

NEWLY DISCOVERED REDOX COFACTORS: Possible Nutritional, Medical, and Pharmacological Relevance to Higher Animals

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ABSTRACT

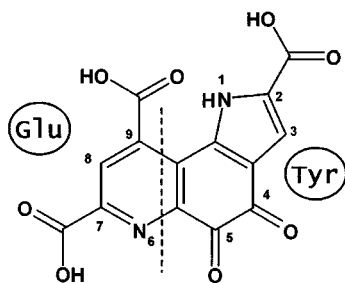
Research spurred by the discovery of pyrroloquinoline quinone (PQQ) in 1979 led to the discovery of four additional oxidation-reduction (redox) cofactors, all of which result from transamification of amino acyl side chains in respective enzymes. These cofactors are (a) topa quinone in copper-containing amine oxidases, enzymes found in nearly all forms of life, including human; (b) lysyl topa quinone of the copper protein lysyl oxidase, an enzyme required for proper cross-linking of collagen and elastin; (c) tryptophan tryptophylquinone of alkylamine dehydrogenases from gram-negative soil bacteria; and (d) the copper-complexed cysteinyl-tyrosyl radical of fungal galactose oxidase. Originally, PQQ was thought to be a covalently bound cofactor in numerous enzymes from eukaryotes and prokaryotes. Today, PQQ is only found as a noncovalent cofactor in bacterial enzymes. The ubiquity of PQQ in the environment and its ready accessibility in the human diet has raised questions concerning its role as a vitamin, or an essential or helpful nutrient. The relevance to nutrition, medicine, and pharmacology of PQQ, topa quinone, lysyl topa quinone, tryptophan tryptophylquinone, the galactose oxidase cofactor, and the enzymes harboring these cofactors are discussed in this review.

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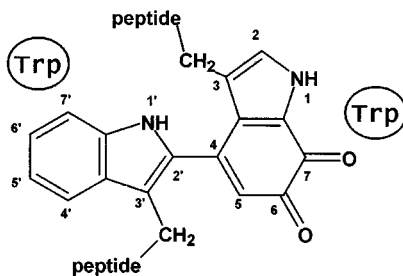
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INTRODUCTION

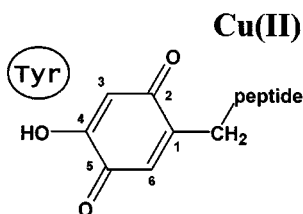
The discovery of a new enzyme cofactor (coenzyme) usually creates a fair amount of excitement and interest among biochemists and nutritionists. Because many cofactors are required nutrients (vitamins) for humans [e.g. ascorbic acid (vitamin C), riboflavin (vitamin B₂), pyridoxal (vitamin B₆)], speculation abounds that the new coenzyme might also fit this bill. This was the case following the 1979 (179) report of the discovery of pyrroloquinonoline quinone (PQQ) (4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, originally called methoxatin) (Figure 1) as the oxidation-reduction (redox) cofactor of an NAD(P)H-independent primary alcohol dehydrogenase from the methylotrophic bacterium, *Pseudomonas* TP1. By the mid-1980s, interest peaked after publication of studies purporting to prove that PQQ was the covalently bound cofactor in numerous enzymes of bacteria and higher organisms, including humans (see Table 2 in Reference 143). Although scientists outside the field believed these assertions, many familiar with the various enzymes remained skeptical. There was little or no preceding evidence that these enzymes contained this type of redox coenzyme. To challenge the published, entrenched notions, incontrovertible evidence to the contrary was required. Because of meticulous work in several laboratories, the requisite evidence materialized. It is now recognized that the proofs of the presence of covalently bound PQQ, or a derivative thereof, were weak and circumstantial and that not a single enzyme from any source harbors covalently bound PQQ. However, several bacterial enzymes use noncovalently bound PQQ as an essential redox cofactor (see Table 1 in Reference 143). Significantly, the ensuing research established the unambiguous structures of several new cofactors: topa quinone (TPQ, also known as 6-hydroxydopa quinone) (93, 113, 206) in copper-containing amine oxidases from mammals, gram-positive and gram-negative bacteria, yeast, fungi, plants, fish, mollusks, etc (145); lysine tyrosylquinone (LTQ) (227) in lysyl oxidase



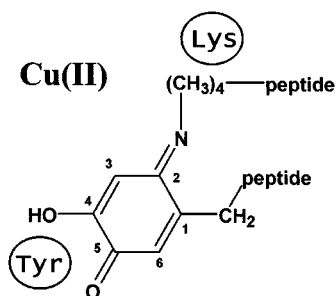
**2,7,9-Tricarboxy-1*H*-pyrrolo-
[2,3-*f*]-quinoline-4,5-dione =
Pyrroloquinoline Quinone (PQQ)**



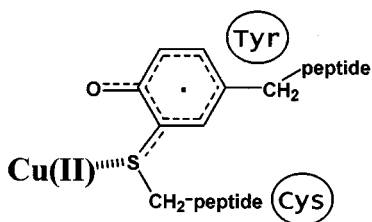
**2',4-Bitryptophan-6,7-dione =
Tryptophan Tryptophylquinone (TTQ)**



**4-Hydroxyphenylalanine-2,5-dione =
Tyrosine-2,5-dione = 6-Hydroxydopa Quinone =
Topa Quinone (TPQ)**



Lysyl Topa Quinone (LTQ)



Galactose Oxidase cofactor

Figure 1 The structures of recently discovered redox cofactors that are derived, more or less, in a direct manner from amino acyl groups in peptides. Systematic names are provided, as are common acronyms. (circles) The three-letter abbreviations for the amino acyl functionalities from which the cofactors originated. Note that PQQ is only known to bind to enzymes noncovalently, whereas the other cofactors are covalently tethered to the polypeptide backbone. The displayed Cu(II) in the amine oxidases is known to be in close proximity to TPQ from X-ray structures (117, 165, 232). It is assumed that the copper in lysyl oxidase is close to LTQ.

isolated from mammalian collagen and elastin; tryptophan tryptophylquinone (TTQ) (146) from NAD(P)H-independent alkylamine dehydrogenases from gram-negative bacteria; and the copper-complexed cysteinyl-tyrosine (CT) cofactor of fungal galactose oxidase (90) (see Figure 1 for the structures of these cofactors). It is not within the scope of this review to provide either details of the cofactor isolations and structural analyses or an extensive history; for these, the reader is referred to other reviews (17, 57, 78, 113, 114, 143–145, 206).

