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FORMATION AND MODE OF ACTION OF FLAVOPROTEINS

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CONTENTS

INTRODUCTION

Knowledge of the biochemical and nutritional aspects of riboflavin (vitamin B_2) has accumulated steadily since its discovery and structural elucidation in the early 1930s. The nutritional significance of riboflavin is dictated by the fact that mammals do not synthesize it and, therefore, must rely on ingestion of foods that contain amounts sufficient to meet metabolic needs. The biological importance of riboflavin is demonstrated by the involvement of flavoproteins, at least peripherally, in every major metabolic pathway and

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in energy production via the respiratory chain. This review provides an overview of our current knowledge of (*a*) the structural variants of riboflavin found in nature, their uptake from foodstuffs, and conversion to active coenzyme forms; (*b*) the noncovalent interactions between the respective coenzymes and apoflavoproteins and their catalytic sequences; (*c*) examples of compounds present in certain foods detrimental to the function of key flavoenzymes; and (*d*) the function of representative flavoenzymes in catalysis of biological redox reactions.

NATURALLY OCCURRING FLAVINS

The flavins found in biological systems include riboflavin [7,8-dimethyl-10-(1'-D-ribityl) isoalloxazine], plus variants of this basic structure (Table 1, Figure 1). The known structural derivatives are thought to be formed by modification of the parent riboflavin structure, with the probable exception of coenzyme F_{420}.

Many microbes and plants synthesize riboflavin, using guanine as precursor and involving reactions similar to those in pteridine biosynthesis (6). Microbes that do not synthesize riboflavin but depend on it for growth have been used to develop sensitive assays for low levels of the vitamin (7). The amount of riboflavin obtained from intestinal flora of mammals is apparently insufficient to meet requirements, even though some vitamin can be absorbed from the large intestine.

Modifications Found on the Ribityl Sidechain

The predominant forms of riboflavin in tissues are the coenzymes FMN (riboflavin 5'-phosphate) and FAD (flavin adenine dinucleotide) (13). The coenzyme forms also account for most flavin in blood; however, as dietary

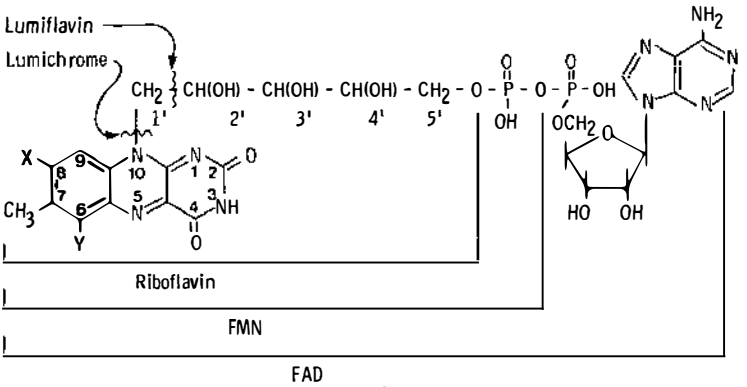


Figure 1 Structures of flavins.

intake of vitamin B_2 increases, higher levels of riboflavin are found (24). Several laboratories (8, 39, 128) have also found that fetal blood contains higher levels of riboflavin than does maternal blood. Human milk contains mainly FAD and FMN (63), whereas milk from other mammals (e.g. bovine) contains mostly riboflavin.

Other phosphorylated forms of riboflavin found in living organisms include riboflavin 2',5'-cyclic phosphate, riboflavin 5'-pyrophosphate, and a riboflavinyl glucoside phosphate (164, 247, 248), although the metabolic function of these forms remains to be elucidated.

Riboflavin analogues in which the 5'-hydroxymethyl group has been oxidized to an aldehyde (riboflavinal) or to a carboxylic acid (riboflavinoic acid) have been named schizoflavins, as they are isolated from *Schizophyllum commune* and are also found in edible Basidiomycetes (250). These flavins appear to participate in the accumulation of L-malate by *S. commune* and to stimulate hydrocarbon fermentation by a *Candida* species.

Glycosides of riboflavin are readily formed by *trans*-glycosidases found in rat liver, plants, and microbes (249, 272). Glycosyl donors include maltose, dextrin, starch, glycogen, and salicin for the riboflavinyl glucosides; lactose and melibiose for riboflavinyl galactosides; and sucrose for riboflavinyl fructoside and glucoside (272). These glycosides are not normally found in significant quantities but do occur in mouse tissues after the animals are loaded with riboflavin and ATP (266). Their metabolic significance remains to be elucidated.

