ATP contamination (65). High concentrations of nucleoside triphosphates (GTP, UTP, and CTP) as well as Mn^{2+} inhibit ATP-sulfurylase activity (66).

APS-kinase

The protein products of cDNAs cloned from the APS-kinase gene of plant (67), fungus (68), bacteria (61), and yeast (69) are functional, and show nearly 50% homology in their deduced amino acid sequences. The amino acid sequences contain two conserved regions that possibly function as purine-nucleotide binding sites and phosphate-transferring groups. Nucleotide binding to APS-kinase is ordered; MgATP binds first, which phosphorylates APS kinase; APS then binds to APS-kinase, which is phosphorylated to form PAPS. PAPS then disassociates from APS-kinase, followed by the disassociation of MgADP. Normally, another APS will occupy the site where PAPS resided, but only after both binding sites are vacant, and subsequent phosphorylation continues (70).

ATP-sulfurylase and APS-kinase coupling

The in vitro rate of PAPS generation from APS is only marginally greater than that from inorganic sulfate even though APS formation appears to be the rate-limiting step in PAPS formation (71). This implies that endogenously synthesized APS may be more readily available to APS-kinase than exogenously supplied APS and that coupling of ATP-sulfurylase with APS-kinase may be the explanation of this phenomenon (71). Further evidence shows that ATP-sulfurylase and APS-kinase activity reside on the same protein complex and that APS is channeled from one active site (ATP-sulfurylase) to another active site (APS-kinase) for the phosphorylation of APS to form PAPS (72).

Brachymorphic mice are unable to form sulfoconjugates at a normal rate (73, 74). This defect is now believed to be caused by a defect in the coupling of ATP-sulfurylase with APS-kinase, decreasing the channeling of APS between the two proteins and subsequently decreasing sulfoconjugation (75).

Kinetics of PAPS synthesis

The reaction catalyzed by ATP-sulfurylase, forming APS, is not favored energetically ($\Delta G' = +11$ kcal/mol), with a V_{max} in the forward and reverse direction of 6.6 and 50 units/mg protein, respectively (64); thus, steady-state concentrations of PAPS within the cell are low (8). Subsequent hydrolysis of the pyrophosphate formed in the first reaction and the rapid utilization of APS in the second reaction relieve energy constraints on the overall reaction. Utilization of APS is rapid due to the high affinity of APS-kinase for APS. Because PAPS inhibits its own synthesis, PAPS consumption or transport out of the cytoplasm causes the reaction to proceed toward PAPS synthesis.

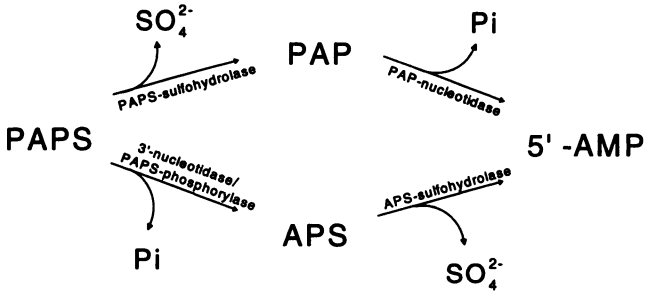
PAPS transport

PAPS is formed in the cytosol where the sulfation of most xenobiotics occurs. However, the sulfation of macromolecules occurs in the lumen of the Golgi apparatus; their sulfation is dependent not only on the synthesis of PAPS, but also on the transport of PAPS from the cytosol into the lumen of the Golgi apparatus (76). The transporter has been identified as a homodimer (77) with a V_{max} of 14 pmol of PAPS translocated $\cdot$ $min^{-1} \cdot$ mg $protein^{-1}$, which is inhibited competitively by PAP (78).

PAPS degradation

PAPS is degraded by two different pathways, both of which lead to the formation of the same end product: 5′-AMP. In the first pathway, PAPS is desulfated by PAPS-sulfohydrolase, forming PAP, which is then dephosphorylated by PAP-nucleotidase to yield 5′-AMP. In the second pathway, PAPS is dephosphorylated by 3′-nucleotidase/PAPS-phosphorylase, forming APS,

which is then desulfated by APS-sulfohydrolase, forming 5′-AMP.