Like PQQ, the cofactors TPQ, LTQ, and TTQ are quinones; thus, all four share some common properties, including similar chemistries and biochemistries. For example, all four are used by enzymes that oxidize amines. Additionally, these quino-cofactors and the CT cofactor of galactose oxidase are derived, in a more-or-less direct manner, from amino acyl groups. Protein-free PQQ is produced in certain gram-positive bacteria in a multistep process involving the condensation of glutamate and tyrosine (86), which are possibly constituents of a precursor peptide of 23–29 amino acyl residues (70–72, 196, 207, 225). The genes for biosynthesis of PQQ have been cloned from several organisms (see 196 and references therein). It appears that at least seven genes are required because seven gene complementation groups have been identified. The genes are gathered in two separate clusters on the *Methylobacterium extorquens* AM1 genome (207). In accord with a new nomenclature, these clusters are *pqqABCDE* and *pqqFG* (see 207 for nomenclature). *PqqA* codes for the putative pre-PQQ peptide. *PqqF* and *pqqG* seem to code for endoproteases, which, hypothetically, are involved in processing the PqqA peptide during its transformation to PQQ (196). *PqqC/D* codes for an O₂- and NAD(P)H-requiring enzyme. When *pqqC/D* is mutated in *M. extorquens* AM1, a PQQ precursor accumulates in the cell and the growth medium. The exact functions of the other *pqq* genes have yet to be elucidated. One of these unidentified genes, *pqqE*, codes for a protein with some similarity to Cnx2 of *Arabidopsis thaliana* (83), MoaA of *Arthrobacter nicotinovorans* (151) and *Escherichia coli*, and NifB of *Enterobacter agglomerans* and *Klebsiella pneumoniae* (207). MoaA and Cnx2 are involved in molybdopterin biosynthesis, and NifB is involved in the biosynthesis of cofactors in nitrogenase. PqqE, MoaA, and NifB all have a CxxxCxYC sequence near the N termini.

TPQ is produced in a self-catalytic process from a specific Tyr residue of the apo-amine oxidase. This process requires enzyme-coordinated copper and O₂ (38, 158, 178, 206). The copper is also required for oxidation of alkylamine substrates in the fully formed oxidases; TPQ is reduced by two electrons from the substrate, and the electrons are then transferred to the copper site, where O₂ is reduced to H₂O₂ (113). Although no evidence exists, it is assumed that LTQ in copper-containing lysyl oxidase is formed in a fashion similar to that formed from a Tyr residue: TPQ is formed and then the quinonoid carbonyl C-2

is attacked by the ϵ -amino group of a lysyl residue, forming the cross-linked imino adduct (113, 227). Because the copper bound in galactose oxidase is coordinated to the tyrosyl moiety of the cross-linked Cys-Tyr cofactor (90), it might be predicted that copper is also involved in a analogous self-catalytic cross-linking of the appropriate Cys and Tyr side groups.

TTQ biosynthesis likely requires external enzymes because amine dehydrogenases do not contain metal ions. It has been hypothesized that the first of the two pre-cofactor Trp residues in the sequence of the small subunit of the dehydrogenases is converted to a tryptophylquinone in an extra-enzymic process. This could be followed by an enzyme-mediated or a self-catalytic cross-linking with the partner Trp in the sequence (146). Methylamine-utilizing (*mau*) gene clusters have been isolated and sequenced from several methylotrophic bacteria (43, 124, 224). Studies of these clusters indicated that at least 12 genes are involved in methylamine utilization. The cluster contains the genes for the large (~ 45 kDa) and small (~ 15 kDa) subunits (*mauB* and *mauA*, respectively) of methylamine dehydrogenase (MADH). Several of the clusters harbor the gene for amicyanin, an in vivo electron acceptor for MADH. In *Paracoccus denitrificans*, the cluster is under the control of a LyrR-type transcription factor (224). Although the function of the proteins encoded by other open reading frames are unknown, *mauG* codes for a protein that has a translated amino acid sequence similar to a diheme cytochrome *c* peroxidase from *Pseudomonas aeruginosa* (43, 63, 176). It has been suggested that the peroxidase is involved at some stage in the transformation of Trp to tryptophylquinone and possibly in the tryptophylquinone/Trp cross-linking process.

PQQ, TPQ, LTQ, TTQ, OR CT, ESSENTIAL NUTRIENTS OR VITAMINS FOR HUMANS?

TTQ, Tryptophan Tryptophylquinone of Bacterial Amine Dehydrogenases, and CT, the Cysteinyl-Tyrosine Cofactor of Galactose Oxidase

An obvious fundamental requirement of a vitamin is that it be widely distributed in and easily accessible from foods. TTQ and CT fail to meet this criterion. Both are produced in situ as integral components of a limited number of enzymes from small groups of organisms. TTQ is found in alkylamine dehydrogenases from soil bacteria (17), and CT exists in galactose oxidase from a few fungi (e.g. *Dactylium dendroides* and *Fusarium* spp.) and possibly in glyoxal oxidase from the fungus *Phanerochaete chrysosporium* (231). It is not anticipated that the range of organisms capable of manufacturing enzymes with these prosthetic groups will grow significantly. Additionally, the only way these cofactors can

be released from their parent proteins is by extensive proteolysis, and they would be released as amino acyl derivatives. Even if these freed cofactors found their way into the human body, there is no known mechanism to incorporate such modified amino acids into nascent polypeptides, although one could fantasize that the amino-acyl derivatives could bind noncovalently to an apo-protein. If human enzymes do contain these cofactors, it is more likely that they are also made in situ. At present, we are led to believe that the availability/accessibility of external TTQ or CT would be so restricted as to have little or no significance for the well-being of humans.

TPQ, Topaquinone of Copper-Containing Amine Oxidases

TPQ is found in copper-containing amine oxidases, quite likely from almost every form of life. Diamine oxidase (DAO, also known as histaminase) has been identified in various human tissues and fluids (145), including decidua, endometrium (84), placenta (87, 125, 153, 162, 241), amniotic fluid (21, 208), pregnancy plasma (65, 209), seminal plasma (85), macrophages (184), intestine (183), lung mast cells (58), kidney (162, 177, 203), endothelial cells, fibroblasts (20), spleen, liver, eosinophil and neutrophil granulocytes, lymph, urine (21), and tumors (125, 171). Other than in pregnant women, the highest levels of DAO are found in the intestines. This enzyme preferentially oxidizes histamine and the diamine putrescine, although it can oxidize the polyamines, spermine and spermidine, and their *N*-acetylated derivatives. The reader is directed elsewhere for aspects of DAO as it relates to inflammation, allergy, ischemia (in its role as histaminase), tissue differentiation, cellular proliferation, cancer, wound healing, catabolism of potentially toxic amine either ingested or produced by enteric bacteria (4, 48, 235), and possibly programmed cell death (apoptosis) by generation of toxic amounts of H_2O_2 via polyamine oxidation (164, 172). The oxidation of histamine, and presumably the levels of histaminase, is significantly decreased in vernal keratoconjunctivitis (1). The gene for human kidney DAO was originally identified as the gene for an amiloride-binding protein (155, 162). The gene maps to human chromosome position 7q34-q36 (22).

In contrast, the roles of the copper-containing monoamine oxidases (MAO) in humans are speculative. Studies of these oxidases are not aided by the confusing nomenclature. Copper-containing amine oxidases can go by the names plasma amine oxidase (PAO), serum amine oxidase, benzylamine oxidase (because this amine is a good substrate even though it is not naturally occurring), clorgyline-resistant amine oxidase (clorgyline is an inhibitor of the flavin-containing mitochondrial MAO), MAO, plasma MAO, and semicarbazide-sensitive amine oxidase (SSAO). To make matters worse, DAO is also semicarbazide sensitive and can oxidize some monoamines (i.e. benzylamine), and SSAO can oxidize histamine (34). Additionally, the SSAOs are enough different from DAO

and "soluble" copper amine oxidase that they may not have TPQ. Based on the stereochemistry of oxidation of various substrates, it was concluded that SSAOs and lysyl oxidase were mechanistically similar, yet distinct from soluble copper-containing amine oxidases. Additionally, the spectra of derivatives of the SSAO quinone cofactor differ from corresponding forms of TPQ bound to native oxidases (163). This suggests that SSAOs have a cofactor related to—but different from—TPQ, as is the case for lysyl oxidase (*vide supra*).

