

- 30 Portnoy ME, Rosenzweig AC, Rae T *et al.* (1999) Structure-function analyses of the ATX1 metallochaperone. *J Biol Chem* 274 (21), 15041–15045.
- 31 Brewer GJ (2001) Copper control as an antiangiogenic anticancer therapy: lessons from treating Wilson's disease. *Exp Biol Med* 226 (7), 665–673.
- 32 Brewer GJ, Merajver SD (2002) Cancer therapy with tetrathiomolybdate: antiangiogenesis by lowering body copper – a review. *Integr Cancer Ther* 1 (4), 327–337.
- 33 Brewer GJ (2003) Tetrathiomolybdate anticopper therapy for Wilson's disease inhibits angiogenesis, fibrosis and inflammation. *J Cell Mol Med* 7 (1), 11–20.
- 34 Folkman J (1971) Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285 (21), 1182–1186.
- 35 Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med* 1 (1), 27–31.
- 36 Brem S (1999) Angiogenesis and cancer control: from concept to therapeutic trial. *Cancer Control* 6 (5), 436–458.
- 37 Raju KS, Alessandri G, Ziche M *et al.* (1982) Ceruloplasmin, copper ions, and angiogenesis. *J Natl Cancer Inst* 69 (5), 1183–1188.
- 38 Ziche M, Jones J, Gullino PM (1982) Role of prostaglandin E1 and copper in angiogenesis. *J Natl Cancer Inst* 69 (2), 475–482.
- 39 Brem SS, Tsanaclis AM, Zagzag D (1990) Anticopper treatment inhibits pseudopodial protrusion and the invasive spread of 9L gliosarcoma cells in the rat brain. *Neurosurgery* 26 (3), 391–396.
- 40 Brem SS, Zagzag D, Tsanaclis AM *et al.* (1990) Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. *Am J Pathol* 137 (5), 1121–1142.
- 41 Brewer GJ, Hedera P, Kluijn KJ *et al.* (2003) Treatment of Wilson disease with ammonium tetrathiomolybdate: III. Initial therapy in a total of 55 neurologically affected patients and follow-up with zinc therapy. *Arch Neurol* 60 (3), 379–385.
- 42 Pan Q, Kleer CG, van Golen KL *et al.* (2002) Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. *Cancer Res* 62 (17), 4854–4859.
- 43 van Golen KL, Bao L, Brewer GJ *et al.* (2002) Suppression of tumor recurrence and metastasis by a combination of the PHSCN sequence and the antiangiogenic compound tetrathiomolybdate in prostate carcinoma. *Neoplasia* 4 (5), 373–379.
- 44 Khan MK, Miller MW, Taylor J *et al.* (2002) Radiotherapy and antiangiogenic TM in lung cancer. *Neoplasia* 4 (2), 164–170.
- 45 Cox C, Merajver SD, Yoo S *et al.* (2003) Inhibition of the growth of squamous cell carcinoma by tetrathiomolybdate-induced copper suppression in a murine model. *Arch Otolaryngol Head Neck Surg* 129 (7), 781–785.
- 46 Pan Q, Bao LW, Kleer CG *et al.* (2003) Antiangiogenic tetrathiomolybdate enhances the efficacy of doxorubicin against breast carcinoma. *Mol Cancer Ther* 2 (7), 617–622.
- 47 Kent MS, Madewell BR, Dank G *et al.* (2004) An anticopper antiangiogenic approach for advanced cancer in spontaneously occurring tumors, using tetrathiomolybdate: a pilot study in a canine animal mode. *J Trace Elem Exp Med* 17 (1), 9–20.
- 48 Brewer GJ, Dick RD, Grover DK *et al.* (2000) Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: Phase I study. *Clin Cancer Res* 6 (1), 1–10.
- 49 Redman BG, Esper P, Pan Q *et al.* (2003) Phase II trial of tetrathiomolybdate in patients with advanced kidney cancer. *Clin Cancer Res* 9 (5), 1666–1672.
- 50 Pan Q, Bao LW, Merajver SD (2003) Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NFκB signaling cascade. *Mol Cancer Res* 1 (10), 701–706.
- 51 Border WA, Noble NA (1994) Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 331 (19), 1286–1292.
- 52 Brewer GJ, Ullenbruch MR, Dick RB *et al.* (2003) Tetrathiomolybdate therapy protects against bleomycin-induced pulmonary fibrosis in mice. *J Lab Clin Med* 141 (3), 210–216.
- 53 Brewer GJ, Dick R, Ullenbruch MR *et al.* (2004) Inhibition of key cytokines by tetrathiomolybdate in the bleomycin model of pulmonary fibrosis. *J Inorg Biochem* 98 (12), 2160–2167.
- 54 Askari FK, Dick RB, Mao M *et al.* (2004) Tetrathiomolybdate therapy protects against concanavalin A and carbon tetrachloride hepatic damage in mice. *Exp Biol Med* 229 (8), 857–863.
- 55 Ma S, Hou G, Dick RD *et al.* (2004) Tetrathiomolybdate protects against liver injury from acetaminophen in mice. *J Appl Res Clin Exp Ther* 4 (3), 419–426.
- 56 Elliott MJ, Maini RN, Feldmann M *et al.* (1993) Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 36 (12), 1681–1690.
- 57 Shanahan JC, St Clair W (2002) Tumor necrosis factor-alpha blockade: a novel therapy for rheumatic disease. *Clin Immunol* 103 (3 Pt 1), 231–242.
- 58 D'Haens G, Van Deventer S, Van Hogezaand R *et al.* (1999) Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. *Gastroenterology* 116 (5), 1029–1034.
- 59 Mease PJ, Goffe BS, Metz J *et al.* (2000) Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet* 356 (9227), 385–390.
- 60 Elnor SG, Elnor VM, Yoshida A *et al.* (2005) Effects of tetrathiomolybdate in a mouse model of retinal neovascularization. *Invest Ophthalmol Vis Sci* 46 (1), 299–303.