Modifications Found on the Flavin Ring

A fraction of the total flavin of mammalian tissues is covalently bound to proteins. Since the discovery that treatment of mitochondrial preparations with proteolytic enzymes releases some of this covalently bound flavin (71), many flavinyl peptides have been characterized (231, 232). The majority of the covalent flavins are attached to cysteinyl, histidyl, or tyrosyl residues via the 8α-position of the isoalloxazine ring, with the exception of the 6-S-cysteinyl flavin of trimethylamine dehydrogenase (242, 243) (See Table 1 and Figure 1).

Recent work (2, 278) has shown that rat liver mitochondria contain four major polypeptides with covalently bound flavin; they have apparent molecular weights corresponding to those of succinate dehydrogenase, monoamine oxidase, sarcosine dehydrogenase, and an unidentified protein (M_r = 44,000). Only one flavinyl-polypeptide, identified as L-gulono-γ-lactone oxidase, was found in rat liver microsomes.

Other riboflavin derivatives modified on the dimethylbenzenoid ring include roseoflavin (8-dimethylamino flavin) and 6- and 8-hydroxy flavins.

Table 1 Naturally occurring flavins

Name	Structure X	Structure Y	Structure Other	Known sources	Ref.
Riboflavin	CH_3	H	▬	Multiple	131, 140
FMN	CH_3	H	5′-phosphate	Multiple	140, 269
FAD	CH_3	H	5′-ADP	Multiple	140, 269
Riboflavin 2′,5′-cyclic phosphate	CH_3	H	2′,5′-cyclic phosphate	*Aspergillus oryzae, Rhizopus oryzae*	247
Schizoflavins	CH_3	H	5′-aldehyde 5′-carboxylic acid	*Schizophyllum commune*	250
Nekoflavin	Unknown (altered ribityl sidechain?)			Cats' eyes, liver	151, 152
Riboflavinyl glycosides	CH_3	H	5′-glycoside	Multiple	272
6-S-Cysteinyl-FMN	CH_3	-S-cysteinyl	5′-phosphate	Trimethylamine dehydrogenase (bacterium W 3A1)	242, 243
8α-S-Cysteinyl-FAD	CH_2-S-cysteinyl	H	5′-ADP	Monoamine oxidase (outer mitochondrial membrane), Cytochrome C_{553} (*chlorobium*)	48, 125
8α-N^1-Histidyl-FAD	CH_2-N^1-histidyl	H	5′-ADP	β-Cyclopiazonate oxidocyclase (*Penicillium cycloplum*), L-gluconо-δ-lactone oxidase (rat liver), thiamin dehydrogenase (unidentified soil bacterium), L-galono-γ-lactone oxidase (yeast), cholesterol oxidase (*schizophyllum*)	48, 123, 124, 126

8α-N^3-Histidyl-FAD	CH_2-N^3-histidyl	H	5′-ADP	D-6-Hydroxynicotine oxidase (*Arthrobacter oxidans*), sarcosin dehydrogenase (*Pseudomonas*) Fumarate reductase, chlorine oxidase Succinate dehydrogenase	48, 193, 224, 261, 271
8α-O-Tyrosyl-FAD	CH_2-O-tyrosyl	H	5′-ADP	*p*-Cresol methylhydroxylase (*Pseudomonas*)	167
6-Hydroxy-FMN	CH_3	OH	5′-phosphate	Glycolate oxidase (pig liver)	158
6-Hydroxy-FAD	CH_3	OH	5′-ADP	Electron-transferring flavoprotein (*Megasphaera elsdenii*)	158
8-Hydroxy-FAD	OH	H	5′-ADP	Electron-transferring flavoprotein (*Megasphaera elsdenii*)	68
Roseoflavin	$N(CH_3)_2$	H	▬	*Streptomycetes davawensis*	114, 200
Coenzyme F_{420}	7,8-didemethyl-8-hydroxy	H	5-carba-5-deaza-5′-phospholactyldiglutamyl	*Methanobacterium* ▬	50
Formylmethylflavin	CH_3	H	10-CH_3CHO	Photodegradation of riboflavin	26
2′- and 4′-lectoriboflavin	CH_3	H	2′- or 4′-lecto	Photodegradation of riboflavin	26
Lumiflavin	CH_3	H	N^{10}-CH_3	Photodegradation of riboflavin (pH > 10)	26
Lumichrome	CH_3	H	N^{10}-H	Photodegradation of riboflavin (pH < 10)	26
2′,5′-anhydroriboflavin	CH_3	H	2′,5′-anhydro	Strong acid	47