The potential exists for differences in PAPS availability and subsequent sulfation based on differences in PAPS degradation. For instance, PAPS-sulfohydrolase activity is higher in fetal and newborn rat livers and in rat placenta compared with that of the adult rat liver, potentially decreasing PAPS availability (79). Also, interruption in the degradation of PAPS may increase PAPS availability and enhance sulfation. For instance, the phosphatase inhibitor phosphonoacetic acid increases in vitro PAPS concentrations of human frontal cortical supematants and increases the sulfation of dopamine, presumably by decreasing the hydrolysis of PAPS (80).

Alterations in PAPS degradation may influence sulfation not only through changes in the availability of PAPS, but by altering the concentrations of intermediates of PAPS degradation: APS and PAP. High concentrations of APS inhibit APS-kinase activity (81); PAP inhibits sulfotransferases (82) and competes with PAPS for transport into the Golgi apparatus. Therefore, changes in APS and PAP concentrations as a result of altered PAPS degradation may also affect sulfation.

SULFATION OF CHEMICALS

Many chemicals that are sulfoconjugated decrease the concentration of PAPS and sulfate. Acetaminophen (AA) has been used extensively to examine the sulfation pathway. AA can undergo three primary biotransformation reactions: sulfation, glucuronidation, and a P-450-catalyzed formation of an electrophilic quinone (the toxic intermediate), which gives rise to the glutathione conjugate. Likewise, salicylamide, phenol, and α-naphthol are extensively sulfoconjugated, and decrease tissue PAPS and sulfate (83).

Sulfation is capacity-limited

When administered to rats at low doses, AA is conjugated predominantly with sulfate. Excretion of AA-sulfate reaches a maximal rate after administration of 1 mmol/kg AA to rats. Further increases in dosage do not result in additional increases in sulfate conjugate formation (**Fig. 2**) (84). In contrast, the amount of AA biotransformed through the glucuronidation and cytochrome P-450-glutathione conjugation pathways increases approximately linearly with dose. Thus, sulfation of AA becomes capacity-limited in rats at low AA doses and glucuronidation becomes the predominant pathway for AA elimination at high doses. In rats, therefore, hepatic sulfation of AA is a high-affinity, low-capacity conjugation reaction, whereas glucuronidation is a low-affinity, high-capacity conjugation reaction (8).

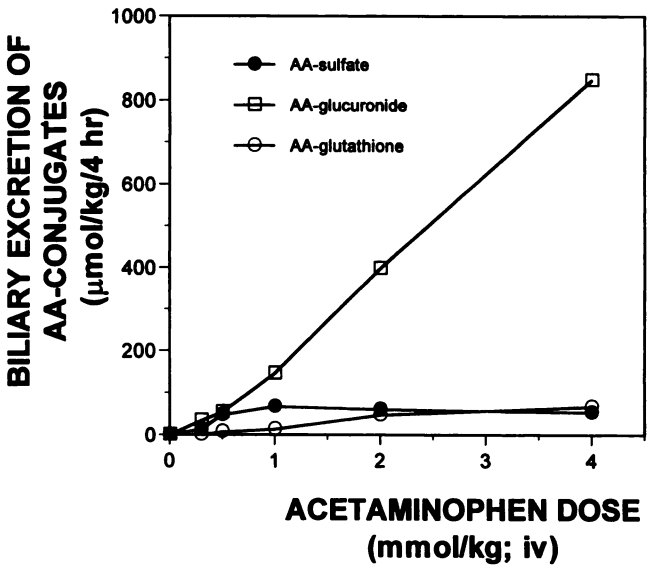


Figure 2. Cumulative excretion of acetaminophen (AA) and AA conjugates into the bile after AA administration. Symbols depict the cumulative biliary excretion of AA-sulfate, AA-glucuronide, AA-glutathione, and total-AA (AA-sulfate+AA-glucuronide+AA-glutathione+unmetabolized AA) 4 h after i.v. administration of various doses of AA (0.3, 0.5, 1, 2, and 4 mmol/kg) to rats. Data previously published (84).

Availability of PAPS as a limiting factor in sulfation

Sulfation of intravenously administered AA takes place predominantly in the liver (85); therefore, hepatic PAPS reflects cosubstrate availability for AA sulfation. In rats, the maximal capacity of AA sulfation occurs concomitantly with a decrease in hepatic PAPS levels (Fig. 2, **Fig. 3**) (86). This becomes even more pronounced at higher AA doses. AA also causes a marked dose-dependent depletion of serum sulfate, suggesting that decreased availability of PAPS results from a reduced supply of its precursor, inorganic sulfate. AA decreases inorganic sulfate not only in the liver, but also in the kidney and serum; it takes more than 12 h for these levels to return to control values after a high dose of AA (87).