### 2.3.14 Trace elements and the liver

*Brent A. Neuschwander-Tetri*

Life would not be possible without a large number of 'trace' elements, each serving critical roles in metabolism and function (Table 1). This chapter reviews the function and relevant biology of trace elements with respect to the liver. Because copper has a special role as a trace element with respect to normal liver function and liver disease, it is covered in more detail in Chapter 2.3.13 and is not discussed further here.

Trace elements are necessary for normal function and are therefore associated with morbid deficiency states. They are also commonly toxic when present in excess, and this chapter will touch briefly on toxicity as it relates to the essential trace elements. Actual values for liver, plasma and total body content of the trace elements that are used to assess deficiency states and toxicity are catalogued in other resources and are not reviewed here [1–4]. Although such values are important in forensic medicine and epidemiological studies, they are rarely relevant to the routine practice of clinical medicine [5]. Accurate

**Table 1** Physiological trace elements in the body.<sup>a</sup>

Element	Function(s)	Causes of deficiency	Deficiency: hepatic consequences	Deficiency: non-hepatic consequences	Changes in liver disease
Arsenic	Uncertain <sup>b</sup>				
Cobalt	Cobalamin (vitamin B12)	Multiple	Fatty liver disease	Multiple	Impaired elimination of vitamin B12 degradation products Hepatic accumulation in chronic liver disease
Chromium	Improves insulin signalling	Defined diet without supplementation	Impaired glucose tolerance	Impaired glucose tolerance	
Fluorine	Uncertain <sup>b</sup>				
Iodine	Thyroid hormone				
Lead	Uncertain <sup>b</sup>				
Manganese	Cofactor for multiple enzymes (e.g. MnSOD, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, arginase, prolidase)	Defined diet without supplementation	Impaired glucose tolerance, elevated ammonia levels, impaired cholesterol synthesis, impaired clotting factor synthesis	Impaired glucose tolerance, dermatitis	Decreased urinary excretion and increased liver concentration in alcoholic cirrhotics [58] Increased levels in globus pallidus in cirrhotics, may cause extrapyramidal symptoms [53]
Molybdenum	Xanthine oxidase Aldehyde oxidase Sulphite oxidase	Defined diet without supplementation	Altered sulphur amino acid metabolism	Not established	
Nickel	Nickel <sup>b</sup> known to have a catalytic role in six bacterial enzymes (including urease), but no eukaryotic enzymes				
Selenium	See Table 2	Dietary protein deficiency Dietary protein solely from Se-deficient areas	Oxidant stress	See text	Low serum levels in chronic liver disease [26]
Silicon	Uncertain <sup>b</sup>				
Tin	Uncertain <sup>b</sup>				
Vanadium	Uncertain <sup>b</sup>				
Zinc	Cofactor for multiple enzymes and transcription factors	Increased urinary loss in cirrhosis Alcoholism Malabsorption (e.g. coeliac disease, inflammatory bowel disease)	Altered ammonia metabolism	Encephalopathy in cirrhotics	Low serum levels in chronic liver disease [26] Low liver levels in alcoholic cirrhosis [58]

<sup>a</sup>Copper is discussed in Chapter 2.3.13.

<sup>b</sup>Certain elements may have physiological roles based on animal studies of specific dietary deficiencies, but requirements and specific functions in humans are not known [5,7].

assessment of trace element levels in organs such as the liver continues to be refined as technologies such as scanning transmission electron microscopic analysis are developed. Nonetheless, the necessity and function of certain elements such as boron, nickel, tin and vanadium continues to be debated (Table 1) because dietary deficiency models in animals sometimes suggest that they are essential, yet their specific functions at the molecu-

lar level have not been established [5,6]. Described below are the functions of major trace elements relevant to the liver with special emphasis on selenium, an element now recognized to play a unique role in human biology. The importance of many trace elements in nutrition and disease was reviewed extensively by an expert panel [7], and the summary below includes many of their key findings.

## Selenium

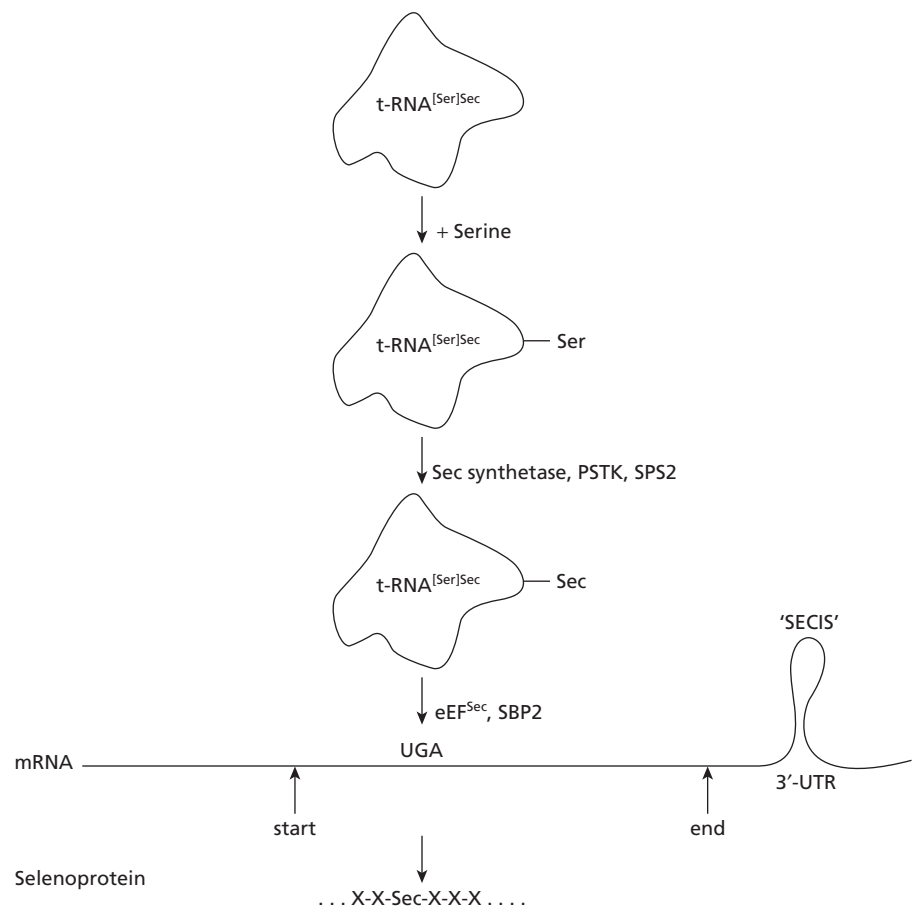
Selenium has a special role in the normal function of the liver and tissues throughout the body. Found directly below sulphur on the periodic table, selenium thus shares many of the chemical properties of sulphur [8]. In fact, most selenium in the body is found in selenoproteins, which contain the amino acid selenocysteine (Sec), a modification in which selenium is found in place of the sulphur atom of cysteine. Failure to produce selenoproteins in the liver causes liver necrosis in mice, attesting to the importance of selenium in normal liver function [9]. Selenium is also incorporated into proteins as selenomethionine from ingested selenomethionine. In contrast to the specific incorporation of Sec in proteins, selenomethionine (Se-met) replaces methionine arbitrarily during translation depending on the relative abundance of Se-met [10]. Although Se-met may exert different local steric effects from methionine, it has not been found to have specific biological functions [11].