The most-studied copper-containing MAO is bovine PAO (93, 155). Recently, a gene for an enzyme that has approximately 80% identity with the bovine PAO nucleotide sequence (Figure 2) was isolated from a human placenta cDNA library (242). Because the nucleotide sequence of the placenta enzyme is identical to a number of human genomic sequence fragments (some are expressed sequence tags) found in GenBank, it was concluded that the placenta oxidase gene maps to human chromosome position 17q21, the same region where the familial breast and ovarian cancer gene, BRCA1, maps (242). The human oxidase may be the one isolated and studied by McEwan over 30 years ago (140, 141). Another cDNA was isolated from a human retina library. It codes for a copper amine oxidase that is similar to the human placenta (88). By fluorescence *in situ* hybridization, it was concluded that this gene also maps to 17q21. As with human mitochondrial MAO-A and -B (these are not quinoprotein amine oxidases but contain the FAD form of riboflavin, vitamin B₂), both of which map to Xp11.23 (121, 230), perhaps the placenta and retina oxidase genes also map close together.

Benzylamine oxidase activities were found in the human brain, heart, aorta, lung, liver, kidney, adrenal gland, digestive tract, and skeletal muscle (123), as well as in amniotic fluid and pharyngeal aspirate (122). Benzylamine oxidase-activity staining was found on native electrophoresis gels for apparently soluble samples from the blood, adrenal gland, spleen, bladder, prostate, lung, liver, kidney, heart, aorta, and brain of humans (174). However, from the staining patterns it can be concluded that the tissue enzymes are similar or the same but that they are different from the plasma enzymes. Confusion enters when it is realized that DAO can also oxidize benzylamine, albeit poorly. A recent review makes a clear distinction between soluble PAO and tissue-bound SSAOs (132). The review also presents a *modus operandi*: using inhibitors to distinguish between SSAOs (i.e. copper-containing MAOs), DAO, and flavin-containing MAO-A and -B.

Membrane-bound SSAO is found in aorta and blood vessel smooth muscle, nonvascular smooth muscle of the uterus, ureter and vas deferens, rat white and brown adipocytes, chondrocytes in rat articular cartilage, and odontoblast of porcine dental pulp. The microsomes seem to have the largest SSAO activity, although low activity is seen in mitochondrial and soluble fractions.

Interestingly, porcine smooth muscle can secrete a soluble SSAO, and antibodies against porcine PAO recognize some component of pig aorta. Although highly purified SSAO (PAO?) from several species has been extensively characterized chemically and biochemically, much less is known about the so-called membrane-bound SSAO. They have a mass of 160–180 kDa and subunit mass of ~90 kDa and they presumably contain 1 atom of copper and 1 equivalent of a quinone cofactor per subunit, although the presence of copper has been questioned (132). In 1997, it was reported that a copper amine oxidase (SSAO?) was purified from rat adipocyte plasma membranes (154). The 3' end of its gene was cloned and sequenced, and the translated, partial N-terminal amino acid sequence shows about 80% identity with bovine PAO and a human placenta amine oxidase (Figure 2). It was concluded that the first 35 amino acids of the rat oxidase constitute a transmembrane anchor. Highly similar N-terminal sequences are seen for the human placenta MAO (242) and the human retina amine oxidase (88), but the N-terminal sequence of bovine PAO has what appears to be a "normal" secretory signal sequence (Figure 2). These observations offer several possibilities. There may be two different, but closely related, copper MAOs in mammals, one a membrane-bound form (SSAO?) and the other a soluble form found in plasma and possibly other tissues and body fluids. It has been observed that the blood of ruminants, rabbits, dogs, and cats contains high levels of PAO. However, human, hamster, and mouse blood contains much less or none, and there does not appear to be PAO in rat or guinea pig blood (59). Thus, alternatively, some species may contain a plasma enzyme (and possibly a membrane-bound form), while other species have only a membrane-bound form. It might be possible that in some species (e.g. humans), a small amount of the membrane-bound form can find its way into the blood after cleavage of the membrane anchor. Obviously, more studies of the type described by Morris et al (154) are required to clarify these issues.

If TPQ exists in enzymes other than the amine oxidases, the expectation is that *in situ* biosynthesis occurs. As with TTQ and CT, release of TPQ can occur only on extensive proteolysis of an oxidase. It is advantageous that TPQ is made *in situ* in the amine oxidases because free TPQ is reactive and a known neurotoxin (93). Although it is clear that these copper amine oxidases are necessary for human well-being, TPQ itself, arising from the transformation of a specific tyrosyl residue in each protein molecule, has no nutritional value. However, a copper deficiency would have a devastating effect because copper is essential for biosynthesis of TPQ in the oxidases. X-linked, recessive, human Menkes syndrome can be a lethal disorder, which is thought to be caused by defects in intestinal mucosa copper transport. This results in decreased serum copper and ceruloplasmin. It was suggested that an altered gene for a copper-transporting ATPase results in Menkes syndrome (210, 226). The

manifestations of the syndrome include hypopigmentation, growth failure, skeletal defects, hypothermia, progressive degeneration of the central nervous system, arterial aneurysms, death in early childhood, cerebellar degeneration, mental retardation, steely short hair, bladder diverticula, and several connective tissue defects (64, 106, 210). X-linked cutis laxa (also known as occipital horn syndrome and Ehlers-Danlos syndrome) is another disorder related to copper metabolism that displays bladder diverticula, slight skin laxity and hyperextensibility, skeletal changes, and occipital horn-like exostose. Patients with Menkes or cutis laxa were shown to have significantly elevated levels of copper in fibroblasts cells due to copper's sequestration by supposedly abnormal metallothionein, and the cells had very low lysyl oxidase activity (148, 149). It has been proposed that the metallothionein gene or a P-type ATPase gene maps to chromosome position Xq12-q13.3 (210). Some of the manifestations of the disease may be a result of increased levels of protein-free Zn^{2+} , which would normally be bound to metallothionein but is prevented from binding by bound Cu^{2+} . Although the copper quinoprotein lysyl oxidase is usually implicated in these syndromes, it may be that some of the effects result from lowered levels of competent copper amine oxidases, such as PAO, DAO, and SSAO.