The sulfation of salicylamide, phenol, and α-naphthol in rats decreases sulfate and PAPS tissue concentrations, as does AA. These three xenobiotics also

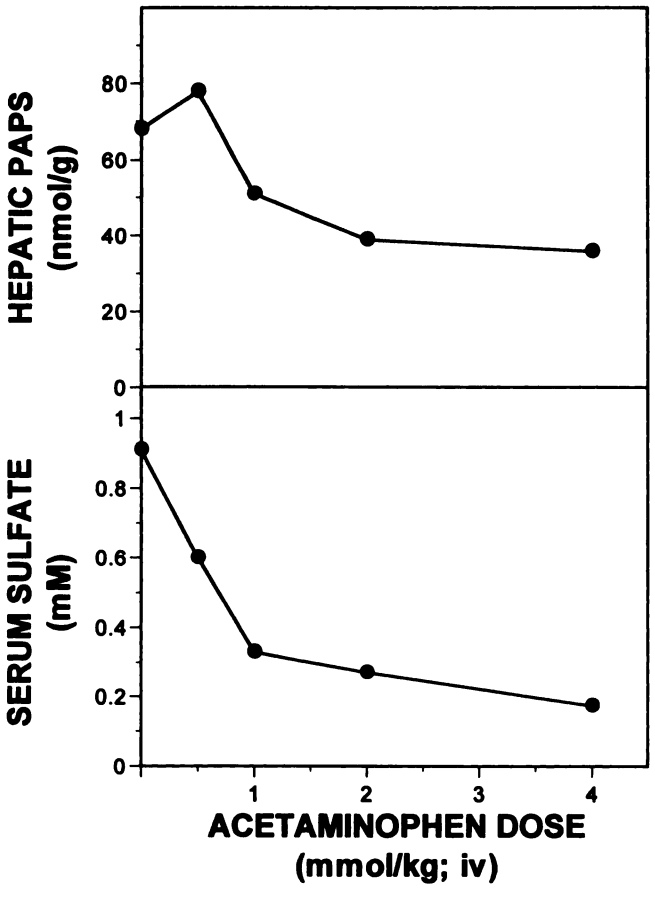


Figure 3. Effects of acetaminophen (AA) on hepatic 3′-phosphoadenosine 5′-phosphosulfate (PAPS) and serum sulfate concentrations. Hepatic PAPS (upper panel) and serum sulfate (lower panel) was determined 2 h after the i.v. administration of AA (0.5, 1, 2, and 4 mmol/kg) to rats. Data previously published (86).

decrease serum sulfate, sulfate urinary excretion, hepatic sulfate, and hepatic PAPS concentrations (83). These findings imply that an increased flux through the sulfation pathway by xenobiotics that are sulfated causes depletion of hepatic PAPS concentrations because of the limited availability of sulfate.

Tissue differences in sulfation capacity

The capacity-limited sulfation of AA in rats appears to be due to reduced availability of PAPS, which in turn is limited by the availability of its precursor, inorganic sulfate. For intravenously administered compounds, the liver appears to be quantitatively the most important site of sulfation because of its high sulfotransferase activities, PAPS concentration, and size. However, most drugs and other xenobiotics enter the body via the oral route. While being absorbed from the intestine, compounds containing hydroxyl groups may be sulfated and/or glucuronidated. In theory, intestinal sulfation of a compound may result in significant presystemic elimination and reduced oral bioavailability. If intestinal sulfation exhibits capacity limitation, saturation of this presystemic elimination mechanism at large doses may result in increased bioavailability. Therefore, to understand the significance and possible implications of intestinal sulfation of a chemical it is desirable to know not only the extent, but also the kinetics, of its intestinal sulfation.

Intestinal conjugation of AA, harmol, and α-naphthol exhibit similar dose-dependent profiles; however, there are marked differences in the kinetics of their glucuronidation and sulfation after oral and systemic administration (88, 89). At low doses, each of these compounds is predominantly glucuronidated in the intestine, and the extent of their sulfation is negligible or small (**Fig. 4**). At higher doses, however, glucuronidation tends to reach a maximal rate whereas sulfation increases in proportion to dose, or even greater than in proportion to dose. As a result, at the highest doses examined the rate of intestinal sulfation of harmol, α-naphthol, and AA approached or even exceeded the rate of glucuronidation.