The story of how Sec becomes incorporated into selenoproteins is one that has challenged the accepted dogma of protein synthesis pathways as it has unfolded over the past three decades. While one possible mechanism of selenium incorporation could be replacement of the sulphur atom in cysteine by

selenium through posttranslational peptide modification, the surprising reality is that Sec is preformed as a unique amino acid and incorporated during translation. The conceptual difficulty this raised is that it contradicted the decades-old paradigm that the 64 possible ribonucleotide triplets of mRNA encode either insertion of 20 specific amino acids or translational termination. Understanding how Sec, now considered to be the 21st amino acid, could be synthesized and incorporated during translation required a revision of this paradigm.

Through a number of elegant experiments conducted over the past two decades, Sec is now recognized to be translationally inserted into nascent peptide chains by a unique transfer RNA,  $t\text{-RNA}^{\text{[Ser]Sec}}$ , which recognizes the UGA codon, a codon canonically identified as a termination signal (Fig. 1). In the context of several proteins acting *in trans* and a specific *cis*-acting hairpin sequence in the 3' untranslated regions of selenoprotein mRNA transcripts, the UGA codon is recognized as the signal for Sec insertion into nascent peptides [12–15]. Genetic deletion of  $t\text{-RNA}^{\text{[Ser]Sec}}$  in mice is embryonically lethal, attesting to the importance of this pathway [9]. Adding to this complexity was the finding that  $t\text{-RNA}^{\text{[Ser]Sec}}$  directly participates in the enzymatic formation of Sec. The t-RNA is first charged with serine, but the bound serine is subsequently converted to Sec while still

**Fig. 1** Synthesis of selenoproteins. Selenocysteine (Sec) is synthesized from the serine (Ser) carbon backbone on  $t\text{-RNA}^{\text{[Ser]Sec}}$ .  $t\text{-RNA}^{\text{[Ser]Sec}}$  is a unique transfer RNA that is required for both Sec synthesis and translational incorporation of Sec into nascent selenoproteins. Sec synthesis is facilitated by an uncharacterized Sec synthetase and selenophosphate synthetase 2 (SPS2). Selenoprotein mRNAs contain an essential stem-loop *cis* element, the Sec insertion sequence (SECIS), in the 3' untranslated region (3' UTR) that is required for the recognition of the UGA codon as a Sec codon rather than a termination signal. Binding of  $t\text{-RNA}^{\text{[Ser]Sec}}$  to the mRNA-ribosomal complex is also facilitated by other unusual factors including a Sec-specific elongation factor ( $e\text{EF}^{\text{Sec}}$ ) and SECIS binding protein 2 (SBP2).



attached to t-RNA<sup>[Ser]Sec</sup> [16]. In no other case is a t-RNA known to participate catalytically in the modification of a bound amino acid. The unique enzymes needed for these steps were first elucidated in bacteria and then defined in eukaryotes, although many details remain to be clarified [12,17]. An interesting recent observation with respect to liver disease is that the antigenic epitope for antisoluble liver antigen (anti-SLA), an antibody associated with autoimmune hepatitis, appears to be t-RNA<sup>[Ser]Sec</sup> [18].

### Biological functions of selenium

The biological functions of selenium are mediated primarily by Sec in selenoproteins. Early tracer studies with <sup>75</sup>Se identified several major selenoproteins that included glutathione peroxidase (GPx) and selenoprotein P (SelP). Until recently, computer algorithms used to identify open reading frames in genes could not accurately identify UGA codons that encode Sec instead of translational termination. However, the recognition of an obligate and unique 3' *cis* element in selenoprotein transcripts led to new computational approaches that subsequently identified a total of 25 putative selenoproteins, now identified as the 'selenoproteome' of the mammalian genome (Table 2) [19]. Reconciling the selenoproteome with previously identified selenoproteins has been complicated by the existence of multiple alternative splice forms of many of the transcripts.

The enzymatic function of selenoproteins typically depends on the presence of a negatively charged deprotonated selenium and, for this reason, the Sec is usually located within the active site. The pK<sub>a</sub> of the selenol group of Sec is substantially higher than the analogous sulphhydryl of cysteine and, as such, is mostly deprotonated (R-Se<sup>-</sup>) at physiological pH. By comparison, the pK<sub>a</sub> of cysteine is lower, and it is mostly protonated (R-SH) at physiological pH. Thus, Sec is a much more facile nucleophile than cysteine at reactive sites of selenoprotein enzymes. Three examples of selenoproteins are described below.

The first selenoprotein to be characterized was glutathione peroxidase 1 (GPx1), an enzyme that catalytically eliminates hydrogen peroxide and lipid hydroperoxides in the cytosol and mitochondria using glutathione as an electron donor. The highest cellular concentrations of GPx1 are found in the liver. Catalase, a non-selenium-dependent enzyme, also eliminates hydrogen peroxide but is confined to peroxisomes and does not participate in the general intracellular defence against oxidant stress. Moreover, catalase does not eliminate lipid hydroperoxides. Although oxidant stress impairs normal cellular functions, overexpression of GPxs may adversely influence normal redox-mediated cellular signalling [20]. For example, mice that overexpress GPx1 in the liver develop features of insulin resistance, suggesting that too much antioxidant defence could have a negative impact on normal insulin signalling [21].