LTQ, Lysyl Topaquinone of Lysyl Oxidase

To date, the quinone cofactor LTQ is only found in lysyl oxidase (LO), an enzyme necessary for cross-linking in collagen and elastin. The enzyme is intimately associated with the connective tissue and, thus, difficult to purify. Pure LO has been obtained from human placenta, chick cartilage, rat skin, piglet skin, and bovine lung and aorta (99). LO is a copper-containing amine oxidase, but there is little amino acid sequence or structural similarities with the amine oxidases PAO, DAO, and SSAO. LO is a single-subunit oxidase of molecular weight 28–32, and the prepro-LO has a molecular weight of 48 (74, 107). Even though LTQ is related to TPQ, LO does not have the pre-cofactor tyrosyl consensus (recognition) amino acid sequence found in all TPQ-containing amine oxidases. Apparently, unprocessed pro-LO can bind copper, and copper incorporation does not require prior glycosylation of the oxidase (116).

The *LO* structural gene maps to human chromosome position 5q23 (77, 135). The *LO* gene has been identified as a tumor-suppressor gene (antioncogene of *ras*), *rrg* (*ras* recision gene) (74, 107). The gene has an INF regulatory factor (IRF)-E transcription regulatory element (TTAAAGTGAAAC), and binding of IRF-1 to this sequence results in transcription of the *LO* gene. IRF-1 has an antioncogenic function in NIH3T3 cells: It suppresses cell transformation by *c-myc* and *fosB*. In addition, the cHa-*ras* oncogene is expressed in *IRF-1*-deficient cells. *IRF-1* maps to chromosome 5q31.1, close to where the *LO* gene maps (see 205). Expression of *LO* cDNA in *ras*-expressing embryonic fibroblast

cell suppresses the mutation in *IFR-1*. It was proposed that regulation of the *LO* gene is involved in the process of malignant cell transformation (74, 205).

A gene has been cloned from human skin fibroblast that has an exon-intron structure, an exon sequence homology, and a tissue-specific expression similar to that of human LO (74, 107). The gene for this LO-like protein (25 kb) is considerably larger than that for LO (14 kb). The deduced amino acid sequences of the two genes are most similar in the last 200 residues of the C termini, which correspond to exons 2–6 of the genes of both proteins. This regions contains the Tyr and Lys residues of the LTQ cofactor. The domains around these two residues are nearly identical (110, 227), and a putative copper-binding domain is identical for LO and the LO-like proteins. Exon 1 of LO codes for a signal sequence, a propeptide sequence, and the first residues of the functional oxidase. The LO-like protein has potential signal sequences at the beginning of the putative coding sequence. However, cleavage at putative endopeptidase cleavage sites between Arg residues would produce mature proteins of 49, 48, or 31 kDa. It is possible that there are multiple forms of LO with slightly different activities or functions. However, different secretory and propeptide sequences may be involved in regulating secretion of the two proteins. A human gene that is identical or similar to the LO-like gene was mapped to chromosome position 15q24-q25 (108).

A lowered level of LO is responsible for many of the manifestations of Menkes syndrome and cutis laxa (vide supra). The LO mRNA is lower in cultured skin fibroblasts cell from Menkes and cutis laxa patients, thus the lowered LO activity is, in part, due to pretranslational control (106). Elevated levels of LO are associated with fibrotic diseases such as atherosclerosis, hypertension, and liver and pulmonary diseases (99, 227).

From the preceding paragraphs, it is obvious that normal levels of a properly functioning LO are important to human health. However, as with TTQ, CT, and TPQ, the protein-free cofactor of LO, LTQ, is of little nutritional value. It is made, in place, from unique Tyr and Lys residues of the polypeptide and would be released from LO on extensive proteolysis. Again, if TPQ exists in enzymes other than LO, or LO-like proteins, it will also be created in situ.

PQQ of Bacterial Dehydrogenases

PQQ is found only in bacterial oxidoreductase and only as a noncovalently bound cofactor, although a great deal of work has been done to prove otherwise (57, 78, 114, 143, 144). This might lead one to believe that PQQ has no nutritional value for humans. Although it is possible PQQ-requiring enzymes exist in humans, for now PQQ cannot be considered an essential nutrient from the standpoint of a coenzyme. However, being readily available in the diet, and because it has redox properties, it may be an important dietary supplement,

akin to other antioxidant factors (see below). For example, there are substances that are important or essential to human health but that so far have not been shown to be cofactors in enzymes. Among these are vitamin E (tocopherols), some carotenoids (139), flavinoids, and plant phenolics [including resveratrol (3,5,4'-trihydroxy-*trans*-stilbene)] (75, 94, 185).

Enzymes containing PQQ as a noncovalently bound cofactor (see Table 1 in 143) are manufactured, often in large quantities, in a variety of microorganisms, including some found in soil, some involved in fermentation, some found in the digestive tract, and some pathogens (e.g. *Acetobacter*, *Acinetobacter*, *Comamonas*, *E. coli*, *Gluconobacter*, *Hyphomicrobium*, *Methylobacterium*, *Methylophilus*, *Norcardia*, *Pseudomonads*, *Salmonella*, *Serratia*, and *Zymomonas*) (17, 57, 136, 143, 144, 221). Not only is PQQ sequestered in various proteins, the following soil bacteria excrete PQQ into the media when grown in the laboratory (7, 9, 10, 147, 212, 213, 216, 221) (values in parenthesis are the largest levels detected): *Acetobacter aceti* (10 µg/liter); *Acetobacter calcoaceticus* (66 µg/liter); *Achromobacter*; *Alteromonas thalassomethanolice* (6.6 mg/liter); *Ancylobacter aquaticus* (1 mg/liter); *Hyphomicrobium* X (1.98 mg/liter); *Hyphomicrobium methylovorum* TK0441 (1 g/liter); *Methanomonas*; *Methylobacterium organophilum* XX (2.64 mg/liter); *Methylobacillus*; *Methylomonas*; *Methylophilus methylotrophus* W3A1 (1.25 mg/liter); *Microcycilus*; *Mycoplasma*; *Norcardia* sp. strain 239 (1.98 mg/liter); *Paracoccus denitrificans* (0.325 mg/liter); *Protaminobacter*; *Protomonas*; *Pseudomonas* P2-3 (40 mg/liter); *Pseudomonas* sp. strain BB1 (1.32 mg/liter); *Pseudomonas aeruginosa* (0.99 mg/liter); *Pseudomonas stutzerii* (3.3 mg/liter); *Pseudomonas putida* biovar B (1.98 mg/liter); *Thiobacillus versutus* (1.98 mg/liter); and *Xanthobacter*. Many of the references describe conditions that increase the yield of excreted PQQ. All these organisms produce PQQ-containing proteins. However, some microorganisms make apo-PQQ forms, e.g. some strains of *E. coli* and *Acinetobacter lwoffii* (221, 223), *Pseudomonas aeruginosa* (223), *Pseudomonas testosteroni*, *Agrobacterium* spp. (221), *Rhizobium meliloti*, *Bradyrhizobium japonicum* (29). It is likely that these organisms import PQQ that is excreted by symbionts, or by other enteric bacteria (e.g. *Klebsiella pneumoniae*) (225) or ingested food, as in the case of *E. coli*. In fact, it has been shown that PQQ has growth-stimulating properties for some microorganisms in culture. Some organisms for which growth improves are *A. aceti* (10, 12, 13); *Acetobacter rancens*; Awarori yeast; *Saccharomyces cerevisiae* (12, 13); *Pseudomonas* sp. VM15C [PQQ is an essential growth factor for this organism (187)]; *Gluconobacter oxydans* IFO 3287 (8); *Agrobacterium radiobacter* (126); *E. coli* (57, 223); and *Comomonas testosteroni* (57, 73). PQQ is a chemotactic attractant for *E. coli* (52), *R. meliloti*, and *B. japonicum* (29). Some strains of *E. coli* produce apo-glucose dehydrogenase (GDH) because of a defect in a gene for