These studies point out the striking difference between systemic and intestinal conjugation. In the intact rat, sulfation capacity is low and is saturable even at low doses. However, the glucuronidation pathway exhibits high maximal capacity, which is not reached even at high dosages. In contrast, in the intestine the relative capacity for sulfation is higher than that for glucuronidation and does not exhibit saturation even at the high doses, whereas the capacity for glucuronidation is limited even at low doses. Therefore, the pattern of conjugation in the intact rat probably reflects conjugation in the liver, because AA perfused at a low dose into isolated rat liver is also preferentially sulfated (85).

Species differences in sulfation capacity

Not only are there differences in the sulfation capacity among tissues, there are species differences in the factors that account for limited sulfation capacity between rats and mice. As noted earlier, the availability of sulfate to form PAPS limits the hepatic sulfation of xenobiotics in the intact rat. In mice, the sulfation of AA is also capacity-limited; however, AA does not decrease hepatic PAPS concentrations as it does in rats (90, 91).

Administration of sodium sulfate to rats and mice also given AA increases their serum sulfate and hepatic PAPS concentrations (91, 92). In rats, sodium sulfate also increases the urinary excretion of AA-sulfate (91). However, in mice, sodium sulfate does not decrease AA serum half-life or clearance or increase the urinary excretion of AA-sulfate, AA-cysteine, or AA-mercapturate. Furthermore, in rats, sulfotransferase activity toward AA is not affected by the administration of AA in vivo whereas in mice it is de-

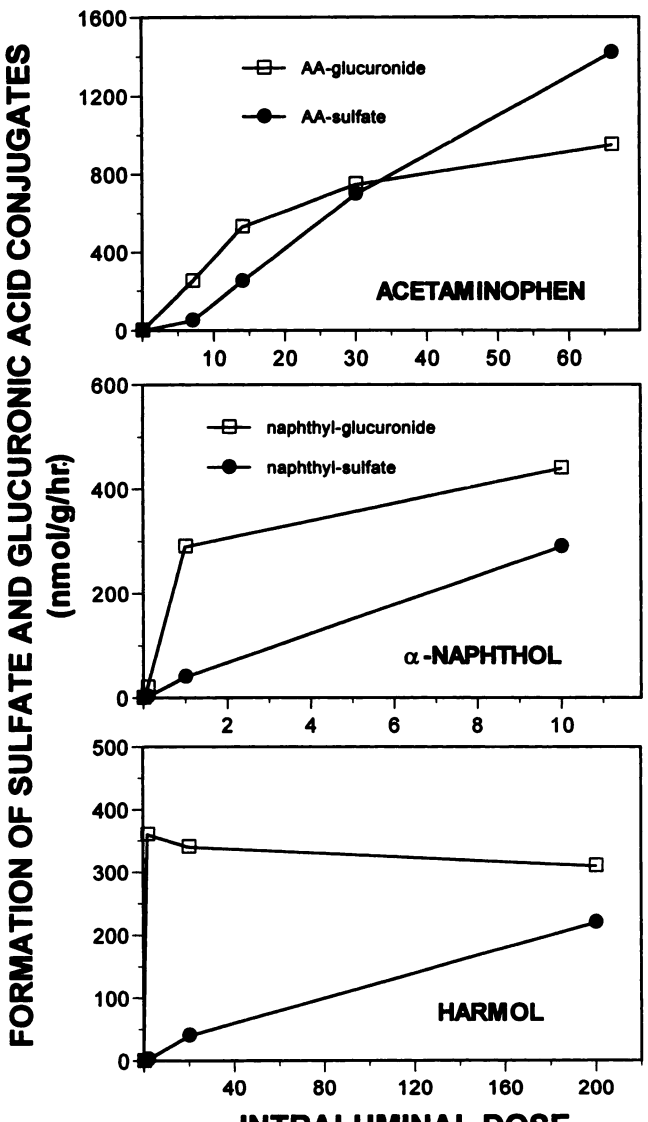


Figure 4. Rates of intestinal conjugation of acetaminophen (AA), α-naphthol, and harmol. Cumulative absorption of the glucuronide and sulfate conjugates of AA (top panel), α-naphthol (middle panel), and harmol (lower panel) from rat in situ isolated intestinal loop system were determined 60 min after administration of their respective parent compound at doses indicated in the graphs. Data previously published (88, 89).

creased (91). These data indicate that in the rat, PAPS and sulfate availability limit sulfation capacity, whereas in mice sulfotransferase activity toward AA limits the maximum rate of sulfoconjugation.

