Invited Review

Distribution and pathophysiologic role of molybdenum-containing enzymes

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Summary. The importance of molybdenum-containing enzymes in the pathophysiology of a number of clinical disorders necessitates a comprehensive understanding of their histological localization and expression. The objectives of this review are to cover such enzymes so far reported and their enzyme- and immunohistochemical localization in various tissues and species, and to discuss their possible pathophysiological effects.

The molybdenum cofactor is essential for the activity of the three molybdenum-containing enzymes, sulfite oxidase, xanthine oxidase and aldehyde oxidase. Sulfite oxidase serves as the terminal enzyme in the pathway of the oxidative degradation of sulfur amino acids, and is also involved in preventing the toxic effects of sulfur dioxide. Biochemical study has revealed a high activity of sulfite oxidase mainly in the liver, heart and kidney with lesser activity observed in other tissues. Subcellular observations have shown that this enzyme is present in the mitochondrial intermembraneous spaces. Xanthine oxidase is the final enzyme in the conversion of hypoxanthine to xanthine, and subsequently, to uric acid. Unlike sulfite and aldehyde oxidases, xanthine oxidase can be converted to xanthine dehydrogenase, and *vice versa*. Xanthine oxidase has been widely investigated for its role in post-ischemic reperfusion tissue injury. Enzyme- and immunohistochemical studies of its localization in various animal species and tissues have shown its ubiquitous distribution in the liver, small and large intestine, lung and kidney, and other tissues. Aldehyde oxidase shares a similar substrate specificity with xanthine oxidase. Although the tissue localization of this enzyme has not been studied as thoroughly as that of xanthine oxidase, aldehyde oxidase is reportedly found in the digestive gland of terrestrial gastropods, the antennae of certain moths as well as the mammalian liver. Recently, the ubiquitous distribution of aldehyde oxidase has been demonstrated in rat tissues. The aldehyde oxidase activity of herbivores exceeds that of carnivores, suggesting a possible role of this enzyme as a protection against the effects of toxic plants. The relationship between the tissue localization of these enzymes and their pathophysiological roles is reviewed.

Key words: Molybdenum-containing enzyme, Sulfite oxidase, Xanthine oxidase, Aldehyde oxidase, Tissue distribution, Histochemistry, Pathophysiology

Overview

The three molybdenum-containing enzymes are sulfite oxidase, xanthine oxidase, and aldehyde oxidase. Essential to their activity is the molybdenum cofactor, a low molecular weight prosthetic group in which the metal is bound to molybdopterin. Although various biological activities of the molybdenum-containing enzymes have been suggested, their tissue or subcellular localization is not completely understood.

Sulfite oxidase serves as the terminal enzyme in the pathway of the oxidative degradation of sulfur amino acids, and is also involved in preventing the toxic effects of sulfur dioxide. Biochemical study has revealed the presence of sulfite oxidase in most tissues of the body. Subcellular observations have shown that this enzyme is present in the mitochondrial intermembraneous spaces. In contrast, xanthine oxidase and aldehyde oxidase are considered as cytosolic enzymes.

Xanthine oxidase is the final enzyme in the conversion of hypoxanthine to xanthine, and subsequently, to uric acid. Unlike sulfite and aldehyde oxidases, xanthine oxidase can be converted to xanthine dehydrogenase, and *vice versa*. Xanthine oxidase has been widely investigated for its role in post-ischemic reperfusion tissue injury (McCord, 1985; Frederiks and Bosch, 1995). Enzyme- and immunohistochemical studies have shown its ubiquitous distribution in the body, suggesting a multifunctional role of this enzyme.

Aldehyde oxidase shares a similar substrate specificity with xanthine oxidase. However, the tissue localization and the physiological role of this enzyme have not been studied as thoroughly as that of xanthine oxidase. The ubiquitous distribution of aldehyde oxidase

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has recently been demonstrated in rat tissues (Moriwaki et al., 1996), suggesting it has physiological roles other than the metabolism of aldehydes.

Sulfite oxidase

Sulfite oxidase (EC 1.8.2.1), a molybdo-hemo-protein, functions as the terminal enzyme in the oxidative degradation of the sulfur amino acids, cysteine and methionine, and also plays a role in preventing the toxic effects of sulfur dioxide. The substrate specificity of sulfite oxidase is strictly confined to sulfite, in marked contrast to the broad substrate specificities of both xanthine and aldehyde oxidase. Sulfite oxidase was first isolated from bovine liver (Cohen and Fridovich, 1971a). It was thereafter purified and characterized from bacteria (Toghrol et al., 1983) as well as from the liver of various species: the fish (Onoue, 1980), rat (Johnson and Rajagopalan, 1977), chicken (Kipke et al., 1989) as well as that of humans (Johnson and Rajopalan, 1976). Its molecular weight is estimated to be about 115,000-120,000 (Cohen and Fridovich, 1971a; Southerland et al., 1978; Onoue, 1980). It is composed of two identical subunits that contain a larger molybdenum and a smaller heme domain (Cohen and Fridovich, 1971b; Kessler and Rajagopalan, 1972; Kessler et al., 1974; Southerland et al., 1978; Onoue, 1980). However, sulfite oxidase from *Thiobacillus novellus* has a smaller monomeric molecular weight of approximately 400,000 (Toghrol et al., 1983). Human sulfite oxidase exhibits a more negative charge and a slightly larger subunit molecular weight than the others, suggesting an evolutionary alteration in its structure with no change in catalytic activity (Johnson and Rajagopalan, 1976). The optimal pH of this enzyme is 8.6 (Cohen and Fridovich, 1971a), 8.7 (Onoue, 1980).

Xanthine oxidase

Xanthine oxidase (EC 1.2.1.37), the final enzyme in the degradation of purines has been widely investigated. This enzyme converts hypoxanthine to xanthine and xanthine to uric acid. However, its substrate is not confined to purines; it oxidizes aldehydes, pyrimidines, pteridines, other heterocyclic nitrogenous compounds and purines, as well as hypoxanthine and xanthine. Unlike sulfite and aldehyde oxidases, xanthine oxidase can be converted to xanthine dehydrogenase, and *vice versa*. Evidence shows that it is involved in the pathogenesis of reperfusion tissue injury by the production of superoxide radicals; superoxide, hydrogen peroxide, or both. Xanthine oxidase has been purified and characterized from the liver of various species: mouse (Carpani et al., 1990), rat (Suleiman et al., 1987) and human (Krenitsky et al., 1986; Moriwaki et al., 1993a), as well as from cow's milk (Hunt and Massey, 1992) and human milk (Abadeh et al., 1992). The molecular weight of xanthine oxidase is estimated to be 300,000 (Suleiman and Stevens, 1987), and consists of

two identical subunits of 150,000 (Krenitsky et al., 1986; Carpani et al., 1990). The optimal pH of human xanthine oxidase is estimated to be 9.0 (Yamamoto et al., 1996).

Aldehyde oxidase

Aldehyde oxidase (EC 1.2.3.1) catalyzes a variety of aldehydes and N-heterocyclics, with a substrate specificity similar to that of xanthine oxidase. Purification of aldehyde oxidase has been reported from the liver of the rabbit (Stell et al., 1989), guinea pig (Yoshihara and Tatsumi, 1985), rat (Ohkubo et al., 1983), hog (Felsted et al., 1973) and human (Johns, 1967). Aldehyde oxidase is a dimeric protein of molecular weight of 270,000-300,000 that consists of two identical subunits (Ohkubo et al., 1983). However, that isolated from potato tubers has a molecular weight of 368,000 with subunits of 37,000 (Rothe, 1975). The optimal pH of aldehyde oxidase is 9.0 for the rabbit enzyme and 10.5 for the bovine enzyme (Felsted et al., 1973).

Investigations of molybdenum-containing enzymes

Amino acid sequences

The amino acid sequences of sulfite oxidase has been evaluated by Garrett and Rajagopalan (1994); that of xanthine oxidase by several investigators: Keith et al., 1987; Amaya et al., 1990; Ichida et al., 1993; Wright et al., 1993; Xu et al., 1994; Glatigny et al., 1995. That of aldehyde oxidase was reported by Li Calzi et al. (1995). The amino acid sequence of human xanthine dehydro-genase has been described by investigators from three different institutions (Ichida et al., 1993; Wright et al., 1993; Xu et al., 1994). The sequence demonstrated by Wright et al. (1993) closely (86% conservation) resembled that of bovine aldehyde oxidase (Li Calzi et al., 1995), and showed less similarity to those of the mouse and rat (49%). It seems likely that the translation product of this cDNA reported by Wright et al. (1993) is not a human xanthine dehydrogenase, but rather is a human homologue of aldehyde oxidase.