Another selenoprotein, thioredoxin reductase (TrxR), plays an important role in most organs in maintaining normal redox status. TrxR-1 is localized to the cytoplasm where, in addition to

its role in thiol redox regulation, it is essential for deoxynucleotide synthesis. Another form, TrxR-2, is located within mitochondria and prevents oxidant injury caused by the constitutive release of superoxide from the mitochondrial electron transport chain. Deletion of TrxR (but not GPx1 or SelP) induces a cellular stress response [22], and human tumour cells treated with tumour necrosis factor (TNF) $\alpha$  undergo increased apoptosis when they lack Sec in TrxR. In addition, non-physiological trace elements such as gold and platinum actively inhibit TrxR [23].

All Sec-containing selenoproteins except SelP contain only one Sec, whereas SelP, thought to serve as a transport protein for selenium, has 10 Sec. Although many tissues express SelP, impaired liver synthesis of SelP reduces serum levels by 75% in mice, indicating that the liver serves a primary role in the synthesis and secretion of this selenium transport protein [9,24]. Evidence that liver-synthesized and secreted SelP is required by other organs is provided by a liver-specific knockout of SelP in mice that demonstrates impaired renal synthesis of selenoproteins [25].

### Deficiency states

Profound liver-specific deficiency of selenoproteins causes liver necrosis and death in genetically modified mice that cannot synthesize selenoproteins in the liver [9]. Dietary selenium deficiency in humans is unlikely to mimic this extreme phenotype, and such overt liver manifestations of selenium deficiency have not been described clinically. However, low selenium levels have been found in patients with chronic liver disease [26–28], and selenium-deficient rodents have higher serum liver enzymes and markers of oxidant stress [29,30]. Studies in cultured cells have shown that selenium deficiency in hepatocytes leads to cell death by oxidative stress, but many malignant hepatocellular carcinoma cell lines survive in culture despite selenium deficiency, possibly indicating the resistance of liver cancer to normal oxidant-mediated mechanisms of cell death.

A diverse diet usually contains sufficient selenium to meet the recommended daily allowance of 55–70  $\mu$ g per day and prevent a deficiency state. However, with the new recognition of multiple Sec-containing proteins, this assertion is being revisited to determine whether some of the previously unknown proteins may be impaired by inadequate selenium status despite a diet containing the currently recommended amount [31,32]. Dietary protein is an important source of selenium. Rats fed a protein-deficient diet had low GPx levels and exhibited increased sensitivity to liver injury induced by carbon tetrachloride [33], abnormalities that could be ameliorated by selenium supplements [34]. Acute alcohol feeding has also been shown to decrease hepatic selenium levels in animal studies, whereas deficiency states in people are usually associated with consuming food produced in a limited geographical region (e.g. certain regions in New Zealand and China) and are associated with growth retardation, defective spermatogenesis, cataracts and cardiomyopathy.

**Table 2** Selenoprotein functions.

Selenoprotein	Function	Comments
GPx1 <sup>a</sup>	Eliminates cytosolic hydrogen peroxide and lipid hydroperoxides; most abundant in the liver	First selenoprotein characterized; also called cytosolic GPx
GPx2	Gastrointestinal mucosal antioxidant, inhibits absorption of lipid hydroperoxides [36]	Originally described in gut mucosa but also found in liver
GPx3	Plasma antioxidant	Secreted glycoprotein, kidneys may be the major source; 'plasma GPx'
GPx4	Eliminates diverse hydroperoxides [59]	May regulate intracellular signalling [60]; 'phospholipid hydroperoxide GPx'
GPx6	Unknown function	Identified by genomic analysis [19]
H	Hypothetical protein	
I	Selenocysteine synthesis	
J	Hypothetical protein	
K	Putative membrane protein	
M	Unknown function [61]	Abundant in brain
N, SEPN1	Possible antioxidant in muscle	Possible role in muscular dystrophy; requires prenylation for function, HMG-CoA reductase inhibitors may interfere and cause myopathy [62]
O	Hypothetical protein	
P, SelP	Selenium storage and transport [24]	10 selenocysteines per molecule, widely expressed in different organs; liver may be the major source of circulating SelP
R, MsrB1	Methionine sulfoxide reductase [63]	
S	Putative membrane protein	
SPS2	Essential for tRNA <sup>[Ser]<sup>Sec</sup></sup> synthesis	Possible selenium donor
T	Hypothetical protein	
Trx1	Catalyses flavin-mediated reduction of oxidized thioredoxin	Localized to cytosol
Trx2	Thioredoxin reduction	Localized to mitochondria
Trx3	Thioredoxin reduction	Localized to testis
V	Hypothetical protein	
W	Probable antioxidant function	Most abundant in cardiac and skeletal muscle
X	Hypothetical protein	mRNA abundant in liver, leukocytes
Z	Hypothetical protein	mRNA abundant in liver, kidney
DI-1 <sup>b</sup>	Catalyses the monodeiodination of thyroxine (T4) to the active thyroid hormone (T3) or inactive form (rT3)	Found in liver, may play a role in liver-specific effects of thyroid hormone
DI-2	Conversion of T4 to T3 and rT3	Not localized to liver
DI-3	Conversion of T4 to rT3	Primarily localized to the CNS
Sep15, 15 kDa protein	Possible role in endoplasmic reticulum protein folding	High expression in liver; localized to the endoplasmic reticulum [35,64]

<sup>a</sup>GPx, glutathione peroxidase (note, GPx5 is not a selenoprotein).

<sup>b</sup>DI, iodothyronine diiodinase [65].

CNS, central nervous system; HMG-CoA, hydroxymethylglutaryl-CoA.

Adapted from refs 19, 12 and 10.