EXPERIMENTAL METHODS TO ALTER SULFATION

Although other pathways of biotransformation such as P450 and glucuronosyltransferase-catalyzed reactions can be increased by induction of their respective enzymes, sulfation cannot be increased in this manner because sulfotransferases are not readily induced. However, pentachlorophenol (93) or 2, 6-dichloro-4-nitrophenol (94) decrease the sulfation of phenolic compounds by competing with substrate for phenolsulfotransferases. In addition, several methods that decrease sulfate availability and/or PAPS synthesis are used to decrease xenobiotic sulfation as well.

ATP-sulfurylase inhibitors in vitro

PAPS formation can be inhibited by substrates that compete with sulfate for ATP-sulfurylase, which catalyzes the first step in PAPS synthesis. Among these alternative substrates are the divalent oxyanions: molybdate (MoO_4^{2-}) (65, 95, 96), selenate (SeO_4^{2-}) (65, 96), tungstate (WO_4^{2-}), chromate (CrO_4^{2-}) (65, 95), and arsenate $(AsHO_4)^{2-}$ (65, 97). These divalent oxyanions form unstable adenosine phosphate complexes (APX) that spontaneously hydrolyze to form AMP in the overall reaction:

$$MgATP + XO_4^{2-} \overset{\text{ATP-sulfurylase}}{\rightleftharpoons} MgPPi + APX$$

$$APX + H_2O \overset{\text{nonenzymatic}}{\rightarrow} AMP + XO_4^{2-}$$

where X = molybdenum, selenium, tungsten, chromium, or arsenic.

In addition, reaction velocities are greater when these divalent oxyanions are substrates for ATP-sulfurylase than when sulfate is the substrate. These higher velocities allow better characterization of

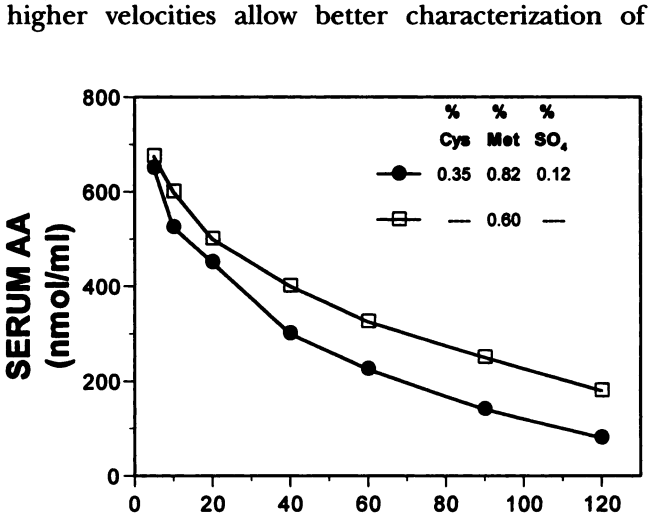


Figure 5. Effects of cysteine- and sulfate-sufficient or-deficient diets on serum concentrations of administered acetaminophen (AA). Rats were fed diets sufficient (●) or deficient (□) in both cysteine (Cys) and inorganic sulfate for 5 days. Serum concentrations of (AA) were determined at the times indicated in the graphs after the administration of AA (0.5 mmol/kg, i.v.). Met = methionine. Data previously published (10).

ATP-sulfurylase kinetics. The reaction velocity can be determined either by measurement of AMP formation or by the generation of Pi from MgPPi after the addition of pyrophosphatase.

Monovalent oxyanions also compete with sulfate for ATP-sulfurylase, thereby inhibiting APS formation. However, they differ from the divalent oxyanions in that APX complexes are not formed. The monovalent oxyanions are: perchlorate (ClO_4^-) (98, 99), nitrate (NO_3^-) (100), chlorite (ClO_3^-), and fluorosulfonate (FSO_3^-) (65).

APS-kinase inhibitors in vitro

APS-kinase catalyzes the formation of PAPS from APS; its activity can be inhibited in vitro by chemical modifiers such as phenylglyoxal or by its substrate APS and its product PAPS (81). Chemical modification of APS-kinase by phenylglyoxal provides structural information, i.e., an essential argininyl residue within the protein (81), whereas inhibition of APS-kinase with endogenous substrate (APS) or product (PAPS) provides information on the mechanism of APS-kinase. Although APS is a substrate for APS-kinase, it disrupts the ordered binding of substrates when in excess. Excess APS occupies the vacant PAPS site, as it would normally, but occupies the site before MgADP departure, thus inhibiting further phosphorylation (70). As previously described, PAPS inhibits its own synthesis by negative product feedback.