Distribution

Biochemical evaluation of distribution of enzyme activity

Several biochemical methods have been used to evaluate the distribution of the activity of the molybdenum-containing enzymes in various animals and higher plants.

Sulfite oxidase

Sulfite oxidase activity in the tissues varies according to species (Johnson and Rajagopalan, 1977). This enzyme is also species-specific (Gunnison et al., 1977; Tejnorová, 1978). Its activity is high in fish liver

and kidney, as compared with that in the heart, spleen, muscle, gill and eye (Onoue, 1980). The distribution of sulfite oxidase activity has been examined in tissue extracts from six mammalian species by spectro-photometry. The liver, kidney and heart showed high sulfite oxidase activity, while that of the brain, lung, spleen and testis was low. Electron paramagnetic resonance spectroscopy demonstrated an absence of sulfite oxidase in the brain, muscle, blood, adipose tissue and thymus of rat (Kessler et al., 1974). The high levels of sulfite oxidase activity in the liver, kidney and heart may be related to the degree of amino acid catabolism in these tissues. Its high activity in the liver may be due to the oxidation of sulfite generated from the catabolism of sulfur amino acids. Its high activity in the kidney may also be involved in the oxidation of sulfite generated by the catabolism of amino acids in this organ, or be circulated in the blood from organs that degrade amino acids. The high activity in the heart may be related to the active metabolism of amino acids in this organ. No differences in the activity of this enzyme were demonstrated among six mammalian species (pig, sheep, cow, horse, rat and rabbit) (Cabré et al., 1990).

Xanthine oxidase

Xanthine oxidase is found in vertebrates such as the mouse, rat, chicken and human. This enzyme exists only as a dehydrogenase form in the chicken. Xanthine oxidase activity has been found in most tissues of the rat. In man, high xanthine oxidase activity has been found only in the liver and small intestine and is undetectable in the heart (Eddy et al., 1987). However, other investigators have claimed the localization of xanthine oxidase activity in the brain and heart (Wajner and Harkness, 1989). In the baboon, xanthine oxidase activity was highest in the duodenum, with some activity being found in the liver and pancreas, while none was found in the lung, stomach, esophagus, tongue, heart or brain (Holmes and Vandeberg, 1986).

Aldehyde oxidase

Aldehyde oxidase activity has been demonstrated in, and purified from potato extract (De Pooter et al., 1989), rabbit, guinea pig, rat, and humans. Its activity shows species differences; eg, being high in the rabbit or baboon liver, and low in that of the rat and dog (Beedham et al., 1987). According to Wurzinger et al., aldehyde oxidase seems to be phylogenetically primitive to xanthine oxidase, with its activity being widely observed among molluscs, crustaceans, insects and vertebrates, except for birds (Wurzinger and Hartenstein, 1974). In contrast, Krenitsky suggested that xanthine oxidase is primordial compared to aldehyde oxidase (Krenitsky, 1978). The latter suggestion seems more likely, since the most primitive species in which aldehyde oxidase has been found is the coelenterate, *Sagartia luciae* (Krenitsky et al., 1974), while xanthine

dehydrogenase was recently found in the fungus, *Aspergillus nidulans* (Glatigny and Scazzochio, 1995). Aldehyde oxidase activity has been detected in such tissues as liver, kidney, duodenum and pancreas of the baboon (Holmes and Vandeberg, 1986), bovine eye ciliary body (Shimada et al., 1989), digestive gland of the terrestrial gastropod (Large and Connock, 1993) and the antenna of certain moths (Teal and Prestwich, 1986; Rybczynski et al., 1989, 1990; Tasayco et al., 1990); the lung, stomach, esophagus, tongue, heart, brain showed no activity (Holmes and Vandeberg, 1986). Aldehyde oxidase activity was examined in the antennae, heads, legs and hemlymph of the moth, *Heliothis virescens* by means of radiochromatographic and spectro-photometric assays. Aldehyde oxidase activity was exhibited primarily in the antennal extracts and, to a lesser degree, in the leg extracts (Tasayco and Prestwich, 1990).

Subcellular fractionation studies of liver xanthine and aldehyde oxidase demonstrated these enzymes to be present mainly in the cytosol, whereas sulfite oxidase was present primarily in the intermembranous space of the mitochondrion as a soluble protein (Wattiaux-De Coninck and Wattiaux, 1971), and to a lesser degree in the cytosol and microsomes (Ito et al., 1977). However, a recent study demonstrated the presence of small amounts of aldehyde oxidase activity, and larger amounts of xanthine oxidase activity, in the mitochondria of the guinea pig liver (Critchley et al., 1992).

Histochemical methods of localizing enzyme activity

As biochemical methods cannot provide information on the precise localization of enzyme activity in the differing cells within a tissue, techniques of histochemical localization such as enzyme-histochemical, immunohistochemical methods and *in situ* hybridization analysis are required to detect mRNA of the enzyme. Although immunohisto-chemical studies cannot differentiate the active and inactive forms of the enzyme, such techniques provide more sensitive and specific enzyme detection than do methods of enzyme-histochemistry. How-ever, the possibility of cross-reactions cannot be completely excluded by immunohistochemical analysis using polyclonal antibody: cross-reactions of rat and human sulfite oxidase polyclonal antibodies to albumin and vitamin D-binding protein, respectively have been reported (Bellissimo and Rajagopalan, 1991). Despite their high sensitivity, the result of immunohistochemical studies for detecting xanthine oxidase protein using polyclonal antibody may not necessarily be correct, since high concentrations of xanthine oxidase antibodies are present in the sera of humans and animals (Bruder et al., 1984). Because of their specificity for a single epitope, monoclonal antibodies offer an advantage over polyclonal anti-bodies.

Table 1. Enzyme-histochemical tissue distribution of molybdenum-containing enzymes.

ENZYME	SPECIES	ORGAN (TISSUE)	REFERENCE
Sulfite oxidase			
	Rat	Heart (mitochondrial membrane, intracristal space)	Ogawa et al., 1968
Xanthine oxidase			
	Rat	Skin, vagina, uterus, penis, liver, oral and nasal cavities, tongue, esophagus, forestomach, small intestine (epithelial cells), adrenal cortex, endothelial cells	Kooij et al., 1992b; Van den Munckhof et al., 1995
	Chicken	Liver, kidney, pancreas, small intestine	McIndoe et al., 1974
	Human	Liver, jejunum	Kooij et al., 1992a
Aldehyde oxidase			
	Rat	Liver, tongue	Moriwaki et al., (unpublished)

Table 2. immunohistochemical tissue distribution of molybdenum-containing enzymes.

ENZYME	SPECIES	ORGAN (TISSUE)	REFERENCE
Sulfite oxidase			
	ND		
Xanthine oxidase			
	Hog	Liver, lactating mammary gland (epithelium)	Jarasch et al., 1981
	Chicken	Liver (hepatocyte, Kupffer cell, endothelial cells lining sinusoids)	Hattori, 1989
	Human	Endothelial cells of various tissues, cardiac, skeletal muscle, macrophage, mast cell, synovial endothelium, epithelium of tongue, esophagus, trachea and stomach, glandular cells of small and large intestine, renal tubules	Stevens et al., 1991; Hellsten-Westing, 1993; Moriwaki et al., 1993a, 1996b
	Rat	Hear (interstitial cell, endothelial cell)	Samra et al., 1991
Aldehyde oxidase			
	Rat	Renal tubules, esophageal, gastric, and small and large intestinal and bronchial epithelium	Moriwaki et al., 1996a

ND: not done.

Enzyme-histochemical methods of localizing enzyme activity (Table 1)

Sulfite oxidase. The localization of sulfite oxidase activity in rat tissues has been studied by electron microscopy (Ogawa et al., 1968) as cytochrome c ferricyanide reductase using the copper ferrocyanide method. Sulfite oxidase activity in the rat heart was observed in the membranous component, and the intra-cristal and outer spaces of the mitochondria. The immunohistochemical localization of sulfite oxidase using polyclonal or monoclonal antibody has not yet been accomplished, although polyclonal antibody has been raised in a rabbit (Bellissimo and Rajagopalan, 1991).