As is true of many other trace elements and vitamins, sub-optimal health attributed to mild and clinically undetected deficiency states has been proposed and is the basis for selenium supplementation as an antioxidant in many multivitamins and other supplements. Disease states that have been attributed to moderately low selenium levels include cancer, susceptibility to viral infections, progression of AIDS, defective spermatogenesis, arthritis, vascular disease, asthma and immune system dysregulation [12,35]. Mice lacking both GPx1 and GPx2 have been found to develop ulcerative colitis and colon cancers, attesting

to the potential importance of selenium enzymes in preventing inflammation and neoplasia [36,37]. On the other hand, the selenium-dependent thioredoxin pathway is antiapoptotic and may favour the survival of malignant cells.

Selenium provided in dietary supplements is typically in forms such as sodium selenite or selenate, although the primary dietary source may be selenomethionine in plant proteins [11,38]. Other organic forms of selenium have been found to be a potentially useful source as well [39] and, despite major differences between selenite supplements and selenate supplements

[40], the best approach to dietary selenium supplementation has not been established.

### Toxicity of selenium

In general, excess selenium intake is rare in humans but is a significant environmental issue for animals. Liver selenium levels are increased in elderly people from the Faeroe Islands where whales are a dietary mainstay, and also in Inuits of the Arctic North American continent [41]. Environmentally, selenium accumulates in the groundwater in specific geographical regions having poor drainage, and wetland birds that dwell in such regions develop selenium toxicity. Cattle and sheep grazing in such regions also develop colourfully named syndromes such as 'blind staggers' and 'alkali disease' attributed to selenium toxicity, although hepatic manifestations of selenium toxicity have not been described.

Routes of selenium excretion have not been fully identified. The liver converts Se-met to Sec, and Sec is converted to serine and selenide, possibly involving an enzyme that specifically removes selenium from Sec [42]. Selenide is di- or trimethylated and then exhaled or excreted [11]. Additionally, a water-soluble carbohydrate conjugate, Se-methyl-N-acetylselenohexosamine, has been identified as a major urinary selenium metabolite in one study [43]. Whether secretory mechanisms can compensate for excessive intake is unknown, although selenium levels do reach a steady-state level during nutritional supplementation with Se-met [11]. The specific role of the liver in these metabolic pathways has not been established.

### Zinc

The liver plays a central role in zinc homeostasis, removing it from albumin in the blood and distributing it to the body as needed. Hormonal stimuli such as glucocorticoids and epinephrine upregulate hepatic zinc uptake by upregulating hepatic metallothionein levels [44]. Zinc serves as an essential cofactor for over 100 enzymes and zinc finger transcription factors that are necessary for intermediary metabolism in the liver and other organs. It serves a major antioxidant function as a cofactor for copper-zinc superoxide dismutase, the first line of defence against cytosolic reactive oxygen intermediates generated in the liver. The specific effects of zinc deficiency on these metabolic steps have not been fully elucidated.

Excretion of zinc is mostly in the stool; biliary secretion is minimal, and most zinc lost in the stool originates from gut mucosa and pancreatic secretions. Cirrhotics have increased urinary zinc loss and can become zinc deficient. Zinc supplements, typically zinc sulphate, 220 mg three times daily in cirrhotics, may be effective in treating portosystemic encephalopathy and muscle cramps [26,45–47], although these data have been disputed [48]. Zinc supplements increase metallothionein expression, which can impair normal copper uptake. This is the desired effect when zinc is used to treat Wilson's

disease, but the importance of impaired copper uptake in cirrhotics taking zinc supplements requires further investigation.

### Chromium

Chromium in its various forms is both an industrial toxin and a commonly used dietary supplement. The toxicity of chromium depends on its redox state. Whereas Cr VI (i.e. chromate) is a known industrial toxin and carcinogen, Cr III is hypothesized to improve insulin sensitivity by enhancing postreceptor insulin signalling [49]. Cr VI is a strong oxidizing agent but is rapidly reduced to Cr III when ingested. The effect of Cr III on insulin signalling is the rationale for its promotion in forms such as chromium picolinate as a dietary supplement to prevent or improve diabetes. It has also been promoted as an aid in weight reduction and as a stimulant for increasing muscle mass, although controlled clinical trials have not confirmed these claims. The uptake of chromium by hepatocytes is facilitated by an anion channel, probably the same channel that mediates the uptake of other anions such as phosphate.

Chromium has the potential to accumulate in the liver of patients with chronic liver disease, and excess dietary supplements should be avoided in this group [7]. One case of severe hepatotoxicity attributed to chromium picolinate use has been reported [50]. It is not secreted into the bile and, thus, cholestasis is not a cause of chromium accumulation. Accumulation in the liver may develop in long-term peritoneal dialysis patients, although the significance of this is uncertain [51].

### Manganese

Manganese is a cofactor for a number of enzymes important for intermediary metabolism, including the hepatic urea cycle enzyme arginase. However, deficiency syndromes and how they affect normal metabolism in the liver are not well described [52]. A provocative finding with respect to fatty liver disease was that manganese-deficient animals developed insulin resistance and glucose intolerance, but clinical studies of supplements have not been found to improve insulin sensitivity or diabetes. As a cofactor for prolinase, an enzyme needed for proline synthesis, and glucosyltransferases, enzymes needed for glycosaminoglycan synthesis, manganese also plays a role in extracellular matrix production and maturation.

Manganese absorbed from food is avidly taken up by the liver and distributed to tissues bound to transferrin and albumin. The mechanisms of absorption appear to be regulated in parallel with iron absorption such that iron deficiency increases manganese absorption from the gastrointestinal tract and iron excess reduces manganese absorption. Excess manganese is secreted into the bile, and some authorities recommend that supplements should be avoided in patients with chronic liver disease to avoid hepatic accumulation [7]. Manganese has been shown to accumulate in the globus pallidus of the brain in patients with cirrhosis [53].

## Cobalt

Cobalt is best known as the catalytic centre of the corrin ring in cobalamin, the active core of vitamin B12. Vitamin B12 is synthesized by gut bacteria and is thus not directly affected by altered hepatic metabolism. However, the liver plays a central role in enterohepatic circulation of vitamin B12 and the disposal of corrinoid metabolites of vitamin B12 through biliary secretion of degradation products. Alterations in vitamin B12 function leading to manifestations of a deficiency state have thus been attributed to altered hepatic metabolism of B12 [54].