Effect of decreasing ATP on PAPS concentrations

The formation of APS, and therefore the synthesis of PAPS, require both ATP and inorganic sulfate. Limiting the availability of ATP can decrease sulfation. Low oxygen concentrations, an adenosyl trapping agent (ethionine), a glycolysis inhibitor (2-deoxyglucose), an uncoupler of oxidative phosphorylation (2,4-dinitrophenol), and mitochondrial electron transport inhibitors (antimycin A, menadione, rotenone) are all capable of decreasing ATP concentrations in isolated hepatocytes, thereby decreasing the sulfation of AA (101, 102) and biphenyl (103).

When uncouplers of oxidative phosphorylation (2,4-dinitrophenol) and electron transport inhibitors (rotenone, antimycin A) are administered in vivo, ATP concentrations are decreased, yet hepatic PAPS concentrations are not (104). Surprisingly, 2,4-dinitrophenol increases hepatic PAPS concentrations, most likely due to an inhibitory effect on the sulfotransferases. Other sulfotransferase inhibitors, 2,6-dichloro-4-nitrophenol and pentachlorophenol, elevate hepatic PAPS concentrations as well (104).

Administration of ethionine and fructose, which results in consumption of cytoplasmic ATP, causes greater decreases in hepatic levels of ATP than inhibitors of mitochondrial energy production. This brings about decreases in PAPS, glutathione, and UDP-glucuronic acid, the cosubstrates for sulfate, glutathione, and glucuronide conjugation, respectively (105). Furthermore, when AA, a substrate for sulfation, glutathione conjugation, and glucuronidation, is administered, the excretion of these conjugates is decreased by prior administration of ethionine and fructose. These results indicate that *1)* steady-state concentrations of PAPS are decreased only with dramatic decreases in ATP, and *2)* decreases in ATP do not selectively impair sulfoconjugation as other conjugation pathways are decreased as well.

Effects of chemicals on ATP-sulfurylase, APS-kinase activity, and PAPS

Whereas some chemicals are used as tools to inhibit PAPS synthesis, some hepatotoxicants also decrease the synthesis of PAPS. For example, cadmium and

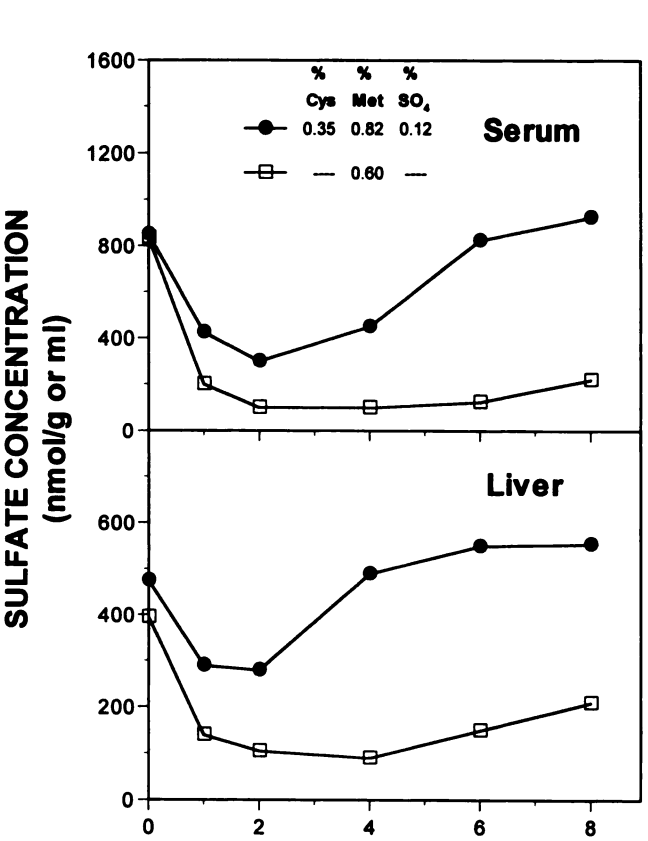


Figure 6. Effects of cysteine- and sulfate-sufficient or -deficient diets on sulfate depletion by administered acetaminophen (AA). Rats were fed diets sufficient (●) or deficient (□) in both cysteine (Cys) and inorganic sulfate for 5 days. Effects of acetaminophen (AA) on the concentration of inorganic sulfate in serum (upper panel) and liver (lower panel) were determined after the administration of AA (0.5 mmol/kg, i.v.) at the times indicated in the graphs. Met = methionine. Data previously published (10).