PQQ biosynthesis. In a glucose-containing medium, the bacteria grow slowly, but eventually fast-growing mutants arise that can produce PQQ for the apoglucose dehydrogenase (28). Cloning a specific gene from *Erwinia herbicola* into *E. coli* presumably leads to the production of PQQ in the later organism (127). Additionally, *pqqE* and *pqqF* cloned from *E. coli* complement PQQ-biosynthesis mutants of *M. organophilum* (211). *K. pneumoniae* has six genes involved in PQQ biosynthesis, and it produces holo-GDH. In order to activate its apo-GDH and to allow the organism to excrete PQQ into the media, all six genes were required to produce enough PQQ in a strain of *E. coli* unable to manufacture this cofactor (225). Cloning *A. calcoaceticus* genes for PQQ biosynthesis into PQQ-minus *E. coli* allowed the latter to produce the cofactor and increased production of GDH (194).

THE CASE FOR PQQ AS AN ESSENTIAL NUTRIENT

That PQQ is a growth factor or essential nutrient for microorganisms is obvious from the above discussion. However, if PQQ is absolutely required for humans, or at least improves health, it must be biosynthesized or easily acquired. To date, there is no evidence to indicate that this quinone is manufactured in our bodies. However, acquisition may occur via enteric bacteria that manufacture oxidoreductases using PQQ as a cofactor. As the bacteria die and lyse in the digestive tract, these enzymes are hydrolyzed, releasing PQQ. Further, some species of *Enterobacteriaceae*, e.g. *E. coli* and *Klebsiella aerogenes* (*K. pneumoniae*) (225), or other intestinal bacteria may excrete PQQ for absorption through the intestinal mucosa and lumen (54). In some studies, it was concluded that the intestinal flora provided little PQQ and that it would need to be made in situ or ingested with food (191, 192).

Although it is not known if plants can biosynthesize PQQ, PQQ excreted by soil bacteria (vide supra) could be absorbed by plant roots. PQQ has been shown to stimulate pollen germination for plant species *Lillium*, *Tulipa*, and *Camellia* (236, 237), and it has been identified in various pistal tissues (237). In addition, PQQ was reported to stimulate the growth of microbial, plant, and animal cells in culture (8). Plant materials are ingested by various animals that provide meat or dairy products for humans. PQQ may be consumed directly from vegetables, fruits, and nuts. In 1985 (14) it was reported that many plant and animal extracts contained PQQ (liver, meat, tomato, tea, carrot, sweet potato, malt extracts, rumen, cane molasses, and corn meal), and supposedly, PQQ from these sources stimulated microorganism growth. Since this report, several groups have reported PQQ in other food, in human and mammalian tissues, in cells and fluids, and in urine and fecal matter (27, 109, 118–120, 167–169, 202). It was demonstrated that radio-labeled PQQ was rapidly absorbed

in the intestines of mice and that most was excreted by the kidney within 24 h. Most of the retained PQQ was localized in the kidney and skin, and most of the PQQ in blood was associated with cells, not plasma (193). It was found, however, that PQQ binds tightly to human serum albumin (3, 217).

Several methods have been used to detect PQQ in biological samples. One of the earliest was a direct reverse-phase chromatographic comparison with authentic PQQ (14, 219) that was purified from bacterial growth media (*vide supra*) (217), synthesized (188 and references therein, 217), or obtained commercially. This method has also been used after stable derivatization of PQQ (219). [Note: A variety of PQQ analogs have been obtained (91, 138, 161, 190, 243, 244), including isotopically labeled forms of PQQ (6, 86, 97, 193)]. Other methods for PQQ analysis and detection are as follows: (a) chromatographic isolation, which is followed by fluorescence measurement (202, 217, 220); (b) extraction, derivatization, and mass spectral or gas chromatographic/mass spectral analysis (36, 118–120); (c) reconstitution of PQQ-dependent apo-enzymes (2, 11, 217); and (d) redox-cycling assay (62). The last method works particularly well as a denaturing-electrophoresis-gel-staining method for protein containing the covalently bound quinone cofactors TPQ, LTQ, and TTQ. This procedure has been severely criticized because, supposedly, ascorbate and riboflavin (vitamin B₂) will also test positive (62, 166, 218).

Other problems are associated with detecting and accurately measuring the levels of PQQ in biological samples. Being a quinone with a redox potential of about 80 mV (normal hydrogen electrode) at pH 7.0, PQQ is electrophilic (143, 144). Thus, it can react with other (nucleophilic) components of the various samples. PQQ is attacked at its 5-position (sometime with the intervention of metal ions such as Cu²⁺) by water, alcohols, ammonia, alkyl- and arylamines, diamines, phenylenediamine, 2,3-diaminenaphthalene, amino acids, acetone, aminoguidine, urea, cyanide, sulfite, thiols, nitroalkanes, dihydronicotine analogs (hydride attack), borate, and carbonyl reagents such as alkyl- and phenylhydrazines, semicarbazide, and 3-methyl-2-benzothiazolinone hydrazone (143 and references therein). PQQ reacts with amino acids to produce oxazole derivatives (220, 222) or imidazolopyrroloquinoline derivatives from the reaction with tryptophan, glycine, tyrosine, and serine (105, 214). These adducts could inhibit or prevent PQQ from reacting with detection reagents (56, 66, 69, 220). PQQ also undergoes acid-promoted tautomeric lactonization (67). PQQ binds tenaciously to laboratory glassware and equipment. Thus, contamination from bacteria, or from pure PQQ used in research, could cause problems in assaying for PQQ in biological samples. Additionally, it is difficult to eliminate PQQ from the liquid-based diets of experimental animals because solutions contain low levels of the cofactor, and there are even trace amounts in domestic water (109). It is recommended that glassware be baked at 560°C for 12 h prior to use and that aqueous solutions be

treated with Amberlyst[®] A21 (50–100 μm , p.A. form, SERVA, Heidelberg, Germany), which tightly binds PQQ (220). With these caveats, many claims concerning PQQ contents and levels in biological samples should be cautiously considered.