## Molybdenum

Molybdenum is an essential cofactor for three enzymes: sulphite oxidase, xanthine oxidase and aldehyde oxidase. Sulphite oxidase is essential for the hepatic metabolism of sulphur-containing molecules such as cysteine and the formation of taurine. Whether molybdenum deficiency impairs the production of the bile acid taurocholic acid or alters the function of bile is not known. The second molybdenum enzyme, xanthine oxidase, metabolizes purines and is required for the elimination of degraded nucleotides. It has also been proposed to be a significant source of superoxide generation during ischaemia-reperfusion injury. Lastly, aldehyde oxidase requires molybdenum as a cofactor. It is known for its role in the hepatic metabolism of ingested ethanol from toxic acetaldehyde to acetate, but it also plays a key role in eliminating other more toxic aldehydes such as the lipid peroxidation product 4-hydroxynonenal.

Despite these known metabolic roles of molybdenum, there are no recognized hepatic effects of molybdenum deficiency or excess. This metal does undergo enterohepatic circulation and crosses cell membranes through an anion channel. It interacts with copper to form thiomolybdate complexes that increase copper elimination and cause copper deficiency. This interaction is the rationale for its use to promote copper excretion in patients with Wilson's disease.

## Toxicity and elimination of trace elements

The liver has a central role in the uptake and disposition of many trace elements, especially the heavy metals [55]. Metallothionein, a cytosolic metal-binding protein composed of 25–30% cysteines, binds heavy metals, prevents their toxic interaction with other cellular components and facilitates their elimination. Metallothionein is especially important in the uptake and detoxification of cadmium [56]. Stress markedly upregulates hepatic metallothionein expression, but the reason for this is uncertain [57]. The liver also has the highest glutathione levels of any organ, and glutathione plays an important role in the biliary secretion of heavy metals, especially copper, cadmium, mercury, lead and zinc [55]. Although cholestasis is

commonly associated with hepatic copper accumulation, the impact of impaired biliary secretion on the disposal of other heavy metals is less certain.

## Summary

Trace elements serve essential metabolic functions in the liver, and the liver plays a central role in the disposition of most trace elements. Much work is needed to identify optimal measures of trace element adequacy and the functional consequences of deficiency states.

## References

- 1 Versieck J (1985) Trace elements in human body fluids and tissues. *Crit Rev Clin Lab Sci* 22, 97–184.
- 2 Aalbers TG, Houtman JP, Makkink B (1987) Trace-element concentrations in human autopsy tissue. *Clin Chem* 33, 2057–2064.
- 3 Iyengar V, Woittiez J (1988) Trace elements in human clinical specimens: evaluation of literature data to identify reference values. *Clin Chem* 34, 474–481.
- 4 Rahil-Khazen R, Bolann BJ, Myking A *et al.* (2002) Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). *J Trace Elem Med Biol* 16, 15–25.
- 5 Linder MC (1984) Other trace elements and the liver. *Semin Liver Dis* 4, 264–276.
- 6 Nielsen FH (1985) The importance of diet composition in ultratrace element research. *J Nutr* 115, 1239–1247.
- 7 Institute of Medicine Food and Nutrition Board (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. Washington, DC: National Academy Press.
- 8 Jacob C, Giles GI, Giles NM *et al.* (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. *Angewandte Chem Int Edn in English* 42, 4742–4758.
- 9 Carlson BA, Novoselov SV, Kumaraswamy E *et al.* (2004) Specific excision of the selenocysteine tRNA<sup>[Ser]<sup>Sec</sup> (*Trsp*) gene in mouse liver demonstrates an essential role of selenoproteins in liver function. *J Biol Chem* 279, 8011–8017.</sup>
- 10 Behne D, Kyriakopoulos A (2001) Mammalian selenium-containing proteins. *Annu Rev Nutr* 21, 453–473.
- 11 Schrauzer GN (2000) Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *J Nutr* 130, 1653–1656.
- 12 Driscoll DM, Copeland PR (2003) Mechanism and regulation of selenoprotein synthesis. *Annu Rev Nutr* 23, 17–40.
- 13 Ramos A, Lane AN, Hollingworth D *et al.* (2004) Secondary structure and stability of the selenocysteine insertion sequences (SECIS) for human thioredoxin reductase and glutathione peroxidase. *Nucleic Acids Res* 32, 1746–1755.
- 14 Diamond AM (2004) On the road to selenocysteine. *Proc Natl Acad Sci USA* 101, 13395–13396.
- 15 Howard MT, Aggarwal G, Anderson CB *et al.* (2005) Recoding