Xanthine oxidase. The localization of xanthine oxidase activity in tissue was previously performed using fixed sections (Sackler, 1966) or in the absence of tissue protectant (Bourne, 1953; Sackler, 1966). However, these methods often caused an inactivation or loss in solution of this enzyme during fixation or incubation, leading to an incorrect interpretation of its localization. A histochemical method for the detection of xanthine oxidase activity was developed by McIndoe et al., who demonstrated xanthine dehydrogenase activity in the liver, kidney, pancreas and small intestine of domestic fowl (McIndoe et al., 1974). The methods were improved by Kooij et al. using unfixed cryostat sections, an incubation medium that contained polyvinyl alcohol as tissue protectant, 1-methoxyphenazine as the intermediate electron carrier, tetranitro blue tetrazolium as the final electron acceptor, and hypoxanthine as the substrate (Kooij et al., 1991). The enzyme-histochemical localization of xanthine oxidase was subsequently investigated (Kooij et al., 1992a,b; Frederiks et al.,

1994). Kooij et al. evaluated the tissue localization of the dehydrogenase and oxidase forms of xanthine oxidoreductase activity in humans (Kooij et al., 1992a) and rats (Kooij et al., 1992b). Those authors demonstrated enzyme activity in the liver and jejunum of humans, supporting the results of previous biochemical studies. In hepatocytes, enzyme activity was mainly observed in the pericentral area and the sinusoidal cells. This finding supports the role of xanthine oxidase as a cause of oxidative stress in the development of mid-zonal liver damage induced by ischemia (Lemasters et al., 1983; Gores et al., 1989). Activity was found in the enterocytes, goblet cells, and lamina propria of the jejunum. In contrast, in the rat, high activity was noted in epithelial cells from the skin, vagina, uterus, penis, liver, the oral and nasal cavities, tongue, esophagus, forestomach and small intestine. Activity was also observed in sinusoidal cells of the liver and adrenal cortex, the endothelial cells in various organs, and the fibroblasts in connective tissue. Similar results have been reported with other enzyme-histochemical methods used in the tissue localization of xanthine oxidase activity, such as the cerium ion capture of H_2O_2 (Gossrau et al., 1990; Frederiks and Marx, 1993). Xanthine oxidase activity has been investigated by electron microscopy in the rat digestive tract by Van den Munckhof et al. who demonstrated such activity in the

cytoplasmic matrix of rat enterocytes and goblet cells and in the duodenal mucus (Van den Munckhof et al., 1995).

Immunohistochemical localization of enzyme activity (Table 2)

Xanthine oxidase. Immunohistochemical localization of xanthine oxidase in tissues has been reported in the bovine liver, the endothelial cells from the lactating mammary gland (Jarasch et al., 1981), and the human synovial endothelium (Stevens et al., 1991). The role of xanthine oxidase in the mammary gland remains to be clarified. Stevens et al. using anti-bovine milk xanthine oxidase, demonstrated human xanthine oxidase antigen in the synovial endothelium, consistent with their previous radioassay results (Allen et al., 1987). This suggests that the endothelial cells may play a major role in the exacerbation of synovitis by producing superoxide radicals. Hattori examined the localization of xanthine dehydrogenase in chicken liver, and reported this

enzyme to be present in the hepatocytes, Kupffer cells and endothelial cells that line the hepatic sinusoids (Hattori, 1989). Hellsten-Westing, using monoclonal antibodies to xanthine oxidase, demonstrated its presence in vascular smooth muscle cells, endothelial cells of capillaries and of smaller vessels in the cardiac and skeletal muscle of humans (Hellsten-Westing, 1993). This author also observed immunoreactivity in macrophage and mast cells. Despite a high activity, the immunohistochemical localization of xanthine oxidase was few in rat tissue other than the liver, until Moriwaki et al. recently demonstrated the ubiquitous presence of this enzyme in rat tissues (Moriwaki et al., 1996a) as shown by enzyme-histochemistry (Kooij et al., 1992b). Using polyclonal antibody to xanthine oxidase and electron microscopy, the enzyme was localized by Jarasch et al. and Ichikawa et al. in the cytoplasm of endothelial cells of capillaries of the bovine heart, small intestine, and hepatocytes (Jarasch et al., 1981), and in the cytoplasm of sinusoidal cells of rat liver (Ichikawa et al., 1992). Using monoclonal antibody to bovine milk
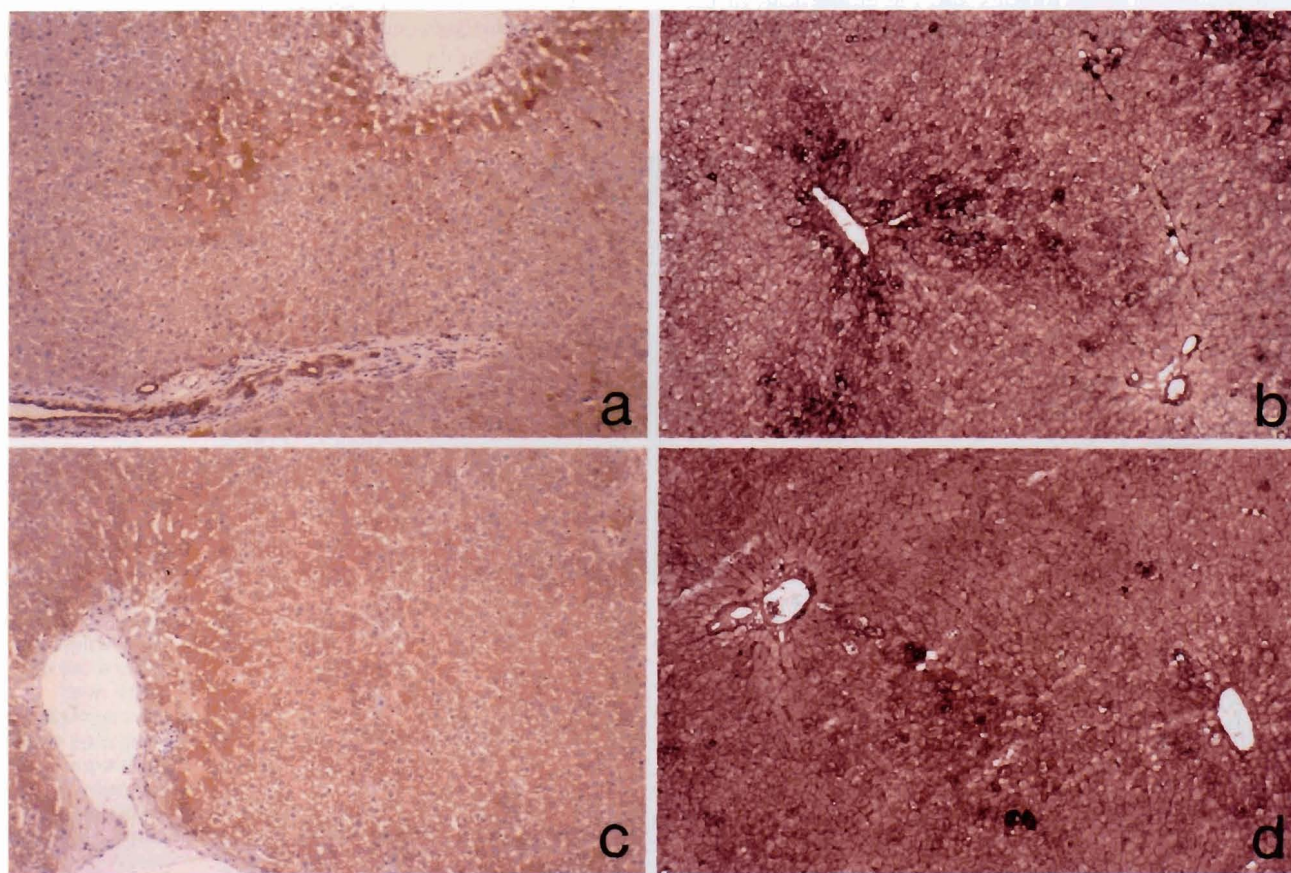

Fig. 1. Enzyme- and immunohistochemical localization of xanthine oxidase and aldehyde oxidase in the rat liver: Enzyme- and immunohistochemical staining show that both enzymes are mainly distributed in the pericentral rather than in the periportal area. a, c. Immunohistochemical staining of xanthine and aldehyde oxidase. b, d. Enzyme-histochemical staining of xanthine and aldehyde oxidase. Enzyme-histochemical analyses of xanthine oxidase and aldehyde oxidase were performed using unfixed cryostat sections 6 μm thick with an incubation medium of 0.1 M Tris-C1 (pH 8.0) that contained 10% polyvinyl alcohol, 0.45 mM phenazine methosulfate, 1.1 mM tetranitro blue tetrazolium, and 0.5 mM hypoxanthine or 0.5 mM p-dimethylaminocinnamaldehyde as substrates. The incubation period was 30 min at 37 °C. x 10

xanthine oxidase, Samra et al. first demonstrated the presence of xanthine oxidase in the interstitial cells and endothelial cells of rat myocardial small vessels (Samra et al., 1991). However, immunohistochemical studies failed to detect the oxidase or the dehydrogenase forms of the enzyme, since neither monoclonal nor the polyclonal antibodies can differentiate these two forms, or the active from the inactive forms. In contrast, enzyme-histochemistry based on the cerium ion capture method detects only the active form of xanthine oxidase (Frederiks and Marx, 1993).