Perhaps the most important investigations into the nutritional benefits of PQQ have been done by Killgore et al (109), Smidt et al (191–193), and Steinberg et al (197). When PQQ was rigorously excluded from chemically defined diets, there were significant effects in mice. When PQQ was included in the diet, or when it was detected as a dietary contaminant or in fecal matter (because of developed resistance to an antibiotic in the diet), the mice grew normally. Some of the manifestations of PQQ-deprived mice were friable skin, mild alopecia, diverticula, and hunched posture. Of 40 PQQ-deprived mice, 8 died (aortic aneurysm, abnormal hemorrhage, etc) by week 8 of deprivation. Only 1 of 33 PQQ-supplemented animals died during the entire experiment. For bred, young, PQQ-deprived female mice, there were either no litters or pups were cannibalized at birth. For normal mice, 90% of pups were weaned successfully. Most striking in the deprived mice was the friable skin, suggesting a decreased maturation or deposition of skin collagen. Collagen extracted from the skin of these mice was twice that extracted from supplemented mice. This suggests a lower amount of collagen cross-linking in the PQQ-deprived animals. If PQQ is biosynthesized by mice, it obviously is not done at a level to prevent abnormal development of the study animals. When these findings were reported in 1989 (109), it was reasonably assumed that lysyl oxidase (LO) contained covalently bound PQQ or a derivative thereof. It was natural to conclude that at least some of the manifestations of PQQ deprivation were due to lowered amounts of active LO. Today, we know that PQQ is not the redox cofactor of LO (*vide supra*). A direct role of PQQ in connective tissue maturation has been suggested (*vide infra*) (186).

In an extension of the dietary studies, it was found that (a) PQQ improves reproductive performance and neonatal growth for BALB/c mice, (b) PQQ affects cell response to concanavalin A and lipopolysaccharides in spleen cells, and (c) interleukin 2 levels were lower in mice fed a PQQ-deficient diet at a time when T-cells proliferate in neonates (197).

POSSIBLE MOLECULAR BASES FOR PROPOSED PHYSIOLOGICAL FUNCTIONS OF PQQ

Because of its redox properties, it has been suggested that PQQ has *in vivo* antioxidant properties and perhaps is involved in oxidative stress in biological tissues (68). For example, PQQ would react with two molecules of superoxide to produce two molecules of O_2 and reduced PQQ (PQQH_2); then PQQH_2 would react with O_2 to form PQQ and H_2O_2 (92, 200). In addition to scavenging

superoxide, PQQ could also scavenge other toxic free radicals, as does vitamin E, β -carotene and carotenoids, vitamin C, flavinoids, conjugated linoleic acid, carnosine, anserine, and phenolic compounds (75). The latter three (along with PQQ) have been called nonessential dietary antioxidants (50). Many phenolic compounds exhibit anticarcinogenic activity (50, 175, 229). Included in this class is newly discovered resveratrol, a phytoalexin found in grapes and other foods (94).

More recently, it was reported that PQQ radical (PQQH \cdot) and PQQH $_2$ might be involved in a dynamic equilibrium between O $_2$ and superoxide. The reaction is pushed from O $_2$ to superoxide by the presence of large amounts of PQQ reductants [e.g. primary alkylamines, thiols, catechols, catecholamine, ascorbic acid, NAD(P)H, etc], high levels of O $_2$, superoxide-oxidizing materials, or superoxide dismutase (167). The equilibrium is probably also affected by pH and the presence of metal ions, such as Cu $^{2+}$, which is known to complex with PQQ and change its physical, chemical, and redox properties (100, 204). PQQ in the presence of NADH and Cu(II) induced damage to DNA (81). Interestingly, free PQQ, Cu $^{2+}$, and O $_2$ oxidize lysyl groups of elastin and collagen in much the same way as LO (186). Is it possible that PQQ and Cu $^{2+}$ complement cross-linking by LO in these connective tissues? If so, the Cu $^{2+}$ deficiencies of Menkes and other syndromes would not only affect LO, they would also affect this hypothetical PQQ/Cu $^{2+}$ /O $_2$ reaction.

PQQ has been found in guinea pig neutrophils. It has been hypothesized that PQQ is involved in the respiratory burst in these cells and in eosinophils, monocytes, and macrophages (27) and that it is involved in superoxide removal in red blood cells. PQQ protects against glutathione depletion and cataract development in chicken embryos that have been oxidatively stressed by administration of glucocorticoid (61, 160).

PQQ is efficiently reduced by a rabbit erythrocyte flavin reductase. The PQQH $_2$ thus formed quickly reduce the higher oxidation states of myoglobin. The reductase is also found in liver and heart and is believed to protect isolated rabbit and rat hearts from reoxygenation injury (238).

PQQ seems to have antitoxin activity. It protects cultured rat cortical neurons from the excitotoxicity of *N*-methyl-D-aspartate (NMDA, a glutamate-receptor agonist) by oxidizing the NMDA receptor redox-modulatory site (5). It was proposed that PQQ inhibits the glutamate-induced free radical-generating response, not by scavenging reactive oxygens species but by oxidizing the NMDA receptor redox center (181). PQQ protects animals from the toxic affects of adriamycin, and it is a neuroprotector in rats with hypoxic/ishemic brain injury (95). (For a more detailed discussion of these issues and other speculations on the human benefits of PQQ, see 44, 61, 167). PQQ inhibits carrageenin-induced rat-paw edema (76) and seems to lower blood and liver acetaldehyde (but not

ethanol) levels after ethanol loading in rats (82). In addition, high levels of PQQ inhibits glucose-dependent insulin release from cultured mouse pancreatic cells (142). PQQ also protects against hepatotoxin-induced liver damage in rats (228). PQQ significantly enhances DNA synthesis in human fibroblasts; PQQ-glycine oxazole, however, had no such effect (156). It was recently reported that imidazopyrroloquinoline (the adduct formed by the reaction of amino acids and PQQ) (105, 214) and derivatives are potent inhibitors of rabbit lens and dog kidney aldose reductases (215).

PQQ was shown to inhibit melanogenesis induced by α -melanocyte stimulating hormone (MSH) in murine B16 melanoma cells (115). It appears that PQQ inhibits MSH-induced tyrosinase activity by reducing the levels of tyrosinase mRNA in the cells, whereas PQQ has no effect on MSH-induced cyclic 3',5'-AMP production. It was hypothesized that PQQ scavenges a reactive oxygen species, which is involved in regulating the mRNA expression.

With all this information, the question lingers as to whether PQQ is an essential nutrient, a beneficial nutrient, or a therapeutic agent (44). PQQ is not known to be a redox cofactor in any human enzyme, although this does not mean that such enzymes do not exist. The functions of PQQ enumerated in the preceding paragraphs could possibly be carried out by other compounds, particularly those with similar physical and chemical properties (e.g. redox properties, highly negatively charged, aromatic, etc), for example, phenolic compounds linoleic acid, carnosine, PQQ isomers and isomers (142), other quinonoid compounds (68), vitamin C and E, etc (75, 192). It has been pointed out that there is an extensive literature on the benefits of cathetins and bioflavonoids on the maturation of connective tissue (192). Additionally, the exact molecular mechanisms of action of PQQ, in almost every case cited, are hypothetical, vague, or unknown. On the other hand, it seems hard to believe that PQQ does not have some specific, essential function. Take the results of the mice fed PQQ-minus diets (109). Any other naturally occurring, common substance that could prevent the effect seen with these so-called PQQ-deficient mice would not be manufactured by the animals (at least at levels to prevent the observed problems), and they, too, would have been unknowingly excluded from the PQQ-minus diet. Because it is extremely difficult to keep PQQ out of consumables, it is reasonable to expect the same of other similar substances. After all, if these were hard to come by, all the mice (and humans) would suffer.