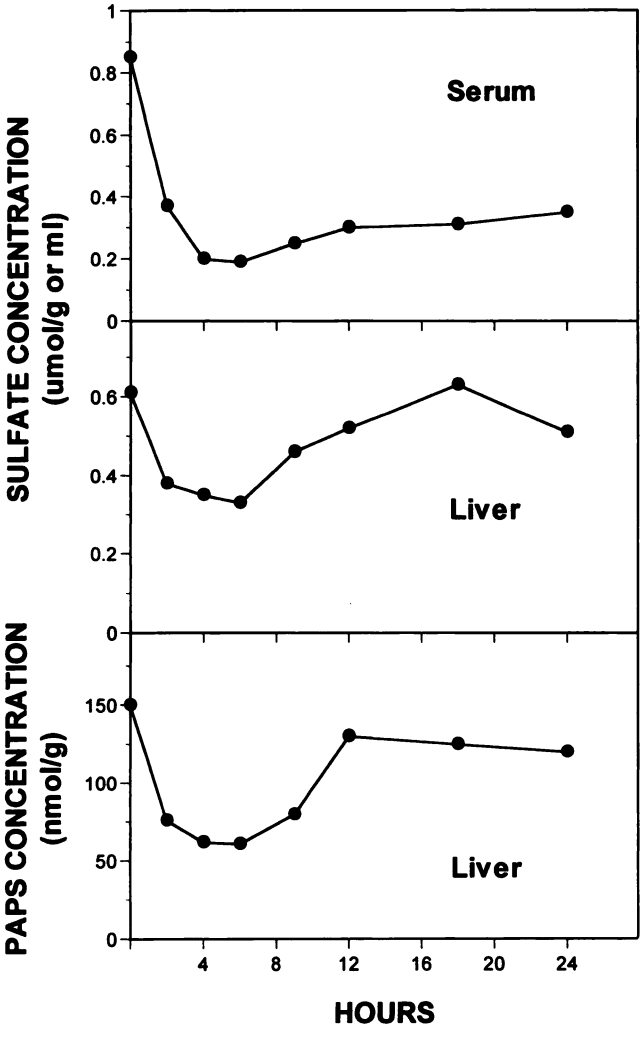


Figure 7. Effect of molybdate on serum and hepatic sulfate concentrations and hepatic 3′-phosphoadenosine 5′-phosphosulfate (PAPS) concentrations. Rats were administered molybdate (15 mmol/kg; p.o.) and serum sulfate (upper panel); hepatic sulfate (middle panel) and hepatic PAPS (lower panel) concentrations were determined at the times indicated in the graphs. Data previously published (112).

thioacetamide decrease both hepatic ATP-sulfurylase and APS-kinase activity, whereas bromobenzene and 1,1-dichloroethylene decrease only APS-kinase activity. Cadmium, thioacetamide, and 1,1-dichloroethylene decrease hepatic PAPS concentrations whereas bromobenzene does not (106).

Decreasing dietary sulfate to inhibit chemical sulfation

Sulfation of chemicals in vivo is sensitive to changes in sulfate availability. Low-protein diets (8% casein) fed to rats for up to 4 days decrease serum sulfate levels by 50%, urinary excretion of sulfate by 50%, and the sulfation of a high-dose of phenol by 45% (107). However, protein restriction is not specific for decreasing sulfate because it also decreases glutathione concentrations and glutathione conjugation of chemicals.

Diets low in sulfate and/or cysteine concentrations can be fed to rats and not cause growth retardation or depletion of glutathione in liver, yet decrease sulfate availability in the body. Diets deficient in either inorganic sulfate or cysteine do not decrease the biotransformation and elimination of AA (0.5 mmol/kg; i.v.), but diets deficient in both sulfate and cysteine result in a 40% reduction in the excretion of AA-sulfate (10). Concomitantly, these rats eliminate AA from the blood at a slower rate (**Fig. 5**). In addition, AA-induced decreases in serum and liver sulfate concentrations are lower in rats on sulfate- and cysteine-deficient diets than in rats with a diet sufficient in sulfur (**Fig. 6**). Thus, the diminished availability of inorganic sulfate reduces sulfation. In addition, whereas AA-induced sulfate depletion is transient in control animals, it is prolonged in rats fed a diet deficient in sulfate and cysteine. Thus, diminished dietary availability of sulfur reduces detoxication and elimination of AA.