While enzyme- and immunohistochemical methods generally tend to be qualitative, quantitative histochemical methods exist for studying xanthine oxidase activity (Frederiks et al., 1994, 1995). One can thus compare tissue xanthine oxidoreductase activity *in situ* in various physiologic and pathologic conditions.

Aldehyde oxidase. The immunohistochemical localization of aldehyde oxidase in tissues has been little studied. One study in the rat (Moriwaki et al., 1996a) showed that aldehyde oxidase, like xanthine oxidase, is ubiquitous in the renal tubules and the epithelium of the esophagus, stomach, intestine and bronchi. It was also found in the cytoplasm of hepatocytes. Aldehyde oxidase was not found in the adrenal gland, spleen,

mesentery or aorta. The tissue distribution of xanthine oxidase and aldehyde oxidase resembles that of superoxide dismutase, the superoxide eliminating enzyme. Therefore, it has been suggested that xanthine oxidase, aldehyde oxidase and superoxide dismutase may serve as a coupled enzyme system to play a physiological, and perhaps, a pathological role in generating and eliminating superoxide radicals. The enzyme-histochemical staining of aldehyde oxidase (unpublished observations) showed the presence of enzyme activity only in the liver and the epithelium of the tongue, with activity absent in stomach, small intestine and large intestine of rat. The discrepancy between these findings and those of immunohistochemistry may be related to the following: 1) the enzyme-histochemical method is less sensitive than immunohistochemistry; the Km value for aldehyde oxidase differs according to the tissue, so that some tissues require a higher concentration of substrate; 2) aldehyde oxidase may be present in the inactive form in certain tissues, as seen with the desulfo form of xanthine oxidase; 3) cross-reactions may exist with xanthine oxidase polyclonal antibody. The enzyme- and immunohistochemical localization of aldehyde oxidase in rat liver showed that it was mainly distributed in the pericentral area, as seen with xanthine oxidase (Fig. 1).
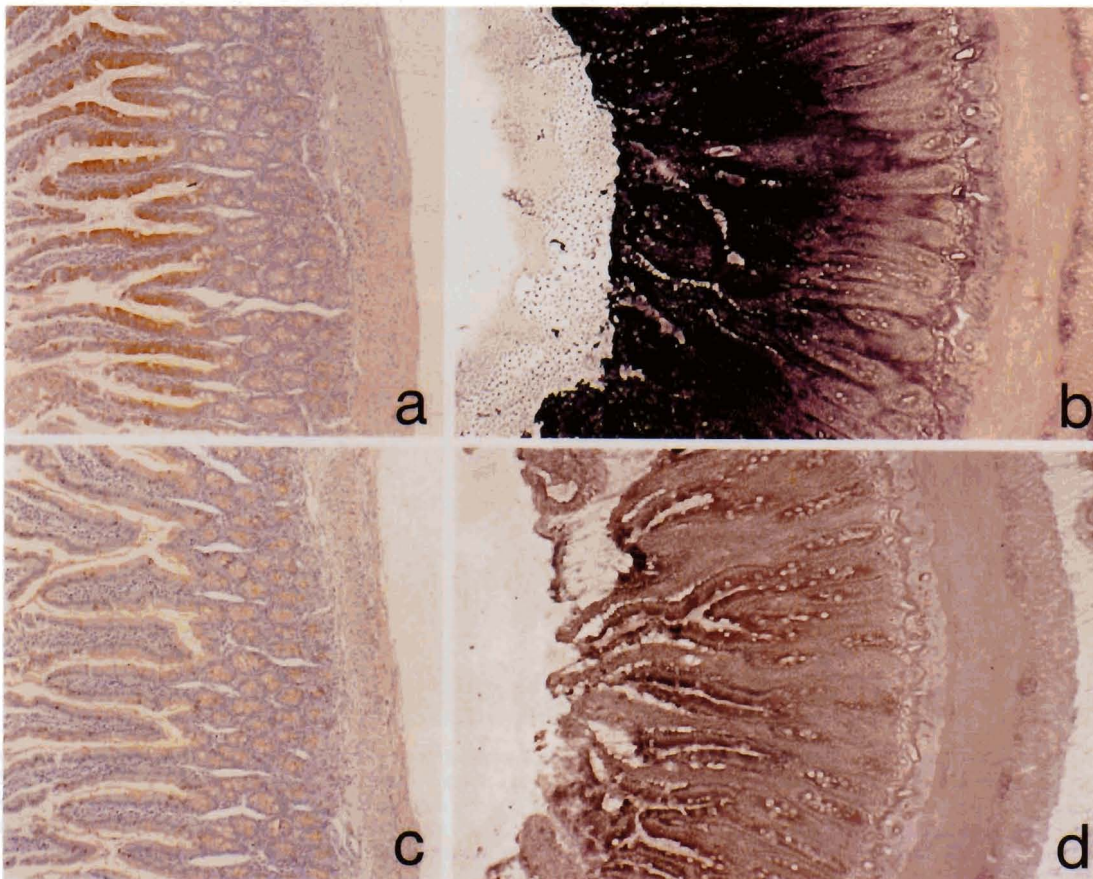

Fig. 2. Enzyme- and immunohistochemical localization of xanthine oxidase and aldehyde oxidase in the small intestine of the rat: Enzyme- and immunohistochemical staining show xanthine oxidase to be distributed in the villi of the epithelia (a, b). Immunohistochemical localization of aldehyde oxidase demonstrated that this enzyme is distributed in the villi of the epithelia, but that little enzyme activity was present (c, d). x 25

Table 3. mRNA expression of molybdenum-containing enzymes.

ENZYME	SPECIES	ORGAN (TISSUE)	REFERENCE
Sulfite oxidase			
	ND		
Xanthine oxidase			
	Horse	Skeletal muscle (capillary endothelium)	Räsänen et al., 1993
	Mouse*	Liver, intestine	Terao et al., 1992
	Rat*	Lung, heart, kidney, liver, skeletal muscle	Moriwaki et al. (unpublished)
	Human*	Heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, intestines, leukocyte	Xu et al., 1994
Aldehyde oxidase			
	ND		

ND: not done; *: northern blot anaysis.

No aldehyde oxidase activity was detected in the small intestine, whereas xanthine oxidase activity was detected in the epithelium of the villi of small intestine (Fig. 2).

Northern blot analysis and *in situ* hybridization of molybdenum-containing enzyme (Table 3)

The expression of mRNA by Northern blot analysis and by *in situ* hybridization techniques has been used to study the synthesis and expression of enzymes or protein molecules. Northern blot analyses of xanthine dehydrogenase mRNA have been performed in the mouse, rat and human. As indicated in Table 3, there are species differences in the expression of xanthine dehydrogenase mRNA in tissues. In contrast to enzyme-histochemistry (Kooij et al., 1992b), human xanthine dehydrogenase mRNA is abundant in the heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes (Xu et al., 1994). Thus, xanthine dehydrogenase may exist in an inactive form in tissues other than the liver or small intestine. Unexpectedly, the signal of xanthine dehydrogenase mRNA is very low in mouse tissues except for the liver and intestine (Terao et al., 1992), a striking contrast to the abundance of xanthine dehydrogenase mRNA in rat tissues (Moriwaki et al., unpublished data).

Räsänen et al. detected xanthine oxidase mRNA in the capillary endothelium of equine skeletal muscle using *in situ* histochemistry (Räsänen et al., 1993), consistent with results of enzyme- and immunohisto-chemical studies. Therefore, xanthine oxidase is more likely to be translated and expressed in endothelial cells. The *in situ* histochemistry of sulfite and aldehyde oxidase has not previously been performed. If *in situ* histochemistry were added to the above approaches, important information could be obtained on the function of sulfite oxidase and aldehyde oxidase.

Functions and clinical significance

Sulfite oxidase

Except its role in the detoxification of sulfite, the actions of sulfite oxidase are unknown. An inverse relationship between sulfite oxidase activity and bisulfite toxicity has been observed in several animal species (Tejnorova, 1978). Patients with a deficiency of molybdenum cofactor exhibit a deficiency of both sulfite oxidase and xanthine oxidase. The deficiency is manifested as severe neurologic abnormalities, dislocation of the ocular lenses, mental retardation, and xanthinuria. One case of an isolated deficiency of sulfite oxidase has been reported (Irreverre et al., 1967). The clinical manifestations of an isolated sulfite oxidase deficiency are the same as those of a molybdenum cofactor deficiency, except for the absence of a deficiency in xanthine oxidase. Less severe cases exhibit an intolerance to foods that are rich in sulfite. Other aspects of sulfite oxidase deficiency are related to the metabolism of sulfite and sulfur dioxide.