MEDICAL, PHARMACOLOGICAL, AND OTHER USES FOR QUINOPROTEINS AND PQQ

It has been proposed that various quinoproteins (proteins containing PQQ, TTQ, TPQ, or LTQ) could be used as biocatalysts to produce research and industrial

amounts of useful chemicals. It was found that pea-seedling DAO could produce a variety of novel phenacyl derivatives from the appropriate diamines (45). Another example has been given for lactate production by 1,2-propanediol oxidation by free-cell suspension of a methylotrophic bacterium. The diol is first converted to lactaldehyde by PQQ-containing methanol dehydrogenase, and then the aldehyde is converted to lactate by an aldehyde dehydrogenase (159). In cases like this, the substrate-reduced quinoprotein dehydrogenase is reoxidized by natural electron acceptors. These are reoxidized by components of the membrane electron transport chain in the cell-free suspension, which reduce O_2 to H_2O . In membrane-free cell extracts or for pure quinoproteins, large levels of a desired chemical could be produced if a convenient natural or artificial electron transport chain were used, for example, a pure or partially pure natural electron acceptor for the quinoproteins, or components of methylotroph membrane electron transport chains (15–17, 49, 136, 137, 144). Another route involves coupling the protein directly or via mediators to an electrode for electrochemical regeneration of substrate-reduced enzyme (37, 47, 80). Along these lines, pure alcohol dehydrogenases (101, 199, 201, 245), methylamine dehydrogenase (129), and GDH s (96, 101, 240) have been used to construct immobilized enzyme-based electrodes. It has been reported that modification of GDH with a cross-linking reagent stabilizes the enzyme (195).

Not only could these enzyme-based electrodes be used to convert cheap feedstock materials into useful chemicals, they could also be designed for use as biosensors (that is, sensors to measure levels of specific substances in biological tissues and fluids) in much the same way as has been done with the flavoprotein, glucose oxidase (GOD) (101, 173). Substrate-reduced GOD is reoxidized by O_2 producing H_2O_2 . Thus, an electrode must either take electrons directly from the reduced flavin (this is difficult to do because the flavin is very deeply buried and protected in the protein) (51, 182), which eliminates the production of H_2O_2 , or the levels of O_2 or H_2O_2 must be measured directly. These aspects complicate the design of the electrode (101, 173), requiring chemical (51, 182) or genetic alterations of GOD, some atypical modifications of the electrode (55), a GOD-to-electrode wiring (233, 234, 240), or the presence of a special mediator (53). The response of a sensor could be limited by the O_2 levels, by low levels of D-glucose, or by interference from other redox-active materials. [Electrochemical detection of H_2O_2 requires a high potential, which could oxidize ascorbate, uric acid, and other components in blood and other samples (101).] The quinoprotein dehydrogenases, on the other hand, are designed to transfer electrons to an external site on their protein surfaces, where natural electron acceptors bind. This means that the quinone redox cofactors are close to, or if buried are able to efficiently transfer electrons to, these sites. It has been shown for a large number of dehydrogenases that efficient electrical communication is obtained

with relatively simple electrode modification (37,80). Therefore, it could be possible to produce a glucose biosensor with PQQ-containing GDH, which would avoid many of the drawbacks of the various types of GOD-based sensors.

It may be advantageous to fabricate sensors using a quinoprotein oxidase (i.e. one containing TPQ or LTQ) to specifically detect amines in biological fluids and tissues. Many of the same methods used for GOD-based electrodes might be applied in these situations. The crystal structures of the copper amine oxidases show that the TPQ cofactor, like the flavin group in GOD, is deeply buried within the proteins (117, 165, 232). Thus, there are difficulties for direct electron transfer to an electrode surface for the amine oxidases, as well as for other copper-containing oxidases (239). With this said, there are reports of a H_2O_2 detection-based biosensor fabricated from pig kidney DAO (33, 134) and bovine PAO (79). These sensors were used for detection of histamine, L-histidine, cadaverine, and putrescine. There also is the potential for development of a galactose oxidase-based biosensor (198).

Even protein-free PQQ has been exploited for interesting electrochemical applications (102–104, 157). For example, a PQQ-mediated GOD electrode was fabricated to measure the levels of D-glucose in fruit juices (128), and PQQ was covalently tethered between a gold-electrode binding molecule and FAD, the latter sequestered in the flavin-binding site of GOD (234). When attached to the electrode surface, this PQQ/FAD wire allowed for a densely packed monolayer of GOD, which gave an unprecedented high rate of glucose electro-oxidation.

PQQ was incorporated into an electrode monolayer, and N^6 -(2-aminoethyl)- NAD^+ was covalently attached to this layer. NAD^+ -dependent lactate dehydrogenase (LDH) was allowed to bind to this monolayer to form an LDH-based electrode. The PQQ allowed for efficient electron transfer between the enzyme and electrode. This could be the basis for a lactate biosensor. Similar work has been done with an NAD^+ -based alcohol dehydrogenase (23).

Although a lot of ink has been given to pharmacological aspects of the copper-containing amine oxidases of humans and other mammals (for example, 21, 39, 40, 130, 132, 235), to my knowledge, not a single clinically useful drug designed to specifically target any of these enzymes is in use today. For the copper MAOs, this stems, in large part, from the fact that their involvement in any physiological process is unknown or ambiguous. On the other hand, DAO has a more specific and defined role. It catabolizes putrescine, spermine, and spermidine, agents known to be involved in various fundamental cellular processes, and DAO has a role as histaminase (see the Introduction section of this review). Unfortunately, for DAO or the copper MAO, it is not known if one, a few, or many forms exist within the human body. Similar oxidases may have different tissue-specific functions, or

different functions in the same tissue (203). For example, there is a soluble MAOs in serum, which seems to be closely related to a membrane-bound form in adipocytes (154). A drug presumably designed to target one form may inhibit other forms to produce undesired side effects. It is also known that substances that interact with these oxidases also interact with other systems (145). (a) Inhibitors of SSAO and mitochondrial FAD-containing MAO are as follows: propargylamine; hydrazines; the antidepressant phenelzine; the antihypertensive agent hydrallazine; the antiarrhythmic drug mexiletin; 4-dimethylamino-2, α -dimethylphenylethylamine (FLA 336); the 2-phenyl-3-haloallylamine analog, (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145); and transcyclopromine. (b) 3-(4-[(3-chlorophenyl)methoxy]phenyl)-5-[(methylamino) methyl]-2-oxazolidinone methane sulfonate (MD 780236) inhibits MAO-B and SSAO (111). (c) Benserazide and carbidopa inhibit SSAO and DOPA decarboxylase. (d) β -Aminopropionitrile inhibits LO and SSAO (132). (e) Oxidation of 2-(3-aminopropylamino)-ethanethiol (WR-1065; used to protect against radiation damage) by a copper-amine oxidase causes intracellular depletion of glutathione (150). (f) Semicarbazide and other carbonyl reagents and β -aminopropionitrile and aminoactonitrile inhibits SSAO, DAO, and LO. (g) Analogs of the diuretic amiloride also inhibit Na⁺ transporting systems and DAO (162). (h) Aminoguanidine inhibits DAO and LO, and antimalarial drugs inhibit DAO (133). (i) 4',6-Diamidino-2-phenylindole inhibits DAO and S-adenosyl-L-methionine decarboxylase (46).