Effect of glutathione depletors on sulfation

Glutathione and sulfate conjugation pathways compete for available cysteine to synthesize their respective cosubstrates; therefore, decreases in cysteine can decrease both cosubstrates for glutathione and sulfate conjugation. However, chemicals used to deplete glutathione may have different effects on cysteine and sulfate concentrations, depending on their mechanism of action. For instance, phorone and diethyl maleate (DEM) are conjugated with glutathione; they deplete hepatic glutathione and increase consumption of cysteine in rats. Therefore, serum inorganic sulfate and hepatic PAPS are decreased, causing decreases in the sulfation of harmol and AA (108, 109). Buthione sulfoximine (BSO), like phorone and DEM, decreases hepatic glutathione concentrations. However, BSO inhibits the first enzyme in glutathione synthesis, spares the use of cysteine in glutathione synthesis, and increases the availability of cysteine for sulfate formation. Consequently, BSO increases the sulfation of AA in rats (109).

Effect of molybdate on sulfation

Molybdate is an analog of sulfate and competes with inorganic sulfate for transport from the intestine (110), renal sulfate reabsorption (111), and sulfate incorporation into APS during the first step of PAPS synthesis (95). Molybdate thereby decreases serum sulfate, hepatic sulfate, and hepatic PAPS concentrations (**Fig. 7**) and markedly decreases the sulfation of AA (**Fig. 8**) (112). Molybdate also decreases the sulfation of glycosaminoglycans in vivo and in vitro (113).

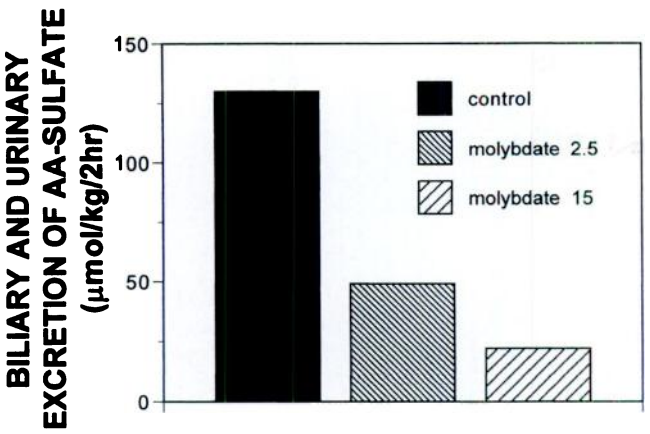


Figure 8. Effect of molybdate on the combined biliary and urinary excretion of acetaminophen (AA) sulfate. Rats were administered molybdate (15 mmol/kg; p.o.) 4 h before administration of AA (1 mmol/kg, i.v.). AA-sulfate concentrations were determined in bile and urine 2 h after AA administration. Bars represent the combined (biliary+urinary) excretion of AA-sulfate during the 2 h period. Data previously published (112).

Toxicity as a consequence of decreasing PAPS and inorganic sulfate availability

Simultaneous exposure to one or more compounds that are extensively sulfated can possibly deplete sulfate and PAPS, thus compromising subsequent sulfation. For instance, AA decreases the sulfation of phenol by 6–35% in rats (107); salicylate decreases the sulfation of patellar glycosaminoglycans by 56% and serum sulfate concentration by 70% in mice (114). Therefore, drugs that lower sulfate availability by being extensively sulfated may hinder in vivo sulfation of glycosaminoglycans in cartilage, which is necessary for normal cartilage integrity (115, 116).

SUMMARY

Sulfation is a capacity-limited process that is dependent on the synthesis of PAPS from inorganic sulfate. PAPS is the obligate cosubstrate for all sulfotransferases and catalyzes the transfer of sulfate to substrates in all higher organisms. PAPS is decreased with the sulfation of high doses of substrates and is the limiting factor in the capacity of sulfation in rats. Several experimental approaches that inhibit the synthesis of PAPS decrease the sulfation of xenobiotics and endogenous molecules, thus offering a means to study sulfation and models for impaired sulfation. FJ

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