Xanthine oxidase

Although the main function of xanthine oxidase appears to be to oxidize hypoxanthine and xanthine to form uric acid, its function requires further study. Xanthine oxidase is involved in the pigmentation of insects by an effect on pteridine metabolism (Watt, 1972). Interest in this enzyme as a source of superoxide radicals has increased since it was implicated in the pathogenesis of reperfusion tissue injury in the kidney, heart and intestine (Granger et al., 1981). The review by Kooij (1994) describes other pathophysiological conditions in which this enzyme is involved. Although some claim that xanthine oxidase is not a source of free radicals in clinical ischemia (Eddy et al., 1987) or in that of the rabbit heart (Downey et al., 1987), it seems to be responsible for reperfusion injury in various tissues (McCord, 1985). However, xanthine oxidase may play a beneficial role by producing either superoxide radicals or uric acid. Tubaro et al. suggested it acted as an antimicrobial agent (Tubaro et al., 1980a,b,c). Van den Munckhof et al. demonstrated electron microscopically the presence of xanthine oxidase activity around bacteria in the epithelium of the esophagus, suggesting its role as a defense against microbial invasion (Van den Munckhof et al., 1995). Superoxide radicals derived from xanthine oxidase may also be involved in the differentiation and/or proliferation of the epithelial cells of small intestine (Kooij et al., 1992b). It has been suggested that uric acid serves as an antioxidant (Ames et al., 1981). Surfaces such as that of the airway are subjected to a variety of oxidant stresses. Nasal secretions contain uric acid (Peden et al., 1990). It is possible that xanthine

oxidase may function as a source of uric acid. The increase of life span and the decrease in age-specific cancer incidence in humans has been attributed to the development of protective systems against oxygen radicals, with uric acid being one such proposed protective system (Ames et al., 1981). This function of uric acid has been reviewed by Becker (1993).

Besides its role in generating oxygen radicals, xanthine oxidase inhibits the growth of *Plasmodium falciparum*, which cannot synthesize purines (Berman et al., 1991). Such findings suggest a new therapeutic approach to treating or preventing malaria infection in humans.

Patients with xanthine oxidase deficiency (xanthinuria) reportedly consist of two subgroups: one that lacks xanthine oxidase and exhibits classic xanthinuria, and the other with a deficiency of xanthine oxidase accompanied by a deficiency of sulfite oxidase. Subjects with xanthine oxidase deficiency are usually asymptomatic; incidental findings include xanthine calculi and myopathy associated with xanthine deposit in the muscle and polyarthritis. Xanthine oxidase deficiency is divided into two pharmacologic subgroups (Kojima et al., 1984). One subgroup does not oxidize allopurinol to oxypurinol or pyrazinamide, an anti-tuberculous agent that inhibits uric acid secretion in the renal tubules, to 5-hydroxypyrazinamide (Yamamoto et al., 1991). This subgroup is thought to have a combined deficiency of the allopurinol-pyrazinamide oxidizing enzyme, or a variant form of xanthine oxidase that fails to oxidize hypoxanthine, xanthine, allopurinol, or pyrazinamide. The other subgroup is able to oxidize allopurinol (Yamanaka et al., 1983; Carpenter et al., 1986) and pyrazinamide to oxypurinol and 5-hydroxy-pyrazinamide, respectively, despite a lack of xanthine oxidase. These findings indicate the existence of an allopurinol-pyrazinamide oxidizing enzyme or a variant form of xanthine oxidase that cannot oxidize hypo-xanthine and xanthine, but can oxidize allopurinol and pyrazinamide. Aldehyde oxidase reportedly oxidizes allopurinol and pyrazinamide to oxypurinol and 5-hydroxypyrazinamide, respectively (Moriwaki et al., 1993b). This subgroup of subjects with xanthine oxidase deficiency may lack aldehyde oxidase activity. The possibility of a combined deficiency of xanthine oxidase and aldehyde oxidase is suggested by an *in vivo* test of aldehyde oxidase activity, which measures the conversion of L-histidine to hydantoin-5-propionic acid (Johnson et al., 1980), or by the conversion of N-methylnicotinamide, a substrate of aldehyde oxidase, to 2- and 4-pyridone (Reiter et al., 1990). The existence of this combined deficiency suggests that the genes for these enzymes are derived from a common progenitor by gene duplication and subsequent genetic modification. No case of isolated aldehyde oxidase deficiency has yet been described. The clinical symptoms of aldehyde oxidase deficiency are difficult to assess, probably because of the instability and low activity of this enzyme in humans. In addition to a congenital deficiency of

these enzymes, an acquired deficiency of sulfite oxidase and xanthine oxidase has been documented in a patient who was receiving total parenteral nutrition (Abumrad et al., 1981). The development of gout has been reported in a subject who received large amounts of molybdenum (Kovalskii et al., 1961).

Aldehyde oxidase

Aldehyde oxidase is a xenobiotic metabolizing enzyme (Beedham, 1985) whose physiological function(s) are not completely understood; for instance its possible involvement in the pathogenesis of tissue reperfusion tissue injury. It has been suggested by some that it may contribute to the production of oxidant in such tissue injury (Jackson et al., 1988), although no direct evidence exists. In contrast, others suggest that aldehyde oxidase is not involved in the post-ischemic tissue reperfusion injury in the rabbit lung, despite the greater activity of this enzyme in the rabbit than in other species, since the inhibition of aldehyde oxidase activity had no significant effect on such injury, in contrast to the inhibition of xanthine oxidase (Adkins and Taylor, 1990). Aldehyde oxidase appears to be more important in herbivores, since it is rich in the liver and intestine, organs that protect against the ingestion of nitrogen-containing heterocyclics. Aldehyde oxidase, together with xanthine oxidase, may present a biochemical barrier to the various toxic substituted pyrimidines present in nature (Krenitsky, 1972, 1974). Several other functions of aldehyde oxidase are suggested by its tissue localization, as well as by the degradation of N-heterocyclics. Aldehyde oxidase has a major role in the synthesis of retinoic acid, active form of vitamin A, from retinal (Huang and Ichikawa, 1994), since this enzyme is identical to retinal oxidase (Tomita et al., 1993). Retinoic acid is involved in the formation of the limb bud and rhombencephalon, the control of epithelial tissue growth, and in cellular differentiation and morphogenesis (Thaller and Eichelle, 1987; Levine and Hoey, 1988). Therefore, aldehyde oxidase may be involved in these processes. The localization of aldehyde oxidase in the epithelium may support that hypothesis. This enzyme may play a major role in the pathogenesis of alcohol-induced liver injury *via* lipid peroxidation, with xanthine oxidase playing a minor role (Shaw and Jayatilleke, 1990). Mira et al. suggested that aldehyde oxidase also acts on NADH to promote a vicious circle that increases the production of superoxide radical, which then increases the hepatotoxicity of alcohol (Mira et al., 1995). Alcoholic liver injury affects primarily the pericentral area of the liver lobule. The enhanced toxicity of ethanol in this area of the liver has been explained by the presence of a relatively low oxygen tension (Lieber, 1984). The oxidation of ethanol exacerbates the preexisting relative hypoxia by increasing the consumption of oxygen. The aldehyde oxidase activity present in the pericentral area may also contribute to the alcoholic liver injury primarily seen in

Table 4. Pathophysiological involvement of molybdenum-containing enzymes.

FUNCTION	REFERENCE
Sulfite oxidase	
Detoxification of sulfite	Cohen and Fridovich, 1971a,b
Xanthine oxidase	
Gout	Pallela and Fox, 1989
Xanthinuria	Yamamoto et al., 1991, 1992
Pigmentation of insects	Watt, 1972
Reperfusion tissue injury	McCord, 1985
Antimicrobial activity	Tubaro et al., 1980a,b,c; Van den Munckhof et al., 1995
Protection against toxic plants (?)	Krenitsky, 1972, 1974
Metabolism of xenobiotics	Beedham, 1985
Proliferation and/or differentiation	Kooij, 1992b
Aldehyde oxidase	
Metabolism of xenobiotics	Beedham, 1985
Protection against toxic plants (?)	Krenitsky, 1972, 1974; Large and Connock, 1993
Degradation of pheromone	Rybcynski et al., 1989, 1990
Alcoholic liver injury	Shaw and Jayatilleke, 1990, Mira et al., 1995
Visual processes	Huang and Ichikawa, 1994
Reperfusion tissue injury (?)	Jackson et al., 1988
Synthesis of retinoic acid	Tomita et al., 1993

The review article by Kooij (1994) covers the details of the pathophysiological role of xanthine oxidase (conditions associated with reactive oxygen species production).

this area. The *Heliothis virescens* moth is the first insect in which aldehyde oxidase activity has been demonstrated in the sensory tissue (Teal et al., 1986). Tasayco et al. demonstrated aldehyde oxidase activity in the extracts of the antenna, and to a lesser extent in the legs, of *Heliothis virescens* (Tasayco and Prestwich, 1990). Rybczynski et al. suggested that in these moths, sensillar aldehyde oxidase modulates the concentration of pheromone in the receptor space, to affect the pheromone-mediated mating behavior (Rybczynski et al., 1989, 1990). The finding of aldehyde oxidase in the digestive gland of plant-eating terrestrial gastropods suggests that this enzyme may be involved in the metabolism of a precursor of, or a breakdown product of, the lignin present in various plants (Large and Connock, 1993).