- elements located adjacent to a subset of eukaryal selenocysteine-specifying UGA codons. *EMBO J* 24, 1596–1607.
- 16 Carlson BA, Xu X-M, Kryukov GV *et al.* (2004) Identification and characterization of phosphoseryl-tRNA<sup>[Ser]<sup>Sec</sup></sup> kinase. *Proc Natl Acad Sci USA* 101, 12848–12853.
  - 17 Mehta A, Rebsch CM, Kinzy SA *et al.* (2004) Efficiency of mammalian selenocysteine incorporation. *J Biol Chem* 279, 37852–37859.
  - 18 Torres-Collado AX, Czaja AJ, Gelpi C (2005) Anti-tRNP<sup>[Ser]<sup>Sec</sup></sup>/SLA/LP autoantibodies. Comparative study using in-house ELISA with a recombinant 48.8 kDa protein, immunoblot, and analysis of immunoprecipitated RNAs. *Liver Int* 25, 410–419.
  - 19 Kryukov GV, Castellano S, Novoselov SV *et al.* (2003) Characterization of mammalian selenoproteomes. *Science* 300, 1439–1443.
  - 20 Arthur JR (2000) The glutathione peroxidases. *Cell Mol Life Sci* 57, 1825–1835.
  - 21 McClung JP, Roneker CA, Mu W *et al.* (2004) Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. *Proc Natl Acad Sci USA* 101, 8852–8857.
  - 22 Mostert V, Hill KE, Burk RF (2003) Loss of activity of the selenoenzyme thioredoxin reductase causes induction of hepatic heme oxygenase-1. *FEBS Lett* 541, 85–88.
  - 23 Nordberg J, Arnér ESJ (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31, 1287–1312.
  - 24 Hill KE, Zhou J, McMahan WJ *et al.* (2003) Deletion of selenoprotein P alters distribution of selenium in the mouse. *J Biol Chem* 278, 13640–13646.
  - 25 Schweizer U, Streckfuss F, Pelt P *et al.* (2005) Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. *Biochem J* 386, 221–226.
  - 26 Loguercio C, De Girolamo V, Federico A *et al.* (2001) Relationship of blood trace elements to liver damage, nutritional status, and oxidative stress in chronic nonalcoholic liver disease. *Biol Trace Elem Res* 81, 245–254.
  - 27 Jain SK, Pemberton PW, Smith A *et al.* (2002) Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. *J Hepatol* 36, 805–811.
  - 28 Aboutwerat A, Pemberton PW, Smith A *et al.* (2003) Oxidant stress is a significant feature of primary biliary cirrhosis. *Biochim Biophys Acta* 1637, 142–150.
  - 29 Matsumoto K-I, Ui I, Satoh K *et al.* (2002) Evaluation of oxidative damage in the liver of selenium-deficient rats. *Redox Rep* 7, 351–354.
  - 30 Moskovitz J, Stadtman ER (2003) Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues. *Proc Natl Acad Sci USA* 100, 7486–7490.
  - 31 Brown KM, Arthur JR (2001) Selenium, selenoproteins and human health: a review. *Public Health Nutr* 4, 593–599.
  - 32 Pagmantidis V, Bermano G, Villette S *et al.* (2005) Effects of Se-depletion on glutathione peroxidase and selenoprotein W gene expression in the colon. *FEBS Lett* 579, 792–796.
  - 33 Gonzalez-Reimers E, Lopez-Lirola A, Olivera RM *et al.* (2003) Effects of protein deficiency on liver trace elements and antioxidant activity in carbon tetrachloride-induced liver cirrhosis. *Biol Trace Elem Res* 93, 127–140.
  - 34 He Y-T, Liu D-W, Ding L-Y *et al.* (2004) Therapeutic effects and molecular mechanisms of anti-fibrosis herbs and selenium on rats with hepatic fibrosis. *World J Gastroenterol* 10, 703–706.
  - 35 Diwadkar-Navsariwala V, Diamond AM (2004) The link between selenium and chemoprevention: a case for selenoproteins. *J Nutr* 134, 2899–2902.
  - 36 Esworthy RS, Aranda R, Martín MG *et al.* (2001) Mice with combined disruption of *Gpx1* and *Gpx2* genes have colitis. *Am J Physiol Gastroint Liver Physiol* 281, G848–G855.
  - 37 Chu FF, Esworthy RS, Chu PG *et al.* (2004) Bacteria-induced intestinal cancer in mice with disrupted *Gpx1* and *Gpx2* genes. *Cancer Res* 64, 962–968.
  - 38 Schrauzer GN (2001) Nutritional selenium supplements: product types, quality, and safety. *J Am Coll Nutr* 20, 1–4.
  - 39 Li L, Xie Y, El-Sayed WM *et al.* (2004) Characteristics of selenazolidine prodrugs of selenocysteine: toxicity, selenium levels, and glutathione peroxidase induction in A/J mice. *Life Sci* 75, 447–459.
  - 40 Mueller AS, Pallauf J, Rafael J (2003) The chemical form of selenium affects insulinomimetic properties of the trace element: investigations in type II diabetic dbdb mice. *J Nutr Biochem* 14, 637–647.
  - 41 Milman N, Laursen J, Byg K-E *et al.* (2004) Elements in autopsy liver tissue samples from Greenlandic Inuit and Danes. V. Selenium measured by X-ray fluorescence spectrometry. *J Trace Elem Med Biol* 17, 301–306.
  - 42 Mihara H, Kurihara T, Watanabe T *et al.* (2000) cDNA cloning, purification, and characterization of mouse liver selenocysteine lyase. Candidate for selenium delivery protein in selenoprotein synthesis. *J Biol Chem* 275, 6195–6200.
  - 43 Kobayashi Y, Ogra Y, Ishiwata K *et al.* (2002) Selenosugars are key and urinary metabolites for selenium excretion within the required to low-toxic range. *Proc Natl Acad Sci USA* 99, 15932–15936.
  - 44 Cousins RJ (1986) Toward a molecular understanding of zinc metabolism. *Clin Physiol Biochem* 4, 20–30.
  - 45 Reding P, Duchateau J, Bataille C (1984) Oral zinc supplementation improves hepatic encephalopathy. Results of a randomised controlled trial. *Lancet* 2, 493–495.
  - 46 Marchesini G, Fabbri A, Bianchi G *et al.* (1996) Zinc supplementation and amino acid-nitrogen metabolism in patients with advanced cirrhosis. *Hepatology* 23, 1084–1092.
  - 47 Kugelmas M (2000) Preliminary observation: oral zinc sulfate replacement is effective in treating muscle cramps in cirrhotic patients. *J Am Coll Nutr* 19, 13–15.
  - 48 Baskol M, Ozbakir O, Coskun R *et al.* (2004) The role of serum zinc and other factors on the prevalence of muscle cramps in non-alcoholic cirrhotic patients. *J Clin Gastroenterol* 38, 524–529.
  - 49 Vincent JB (2000) Quest for the molecular mechanism of chromium action and its relationship to diabetes. *Nutr Rev* 58, 67–72.
  - 50 Cerulli J, Grabe D, Gauthier I *et al.* (1998) Chromium picolinate toxicity. *Ann Pharmacother* 32, 428–431.
  - 51 Borguet F, Wallaey B, Cornelis R *et al.* (1996) Transperitoneal absorption and kinetics of chromium in the continuous ambulatory peritoneal dialysis patient – an experimental and mathematical analysis. *Nephron* 72, 163–170.
  - 52 Keen CL, Ensuna JL, Watson MH *et al.* (1999) Nutritional aspects of manganese from experimental studies. *Neurotoxicology* 20, 213–223.
  - 53 Spahr L, Butterworth RF, Fontaine S *et al.* (1996) Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. *Hepatology* 24, 1116–1120.
  - 54 Lambert D, Benhayoun S, Adjalla C *et al.* (1997) Alcoholic cirrhosis and cobalamin metabolism. *Digestion* 58, 64–71.