Roseoflavin is formed from riboflavin by *Streptomyces davawensis* (153). It is readily phosphorylated by the rat liver flavokinase (177), is a bacteriostatic agent (114), has an antagonistic effect upon the growth-promoting activity of riboflavin for rats (200), and is toxic for poultry (70). Less is known about the 6- and 8-hydroxy flavins; the latter may not be a riboflavin antagonist in animals because it is not phosphorylated by flavokinase nor does it inhibit the phosphorylation of riboflavin (177).

The coenzyme F_{420} isolated from *Methanobacterium* strain M.o.H. differs considerably from riboflavin, having both the ring substituents shown in Table 1 and Figure 1 and a lactyl diglutamyl sidechain that extends beyond the ribityl phosphate at position 10 (50); hence, it may not qualify as a true riboflavin derivative. The related synthetic analogue, 5'-deazariboflavin (191), is a potent anticoccidial riboflavin antagonist (70).

Chemical and Biological Degradation Products

Photodegradation of the ribityl sidechain yields potentially antivitaminic (160) molecules such as formylmethylflavin, lumiflavin, and lumichrome (26). The losses of the vitamin can be extensive; for example, exposure of milk to sunlight for 4 h destroys 71% of the riboflavin (89). Evidence for photodegradation in vivo has been obtained for newborns during phototherapy for hyperbilirubinemia (87, 216). Heating samples that contain riboflavin in acid may yield 2',5'-anhydro-riboflavin (47) and, in base, 1,2-dihydro-6,7-dimethyl-2-keto-1-D-ribityl-3-quinoxaline carboxylic acid plus urea (246). The flavin coenzymes are especially susceptible to both chemical and enzymatic hydrolysis to form riboflavin. Furthermore, groups on the ribityl hydroxyls can migrate or cyclize to produce contaminants such as riboflavin 4'-phosphate, riboflavin 4',5'-cyclic phosphate, or the 4',5'-cyclic phosphate of FAD (164).

The major excretory flavin catabolite in mammals is riboflavin. Much smaller amounts of sidechain degradation products, such as lumichrome, 10-formylmethylflavin, 10-(2'-hydroxyethyl) flavin, and ring-altered compounds, are found and may in part be formed by intestinal microorganisms (163, 254). 8α-Flavin peptides are excreted both in an unaltered form and in an altered form with modifications of the amino acid moiety (decarboxylation) and of the flavin (presumably some sidechain cleavage) (34). In addition, 8α-S-(cysteinyl)riboflavin has been found to be converted to its sulfoxide and sulfone forms (34). Some degradation of the 8α-(amino acid) flavins to 8-formyl flavin is expected, based on model chemical observations (34, 232).

TRANSPORT AND METABOLISM

Uptake and Excretion

Flavins are consumed primarily in complexes with food proteins. Riboflavin is obtained directly from foods rich in the underivatized vitamin, such as bovine milk and eggs, upon gastric acidification and gastric and intestinal proteolysis. The coenzymatic forms are similarly released from flavoenzymes and are hydrolyzed to riboflavin by relatively nonspecific pyrophosphatases and phosphatases. Flavin glycosidases are cleaved by glycosidases in the gut. Some portion of the 8α-(amino acid) riboflavins, released by proteolysis of flavoproteins containing covalently bound flavin, is absorbed, although neither 8α-(cysteinyl)- nor 8α-(histidyl)-riboflavin has significant vitaminic activity (163).