The proposed pathophysiological functions of the molybdenum-containing enzymes are summarized in Table 4.

Conclusions

Considerable evidence suggests that xanthine oxidase, as well as aldehyde oxidase, have numerous roles. However, the enzyme- and immunohistochemical localization of sulfite oxidase and aldehyde oxidase have not been extensively studied. To elucidate the exact function(s) of these enzymes, it is imperative to establish their tissue or subcellular localization. Little is known about the tissue expression of mRNA of the molyb-

denum-containing enzymes by *in situ* hybridization techniques. Therefore, the various pathophysiological functions of these enzymes remain to be clarified.

The clinical issue of the cause of xanthinuria has to be resolved; that is, whether it results from a combined deficiency of xanthine oxidase and aldehyde oxidase, or from a mutant form of xanthine oxidase that can oxidize allopurinol and pyrazinamide. It is also unclear whether or not isolated aldehyde oxidase deficiency exists, or the importance of aldehyde oxidase deficiency. Genetic studies, including the cloning of human aldehyde oxidase cDNA, and studies using aldehyde oxidase gene knock-out mouse, are required to answer this question.

References

Abadeh S., Killacky J., Benboubetra M. and Harrison R. (1992). Purification and partial characterization of xanthine oxidase from human milk. Biochim. Biophys. Acta 1117, 25-32.

Abumrad N.N., Schneider A.J., Steel D. and Rogers L.S. (1981). Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. Am. J. Clin. Nutr. 34, 2551-2559.

Adkins W.K. and Taylor A.E. (1990). Role of xanthine oxidase and neutrophils in ischemia-reperfusion injury in rabbit lung. J. Appl. Physiol. 69, 2912-2918.

Allen R.E., Outhwaite J.M., Morris C.J. and Blake D.R. (1987). Xanthine oxidoreductase is present in human synovium. Ann. Rheum Dis. 46, 843-845.

Amaya Y., Yamazaki K., Sato M., Noda K., Nishino T. and Nishino T. (1990). Proteolytic conversion of xanthine dehydrogenase from the NAD-dependent type to the O_2-dependent type. J. Biol Chem. 265, 14170-14175.

Ames B.N., Cathcart R., Scwiers E. and Hochstein P. (1981). Uric acid provides an antioxidant defence in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc. Natl. Acad. Sci. USA 78, 6858-6862.

Becker B.F. (1993). Towards the physiological function of uric acid. Free Radic. Biol. Med. 14, 615-631.

Beedham C. (1985). Molybdenum hydroxylases as drug-metabolising enzymes. Drug. Metab. Rev. 16, 119-156.

Beedham C., Bruce S.E., Critchley D.J., Al-Tayib Y. and Rance D.J. (1987). Species variation in hepatic aldehyde oxidase activity. Eur. J. Drug Metab. Pharmacokinet. 12, 307-310.

Bellissimo D.B. and Rajagopalan K.V. (1991). Rat sulfite oxidase antibodies cross-react with two gene family-related proteins: albumin and vitamin D-binding protein. Arch Biochem. Biophys. 291, 168-175.

Berman P.A., Human L. and Freese J.A. (1991). Xanthine oxidase inhibits growth of Plasmodium falciparum in human erythrocytes in vitro. J. Clin. Invest 88, 1848-1855.

Bourne G.H. (1953). Histochemistry of xanthine oxidase. Nature 172, 193-195.

Bruder G., Jarasch E.D. and Heid H.W. (1984). High concentration of antibodies to xanthine oxidase in human and animal sera, molecular characterization. J. Clin. Invest. 74, 783-794.

Cabré F., Marin C., Cascante M. and Canela E.I. (1990). Occurrence and comparison of sulfite oxidase activity in mammalian tissues. Biochem Med. Metab. Biol. 43, 159-162.

Carpani G., Racchi Ghezzi P., Terao M. and Garaittini E. (1990).

Purification and characterization of mouse liver xanthine oxidase. Arch. Biochem. Biophys. 279, 237-241.

Carpenter T.O., Lebowitz R.L., Nelson D. and Bauer S. (1986). Hereditary xanthinuria presenting in infancy with nephrolithiasis. J. Pediatr. 109, 307-309.

Cohen H.J. and Fridovich I. (1971a). Hepatic sulfite oxidase. Purification and properties. J. Biol. Chem. 246, 359-366.

Cohen H.J. and Fridovich I. (1971b). Hepatic sulfite oxidase. The nature and function of the heme prosthetic groups. J. Biol. Chem. 246, 367-373.

Critchley D.J.P., Rance D.J. and Beedham C. (1992). Subcellular localisation of guinea pig hepatic molybdenum hydroxylases. Biochem. Biophys. Res. Commun. 185, 54-59.

De Pooter H.L., Huang Q.L. and Schamp N.M. (1989). Isolation of aldehyde oxidase (EC 1.2.3.1) from potato extracts by preparative PAGE. Prep. Biochem. 19, 61-67.

Downey J.M., Miura T., Eddy L.J., Chambers D.E., Mellert T., Hearse D.J. and Yellon D.M. (1987). Xanthine oxidase is not a source of free radicals in the ischemic rabbit heart. J. Mol. Cell. Cardiol. 19, 1053-1060.

Eddy L.J., Stewart J.R., Jones H.P., Engerson T.D., McCord J.M. and Downey J.M. (1987). Free radical-producing enzyme, xanthine oxidase, is undetectable in human hearts. Am. J. Physiol. 253, H709-H711.

Felsted R.L., Chu A.E-Y. and Chaykin S. (1973). Purification and properties of aldehyde oxidases from hog and rabbit livers. J. Biol. Chem. 248, 2580-2587.

Frederiks W.M. and Bosch K.S. (1995). The role of xanthine oxidase in ischemic/reperfusion damage of rat liver. Histol. Histopathol. 10, 111-116.

Frederiks W.M. and Marx F. (1993). A histochemical procedure for light microscopic demonstration of xanthine oxidase activity in unfixed cryostat sections using cerium ions and a semipermeable membrane techniques. J. Histochem. Cytochem. 41, 667-670.

Frederiks W.M., Bosch K.S., Van den Munckhof R.J.M. and van Noorden C.J.F. (1994). A quantitative histochemical study of xanthine oxidase activity in rat liver using the cerium capture method in the presence of polyvinyl alcohol. J. Histochem. Cytochem. 42, 1091-1097.

Frederiks W.M., Bosch K.S. and Kooij A. (1995). Quantitative in situ analysis of xanthine oxidoreductase activity in rat liver. J. Histochem. Cytochem. 43, 723-726.

Garrett R.M. and Rajagopalan K.V. (1994). Molecular cloning of rat sulfite oxidase. J. Biol. Chem. 269, 272-276.

Glatigny A. and Scazzocchio C. (1995). Cloning and molecular characterization of hxA, the gene coding for the xanthine dehydrogenase (Purine hydroxylase I) of Aspergillus nidulans. J. Biol. Chem. 270, 3534-3550.

Gores G.J., Flarsheim C.E., Dawson T.L., Nieminen A., Herman B. and Lemasters J.J. (1989). Swelling, reductive stress, and cell death during chemical hypoxia in hepatocytes. Am. J. Physiol. 257, C347-C354.

Gossrau R., Frederiks W.M. and van Noorden C.J.F. (1990). Histochemistry of reactive oxygen-species (ROS)-generating oxidases in cutaneous and mucous epithelia of laboratory rodents with special reference to xanthine oxidase. Histochemistry 94, 539-544.

Granger D.N., Rutili G. and McCord J.M. (1981). Superoxide radicals in feline intestinal ischemia. Gastroenterology 81, 22-29.

Gunnison A.F., Bresnahan C.A. and Palmes E.D. (1977). Comparative sulfite metabolism in the rat, rabbit and rhesus monkey. Toxicol. Appl. Pharmacol. 42, 99-109.

Hattori Y. (1989). Localization of xanthine dehydrogenase in chicken liver. Acta Histochem. Cytochem. 22, 617-624.

Hellsten-Westing Y. (1993). Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. Histochemistry 100, 215-222.