- 55 Ballatori N (1991) Mechanisms of metal transport across liver cell plasma membranes. *Drug Metab Rev* 23, 83–132.
- 56 Klaassen CD, Liu J (1998) Metallothionein transgenic and knock-out mouse models in the study of cadmium toxicity. *J Toxicol Sci* 23 (Suppl 2), 97–102.
- 57 Jacob ST, Ghoshal K, Sheridan JF (1999) Induction of metallothionein by stress and its molecular mechanisms. *Gene Expr* 7, 301–310.
- 58 Rodriguez-Moreno F, Gonzalez-Reimers E, Santolaria-Fernandez F *et al.* (1997) Zinc, copper, manganese, and iron in chronic alcoholic liver disease. *Alcohol* 14, 39–44.
- 59 Ran Q, Liang H, Gu M *et al.* (2004) Transgenic mice overexpressing glutathione peroxidase 4 are protected against oxidative stress-induced apoptosis. *J Biol Chem* 279, 55137–55146.
- 60 Imai H, Nakagawa Y (2003) Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic Biol Med* 34, 145–169.
- 61 Korotkov KV, Novoselov SV, Hatfield DL *et al.* (2002) Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element. *Mol Cell Biol* 22, 1402–1411.
- 62 Baker SK (2005) Molecular clues into the pathogenesis of statin-mediated muscle toxicity. *Muscle Nerve* 31, 572–580.
- 63 Kim H-Y, Gladyshev VN (2004) Methionine sulfoxide reduction in mammals: characterization of methionine-R-sulfoxide reductases. *Mol Biol Cell* 15, 1055–1064.
- 64 Korotkov KV, Kumaraswamy E, Zhou Y *et al.* (2001) Association between the 15-kDa selenoprotein and UDP-glucose:glycoprotein glucosyltransferase in the endoplasmic reticulum of mammalian cells. *J Biol Chem* 276, 15330–15336.
- 65 Beckett GJ, Arthur JR (2005) Selenium and endocrine systems. *J Endocrinol* 184, 455–465.

### 2.3.15 Hepatic metabolism of drugs

Chris Liddle and Catherine A.M. Stedman

#### General concepts

Drug metabolism is the process by which drug molecules are chemically altered, usually to more polar metabolites that exhibit increased water solubility to allow elimination in urine or bile and/or increased access to excretory transporters. The liver is quantitatively and qualitatively the most important site of drug metabolism, although extrahepatic metabolism of drugs is also well recognised, both in the gastrointestinal mucosa and by circulating enzymes such as esterases. Drug metabolism is likely to be a byproduct of metabolic pathways that metabolize endogenously synthesized compounds (endobiotics) such as steroids, sterols, bile acids and eicosanoids. This is supported by the observation that hepatic enzymes involved in drug metabolism often have these endobiotic compounds as substrates, and that some hepatocellular transporters that handle bile acids also transport drugs [1,2].

One of the most surprising features of hepatic drug-metabolizing pathways is their ability to cope with a seemingly

endless array of drug substrates. Thus, many of the newer fully synthetic drugs that have no close structural counterparts in nature are successfully metabolized by the liver and irreversibly removed or ‘cleared’ from the body. This broad substrate recognition is achieved by the presence of multiple drug-metabolizing enzymes in the hepatocyte, and many can metabolize multiple substrates. For example, the calcium channel blocker nifedipine is almost entirely metabolized by the cytochrome P450 (P450) CYP3A4, an enzyme with very broad substrate recognition that is responsible for the metabolism of some 50% of all therapeutic drugs. In contrast, the benzodiazepine diazepam is metabolized by P450s belonging to the CYP2C, 2D and 3A subfamilies to several primary metabolites, some retaining pharmacological activity. Thus, individual drugs may be metabolized in very different ways, and variation in the expression of individual enzymes and transporters for genetic or other reasons may have complex effects, which may sometimes be difficult to model and predict in advance.

#### Central role of the liver in drug metabolism

In terms of both gross anatomy and microstructure, the liver is ideally designed as a drug clearance organ (Fig. 1). Most foreign chemical compounds (xenobiotics), including therapeutic drugs, enter the body by absorption from the gastrointestinal tract. As covered in Chapter 2.1.1 of this book, venous drainage from most of the gastrointestinal tract is via the portal vein to the liver. Along with drug-metabolizing enzymes and drug transporters expressed in the intestinal mucosa, the liver provides an effective barrier that prevents xenobiotics from entering the systemic circulation. The fraction of an absorbed drug metabolized on this initial postabsorptive pass through the liver is termed ‘first-pass clearance’ and, factored together with the fraction of the drug absorbed from the gastrointestinal tract, determines the ‘bioavailability’ or the fraction of an orally administered drug that reaches the systemic circulation as intact drug [3]. For some drugs, the extent of first-pass clearance can be so great that oral administration is largely ineffective; for example, glyceryl trinitrate and lidocaine (lignocaine).

Within the hepatic lobular architecture, drug metabolism is not evenly distributed. Some important drug-metabolizing P450s such as CYP3A4 are predominantly expressed in pericentral (zone 3) hepatocytes [4]. One reason for this functional specialization may be that metabolism can give rise to highly toxic electrophilic intermediates, which may lead to cell death. With metabolic segregation, only pericentral necrosis occurs, rather than massive necrosis if highly reactive molecules cannot be successfully detoxified.

#### Drug-metabolizing enzymes

Phase I metabolism refers to a basic structural alteration of a drug molecule, whereas in phase II metabolism, a water-soluble