Since the copper amine oxidases are of fundamental importance, it is not certain that inhibiting them would be beneficial. There is a seemingly necessary rise in levels of DAO in developing tissue, tissue differentiation, tumors, healing wounds, etc. In cancer cells, elevated DAO level (4) is likely a response to the increased levels of polyamines and putrescine. It is possible that this is a defense mechanism in precancerous cells or the early stages of tumor formation, whereby increased levels and oxidation of the amines produce toxic levels of aldehydes and H₂O₂ in an attempt to kill cells or limit cellular proliferation. On the other hand, the increase in these amines, in part, may cause tumor formation. Could it be that if the elevated levels of DAO are not high enough to maintain polyamine homeostasis, cancer develops? It was found that Sendai virus envelope-encased DAO or PAO injected into transformed fibroblast induced severe cellular damage (19). Additionally, when DAO bound to concanavalin A Sepharose was injected intraperitoneally into mice Ehrlich ascites tumors, tumor growth was inhibited (4). Further, exposure of hamster ovary cell to bovine serum amine oxidase and spermine was cytotoxic (18).

Exposure of human immunodeficiency virus type-1 (HIV-1) to spermidine, PAO, and myeloperoxidase in the presence of Cl⁻ caused rapid inactivation of

the virus. Because semen contains high levels of spermine, spermidine, and putrescine, it was suggested that their oxidation by an amine oxidase would produce H_2O_2 , which in the presence of Cl^- and myeloperoxidase could have important viricidal activity in semen and the vaginal canal (112). Thus arises the intriguing notion that high levels of endogenous amines are released in response to viral, bacterial, and/or fungal infection, or in precancerous cells, for specific oxidation by an amine oxidase. The production of H_2O_2 , either alone or in concert with other agents, is the body's attempt to inactivate invaders or cancer.

It has been documented that free radicals and H_2O_2 are released in response to agonist binding to a cell surface, i.e. they are intracellular second messengers (41). This is particularly interesting because many SSAOs are membrane bound (132, 154). As a second messenger, H_2O_2 may regulate transcription, metabolic pathways, smooth muscle relaxation, cell proliferation, etc (41). Other roles for H_2O_2 are stimulating $PGF_{2\alpha}$ formation in the brain, relaxation of isolated aorta, vasodilation of cremasteric arterioles, vasoconstriction in isolated perfused lung, transmembrane signaling in white adipose tissue, pulmonary artery relaxation by activation of guanylate cyclase, and stimulating insulin receptor kinase (39).

Some of the studies cited above suggest that the copper-amine oxidases themselves could be used as therapeutic agents. For example, the purified enzymes could be injected into tumors. Perhaps someday PQQ could also be used clinically, possibly in maladies resembling those seen in the PQQ-deficient mice, or in individuals with connective tissue or developmental problems. Whether PQQ will ever be used as a general dietary supplement (as is the case for vitamins C and E, β -carotene, melatonin, etc) remains to be seen. Research papers on some of the other antioxidants (e.g. vitamin C) number in the thousands. This outnumbers, by orders of magnitude, the papers that have been published on the nutritional aspects of PQQ. Even in the case of a well-studied nutrient like vitamin C, there is still much controversy concerning its benefits (25, 75).

In contrast to some of the studies cited above, it was proposed that the products of amine oxidase oxidation of putrescine and polyamines (aldehydes, H_2O_2 , and oxygen radicals) were considered to be cofactors in the development of cervical cancer. These substances were labeled *in vivo* mutagens, cytotoxins, and immunosuppressants. Women with high levels of copper amine oxidases in cervical mucosa might have an increased risk of developing cancer, particularly with partners with high levels of the amines in their seminal fluid (60). Other purported negative aspects of H_2O_2 are breaking DNA strands, induction of chromatid aberration, and inducing programmed cell death (60, 81, 164, 172).

In addition to inhibitors of the copper amine oxidases, it may be advantageous to identify activators. This could be useful in maladies where increased activity would be beneficial, as, for example, in tumor cells. To my knowledge, there

is only a single report of activation of a copper amine oxidase: the methyl derivative of 3*H*-imidazo[4,5-*f*]quinoline activates PAO (42).

Some drugs are oxidized by the copper amine oxidases, thus reducing their efficacy. For example, some of the MAO inhibitors (e.g. kojic amine and the oxazolidine MD 220661) are oxidized (39). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could be oxidized in the blood by PAO to MPP⁺ (1-methyl-4-phenylpyridinium) (26). Being a neutral compound, MPTP passes through the blood-brain barrier, whereas MPP⁺ cannot. In the brain, MPTP is oxidized to MPP⁺ by MAO. MPP⁺ destroys nigrostriatal cells, resulting in Parkinsonism (189). Interestingly, pyridoxamine (a form of vitamin B₆) inhibits SSAO (35).

SSAO oxidizes endogenous amines (e.g. methylamine that is produced on metabolism of dietary choline, lecithin, and creatinine by enteric microorganisms, and aminoacetone formed in the metabolism of threonine by threonine dehydrogenase, or from glycine via aminoacetone synthetase), and xenobiotic alkylamines (e.g. allylamine) (130, 131). Methylamine, aminoacetone, and allylamine are converted to the toxins formaldehyde, methylglyoxal, and acrolein, respectively. It was found that SSAO inhibitors increase the levels of methylamine in urine (131).

To date, less work has been done on the pharmacology of LO. The level of LO is marked increased in fibrotic tissue, and LO is released into the blood of experimental animals after induced liver fibrosis. This increase may be a sensitive marker for liver fibrosis in humans. Use of a highly specific LO inhibitor could be use to treat fibrosis. Several inhibitors of LO are β -propionitrile, 2-bromoethylamine, 2-nitroethylamine, *p*-chlorobenylamine, and (*R,R*)-1,2-diaminocyclohexane (99).

The final discussion in this section deals with the various amine oxidases as markers for different pathologies. As mentioned, the LO level may be a good indicator for liver fibrosis. One report has attempted to define the role of DAO in various diseases: that is, whether decreases in DAO activity are a necessary determinant (type 1), a sufficient determinant (type 2), a contributing condition (type 3), or something other (type 4 to 6) for a disease (180). It has been offered that DAO could be used as a clinical tumor marker and a marker of intestinal mucosal integrity (171). The levels of DAO, PAO, and SSAO change in potentially measurable way in a variety of conditions. For DAO, changes are caused by pregnancy; heparin treatment; various carcinomas, cancers, and malignancies (24); chronic renal failure; burns; anaphylactic shock; cystic fibrosis; bronchial asthma; and various allergic conditions. For PAO, changes are caused by menstruation, chronic liver disease, congestive heart failure, hyperthyroidism, diabetes mellitus, acromegaly, Addison's disease, carcinoid syndrome, progressive systemic sclerosis, malignancies, burns,

rheumatic fever, estrogen treatment (30), long-term treatment of Parkinsonian patients with L-dopa (89), and congestive heart failure (32). For SSAO, changes are caused by pregnancy toxemia, abortion, neoplasia, cystic fibrosis, infertility, uremia, bowel ischemia (235), and diabetes mellitus (31).

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