Holmes R.S. and Vandeberg J.L. (1986). Aldehyde dehydrogenase, aldehyde oxidase and xanthine oxidase from baboon tissues: phenotypic variability and subcellular distribution in liver and brain. Alcohol 3, 205-214.

Huang D-Y. and Ichikawa Y. (1994). Two different enzymes are primarily responsible for retinoic acid synthesis in rabbit liver cytosol. Biochem. Biophys. Res. Commun. 205, 1278-1283.

Hunt J. and Massey V. (1992). Purification and properties of milk xanthine dehydrogenase. J. Biol. Chem. 267, 21479-21485.

Ichida K., Amaya Y., Noda K., Minoshima S., Hosoya T., Sakai O., Shumizu N. and Nishino T. (1993). Cloning of the cDNA encoding human xanthine dehydrogenase (oxidase); structural analysis of the protein and chromosomal location of the gene. Gene 133, 279-284.

Ichikawa M., Nishino T., Nishino T. and Ichikawa A. (1992). Subcellular localization of xanthine oxidase in rat hepatocytes: high-resolution immunoelectron microscopic study combined with biochemical analysis. J. Histochem. Cytochem. 40, 1097-1103.

Irreverre F., Mudd S.h., Heizer W.D. and Laster L. (1967). Sulfite oxidase deficiency: studies of a patient with mental retardation, dislocated ocular lenses, and abnormal urinary excretion of S-sulfo-L-cysteine, sulfite, and thiosulfate. Biochem. Med. 1, 187-217.

Ito A., Kuwahara T., Mitsunari Y. and Omura T. (1977). Distribution of hepatic sulfite oxidase among subcellular organelles and its intraorganelle localization. J. Biochem. 81, 1531-1541.

Jackson R.M., Veal C.F., Alexander C.B., Brannel A.L. and Fulmer J.D. (1988). Re-expansion pulmonary edema. Am. Rev. Resp. Dis. 137, 1165-1171.

Jarasch E.D., Grund C., Bruder G., Heid H.W., Keenan T.W. and Franke W.W. (1981). Localization of xanthine oxidase in mammary-gland epithelium and capillary endothelium. Cell 25, 67-82.

Johns D.G. (1967). Human liver aldehyde oxidase: differential inhibition of oxidation of charged and uncharged substrates. J. Clin. Invest. 46, 1492-1505.

Johnson J.L. and Rajagopalan K.V. (1976). Purification and properties of sulfite oxidase from human liver. J. Clin. Invest. 58, 543-550.

Johnson J.L. and Rajagopalan K.V. (1977). Tryptic cleavage of rat liver sulfite oxidase. J. Biol. Chem. 252, 2017-2025.

Johnson J.L., Waud W.R., Rajagopalan K.V., Duran M., Beemer F.A. and Wadman S.K. (1980). Inborn errors of molybdenum metabolism: combined deficiencies of sulfite oxidase and xanthine dehydrogenase in a patient lacking the molybdenum cofactor. Proc. Natl. Acad. Sci. USA 77, 3715-3719.

Keith R.P., Riley M.J., Kreitman M., Lewontin R.C., Curtis D. and Chambers G. (1987). Sequence of the structural gene for xanthine dehydrogenase (rosy lucus) in Drosophila melanogaster. Genetics 116, 67-73.

Kessler D.L. and Rajagopalan K.V. (1972). Purification and properties of sulfite oxidase from chicken liver. J. Biol. Chem. 247, 6566-6573.

Kessler D.L., Johnson J.L., Cohen H.J. and Rajagopalan K.V. (1974). Visualization of hepatic sulfite oxidase in crude tissue preparations by electron paramagnetic resonance spectroscopy. Biochim. Biophys. Acta 334, 86-96.

Kipke C.A., Enemark J.H. and Sunde R.A. (1989). Purification of prosthetically intact sulfite oxidase from chicken liver using a modified procedure. Arch. Biochem. Biophys. 270, 383-390.

Kojima T., Nishina T., Kitamura M., Hosoya T. and Nishioka K. (1984). Biochemical studies on the purine metabolism of four cases with hereditary xanthinuria. Clin. Chim. Acta 137, 189-198.

Kooij A. (1994). A re-evaluation of the tissue distribution and physiology of xanthine oxidoreductase. Histochem. J. 26, 889-915.

Kooij A., Frederiks W.M., Gossrau R. and van Noorden C.J.F. (1991). Localization of xanthine oxidoreductase activity using the tissue protectant polyvinyl alcohol and final electron acceptor tetranitro BT. J. Histochem. Cytochem. 39, 87-93.

Kooij A., Schijns M., Frederiks W.M., van Noorden C.J.F. and James J. (1992a). Distribution of xanthine oxidoreductase activity in human tissues- a histochemical and biochemical study. Virchows Arch. (B) 63, 17-23.

Kooij A., Bosch K.S., Frederiks W.M. and van Noorden C.J.F. (1992b). High levels of xanthine oxidoreductase in rat endothelial, epithelial and connective tissue cells. Virchows Arch. (B) 62, 143-150.

Kovalskii V.V., Yarovaya G.A. and Shamavonyan D.M. (1961). The change in purine metabolism of humans and animals under the conditions of molybdenum biochemical provinces. ZK Obshch. Biol. 22, 179-191 (in Russian).

Krenitsky T.A. (1978). Aldehyde oxidase and xanthine oxidase-functional and evolutionary relationships. Biochem. Pharmacol. 27, 2763-2764.

Krenitsky T.A., Neil S.M., Elion B.B. and Hitchings G.H. (1972). A comparison of the specificities of xanthine oxidase and aldehyde oxidase. Arch. Biochem. Biophys. 150, 585-599.

Krenitsky T.A., Tuttle J.V., Cattau E.L. and Wang P. (1974). A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. Comp. Biochem. Physiol. 49B, 687-703.

Krenitsky T.A., Spector T. and Hall W.W. (1986). Xanthine oxidase from human liver: purification and characterization. Arch. Biochem. Biophys. 247, 108-119.

Large A.T. and Connock M.J. (1993). Aromatic alcohol oxidase and aldehyde oxidase activities in the digestive gland of three species of terrestrial gastropod (Helix aspersa, Arion ater, Limax flavus). Comp. Biochem. Physiol. 104B, 489-491.

Lemasters J.J., Stemkowski C.J., Sungchul J.I. and Thurman R.G. (1983). Cell surface changes and enzyme release during hypoxia and reoxygenation in the isolated, perfused rat liver. J. Cell Biol. 97, 778-786.

Levine M. and Hoey T. (1988). Homeobox proteins as sequence-specific transcription factors. Cell 55, 537-540.

Li Calzi M., Raviolo C., Ghibaudi E., De Gioia L., Salmona M., Cazzaniga G., Kurosaki M., Tarao M. and Garattini E. (1995). Purification, cDNA cloning, and tissue distribution of bovine liver aldehyde oxidase. J. Biol. Chem. 270, 31037-31045.

Lieber C.S. (1984). Alcohol and the liver. 1984 update. Hepatology 4, 1243-1260.

McCord J.M. (1985). Oxygen-derived free radicals in postischemic tissue injury. N. Engl. J. Med. 312, 159-163.

McIndoe W.M., Wight P.A.L. and MacKenzie G.M. (1974). Histochemical demonstration of xanthine dehydrogenase in the tissues of the domestic fowl. Histochem. J. 6, 339-345.

Mira L., Maia L., Barreira L. and Manso C.F. (1995). Evidence for free radical generation due to NADH oxidation by aldehyde oxidase

during ethanol metabolism. Arch. Biochem. Biophys. 318, 53-58.

Moriwaki Y., Yamamoto Y., Suda M., Nasako Y., Takahashi S., Agbedana O.E., Hada T. and Higashino K. (1993a). Purification and immunohistochemical tissue localization of human xanthine oxidase. Biochim. Biophys. Acta 1164, 327-330.

Moriwaki Y., Yamamoto T., Nasako Y., Takahashi S., Suda M., Hiroishi K., Hada T. and Higashino K. (1993b). In vitro conversion of pyrazinamide and allopurinol by rat liver aldehyde oxidase. Biochem. Pharmacol. 46, 975-981.

Moriwaki Y., Yamamoto T., Yamaguchi K., Takahashi S. and Higashino K. (1996a). Immunohistochemical localization of aldehyde and xanthine oxidase in rat tissues using polyclonal antibodies. Histochem. Cell Biol. 105, 71-79.

Moriwaki Y., Yamamoto T., Yamaguchi K., Suda M., Yamakita J., Takahashi S. and Higashino K. (1996b). Immunohistochemical localization of xanthine oxidase in human tissues. Acta Histochem. Cytochem. 29, 153-162.

Ogawa K., Sato T. and Mayahara H. (1968). The site of ferricyanide reduction by reductases within mitochondria as studied by electron microscopy. J. Histochem. Cytochem. 16, 49-57.

Ohkubo M., Sakiyama S. and Fujimura S. (1983). Purification and characterization of N_1-methylnicotinamide oxidases I and II separated from rat liver. Arch. Biochem. Biophys. 221, 534-542.

Onoue Y. (1980). Sulfite oxidase from Merluccius productus. Biochim. Biophys. Acta 615, 48-58.

Pallela T.D. and Fox I.H. (1989). Hyperuricemia and gout. In: The metabolic basis of inherited disease. Scriver C.R., Beaudet A.L., Sly W.S. and Valle D. (ed). McGraw-Hill. New York. pp 965-1006.

Peden D.B, Hohman R., Brown M.E., Mason R.T., Berkebile C., Fales H.M. and Kaliner M.A. (1990). Uric acid is a major antioxidant in human nasal airway secretions. Proc. Natl. Acad. Sci. USA 87, 7638-7642.

Räsänen L.A., Karvonen U. and Pösö A.R. (1993). Localization of xanthine dehydrogenase mRNA in horse skeletal muscle by in situ hybridization with digoxigenin-labelled probe. Biochem. J. 292, 639-641.

Reiter S., Simmonds H.A., Zöllner N., Braun S.L. and Knedel M. (1990). Demonstration of a combined deficiency of xanthine oxidase and aldehyde oxidase in xanthinuric patients not forming oxipurinol. Clin. Chim. Acta 187, 221-234.

Rothe G.M. (1975). Intracellular localization and some properties of two aldehyde oxidase isoenzymes (EC1.2.3.1) in potato tubers (Solanum tuberosum). Biochem. P°hysiol. Pflanzen 167, 411-418.

Rybczynski R., Reagan J. and Lerner M.R. (1989). A pheromone-degrading aldehyde oxidase in the antennae of the moth Manduca sexta. J. Neurosci. 9, 1341-1353.

Rybczynski R., Vogt R.G. and Lerner M.R. (1990). Antennal-specific pheromone-degrading aldehyde oxidases from the moths Antheraes polyphemus and Bombyx mori. J. Biol. Chem. 265, 19712-19715.

Sackler M.L. (1966). Xanthine oxidase from liver and duodenum of the rat. Histochemical localization and electrophoretic heterogeneity. J. Histochem. Cytochem. 14, 326-333.

Samra Z.Q., Oguro T., Fontaine R. and Ashraf M. (1991). Immunocytochemical localization of xanthine oxidase in rat myocardium. J. Submicrosc. Cytol. Pathol. 23, 379-390.

Shaw S. and Jayatilleke E. (1990). The role of aldehyde oxidase in ethanol-induced hepatic lipid peroxidation in the rat. Biochem. J. 268, 579-583.

Shimada S., Mishima H.K., Nikaido H., Kitamura S. and Tatsumi K.

(1989). Purification of aldehyde oxidase from bovine ciliary body. Curr Eye Res. 8, 721-726.

Southerland W.M., Winge D.R. and Rajagopalan K.V. (1978). The domains of rat liver sulfite oxidase. J. Biol. Chem. 235, 8747-8752.

Stell J.G.P., Warne A.J. and Wooley C.L. (1989). Purification of rabbit liver aldehyde oxidase by affinity chromatography on benzamidine sepharose 6B. J. Chromatogr. 475, 363-372.

Stevens C.R., Benboubetra M., Harrison R., Sahinoglu T., Smith E.C. and Blake D.R. (1991). Localization of xanthine oxidase to synovial endothelium. Ann. Rheum. Dis. 50, 760-762.

Suleiman S.A. and Stevens J.B. (1987). Purification of xanthine dehydrogenase from rat liver: a rapid procedure with high enzyme yields. Arch. Biochem. Biophys. 258, 219-225.

Tasayco M.L. and Prestwich G.D. (1990). Aldehyde-oxidizing enzymes in an adult moth: in vitro study of aldehyde metabolism in Heliothis virescens. Arch. Biochem. Biophys. 278, 444-451.

Teal P.E.A., Tumlinson J.H. and Heath R.R. (1986). Chemical and behavioral analysis of volatile sex pheromone components related by calling Heliothis virescens (F.) Females (Lepidoptera: Noctuidae). J. Chem. Ecol. 12, 107-126.

Tejnorová I. (1978). Sulfite oxidase activity in liver and kidney tissue in five laboratory animal species. Toxicol. Appl. Pharmacol. 44, 251-256.

Terao M., Cazzaniga G., Ghezzi P., Bianchi M., Falciani F., Perani P. and Garattini E. (1992). Molecular cloning of a cDNA coding for mouse liver xanthine dehydrogenase. Biochem. J. 283, 863-870.

Thaller C. and Eichele G. (1987). Identification and spatial distribution of retinoids in the developing chick limb bud. Nature 327, 625-628.

Toghrol F. and Southerland W.M. (1983). Purification of Thiobacillus novellus sulfite oxidase. J. Biol. Chem. 258, 6762-6766.

Tomita S., Tsujita M. and Ichikawa Y. (1993). Retinal oxidase is identical to aldehyde oxidase. FEBS Lett. 336, 272-274.

Tubaro E., Lotti B., Cavallo G., Croce C. and Borelli G. (1980a). Liver xanthine oxidase increase in mice in three pathological models. Biochem. Pharmacol. 29, 1939-1943.

Tubaro E., Lotti B., Santiangeli C. and Cavallo G. (1980b). Xanthine oxidase increase in polymorphonuclear leukocytes and macrophages in mice in three pathological situations. Biochem. Pharmacol. 29, 1945-1948.

Tubaro E., Lotti B., Santiangeli C. and Cavallo G. (1980c). Xanthine oxidase: an enzyme playing a role in the killing mechanism of poly-

morphonuclear leukocytes. Biochem. Pharmacol. 29, 3018-3020.

Van den Munckhof R.J.M., Vreeling-Sindelarova H., Schellens J.P.M., van Noorden C.J.F. and Frederiks W.M. (1995). Ultrastructural localization of xanthine oxidase activity in the digestive tract of the rat. Histochem. J. 27, 897-905.

Wajner M. and Harkness R.A. (1989). Distribution of xanthine dehydro-genase and oxidase activities in human and rabbit tissues. Biochim. Biophys. Acta 991, 79-84.

Watt W.B. (1972). Xanthine dehydrogenase and pteridine metabolism in Colias butterflies. J. Biol. Chem. 247, 1445-1451.

Wattiaux-De Coninck S. and Wattiaux R. (1971). Subcellular distribution of sulfite cytochrome c reductase in rat liver tissue. Eur. J. Biochem. 19, 552-556.

Wright R.M., Vataitis G.M., Wilson C.M., Repine T.B., Terada L.S. and Repine J.E. (1993). cDNA cloning, characterization, and tissue-specific expression of human xanthine dehydrogenase/xanthine oxidase. Proc. Natl. Acad. Sci. USA 90, 10690-10694.

Wurzinger K-H. and Hartenstein R. (1974). Phylogeny and correlations of aldehyde oxidase, xanthine oxidase, xanthine dehydrogenase and peroxidase in animal tisues. Comp. Biochem. Physiol. 49B, 171-185.

Xu P., Huecksteadt T.P., Harrison R. and Hoidal J.R. (1994). Molecular cloning, tissue expression of human xanthine dehydrogenase. Biochem. Biophys. Res. Commun. 199, 998-1004.

Yamamoto T., Kario K., Suda M., Moriwaki Y., Takahashi S. and Higashino K. (1991). A case of xanthinuria: a study on the metabolism of pyrazinamide and allopurinol. Jpn. J. Med. 30, 430-434.

Yamamoto T., Moriwaki Y., Suda M., Takahashi S., Hada T., Nanahoshi M., Agbedana E.O. and Higashino K. (1992). An immunoreactive xanthine oxidase protein-possessing xanthinuria and her family. Clin. Chim. Acta 208, 93-98.

Yamamoto T., Moriwaki Y., Takahashi S., Tsutsumi Z., Yamakita J., Nasako Y., Hiroishi K. and Higashino K. (1996). Determination of human plasma xanthine oxidase activity by high-performance liquid chromatography. J. Chromatogr. Biomed. 681, 395-400.

Yamanaka H., Nishioka K., Suzuki T. and Kohno K. (1983). Allopurinol metabolism in a patient with xanthine oxidase deficiency. Ann. Rheum. Dis. 42, 684-686.

Yoshihara S. and Tatsumi K. (1985). Guinea pig liver aldehyde oxidase as a sulfoxide reductase: its purification and characterization. Arch. Biochem. Biophys. 242, 213-224.