Riboflavin is absorbed by man in a site-specific (proximal small intestine), saturable transport system. The kinetics of uptake of ingested riboflavin have been studied by following the concentrations of this molecule in plasma and urine (106). Uptake by normal adults was rapid and proportional to dose, until leveling off at 25 mg. Ingestion of riboflavin with other food increases the amount taken up, probably by decreasing the rate of intestinal transit (102, 106). It has also been observed (17) that riboflavin absorption is increased by exposure to γ-radiation, which decreases the rate of gastric emptying. The presence of bile salts increases uptake (106, 155); furthermore, impaired riboflavin absorption is observed in children with biliary obstruction (107). Although bowel disorders might generally decrease uptake, children with gastroenteritis have normal levels of riboflavin (4). Other vitamins and most drugs examined to date do not appear to inhibit transport (106, 137).

The characteristics of uptake change with age. The urinary excretion of riboflavin by newborn infants is slow and prolonged, reflecting sustained intestinal absorption (101, 106). The cumulative amount excreted in urine, however, is similar to that for older infants (6% of the dose). The urinary recovery increases to 12% by 40 years of age, reflecting primarily changes in intestinal transit rate (106). Some absorption of riboflavin from the large intestine can occur (237). The amount of riboflavin that appears in blood after instillation through a catheter into the large intestine was, at most, 25% of that from an oral dose.

Results from experiments on riboflavin absorption by rat intestine in vitro have been contradictory (36, 106, 219). Some of the confusion may arise from absorption at low doses by specialized processes and at higher concentrations by passive diffusion. The active transport of riboflavin, measured with isolated perfused segments of rat small intestine, occurs maximally at

the lowest riboflavin concentration examined, 0.2 μM (168). Absorption by intestine in vivo is saturable and Na^+-dependent ($K_t = 3$ μM) (212). Recent studies (W. B. Im, A. H. Merrill Jr., R. G. Faust, unpublished observations) of riboflavin uptake by purified brush border membrane vesicles from rat jejunum and kidney cortex indicate that a Na^+-dependent transport system, like those for glucose and amino acids (94), may be present in the kidney.

Few studies have been conducted on the mechanisms of riboflavin uptake from blood by tissues. Injection of flavin coenzymes, double-labeled in the isoalloxazine ring ([^{14}C]) and phosphate ([^{32}P], FMN) or adenine ([^{3}H], FAD) groups, yielded incorporation of only radiolabel from the isoalloxazine ring into coenzymes in the liver (195). Hence, these molecules are rapidly hydrolyzed in the body, and presumably, it is free riboflavin that traverses cell membranes. Riboflavin antagonists such as galactoflavin and isoriboflavin, which are neither substrates nor inhibitors for flavokinase (164, 177, 210), may inhibit cellular transport of riboflavin. The isolated choroid plexus actively accumulates riboflavin against a concentration gradient with an apparent K_t of 78 μM (238).

Riboflavin is excreted unchanged in the urine and, in small amounts, in feces. Renal excretion in man involves glomerular filtration, tubular secretion, and tubular reabsorption (106). Renal clearance is relatively constant at low serum flavin concentrations and increases to a maximum at flavin plasma levels of 1 μg/ml (105). Probenecid, which inhibits a number of renal transport processes, also inhibits renal clearance of riboflavin in human subjects (103, 106). Various drugs, natural substances, and physiological conditions influence the urinary excretion of riboflavin (106). Some biliary excretion as well as recycling by enterohepatic circulation probably occurs; however, for man, this route accounts for a negligible portion of the flavin excreted (106).

Association with Binding (Carrier) Proteins

Because riboflavin is primarily present in biologic fluids and tissues in coenzymatic forms, it is presumed that apo-flavoenzymes are responsible for the binding and retention of this vitamin in the body and that excess free riboflavin should be excreted. Although subject to verification by further studies, the available data on flavin levels in tissues support these assumptions.

Since it appears that riboflavin, not the coenzymes, is the major form of flavin transported into cells, it is of interest to know the extent of its binding by plasma proteins. The binding of 1.3 μM riboflavin to whole plasma, individual plasma proteins at physiological concentrations, and reconstituted plasma has been measured by equilibrium dialysis (104). Binding

by albumin (K_D = 0.77 mM at 37 C) accounted for most of the riboflavin bound in whole plasma (42%), although other protein fractions (β-globulin > α-globulin > γ-globulin > fibrinogen) also bound significant amounts. Analysis of the riboflavin content of human plasma proteins resulted in the following distribution: β-globulin > α-globuin > γ-globulin > β-lipoprotein > albumin (60). Although some of the riboflavin measured in the latter study may have been in coenzymatic forms, riboflavin binding by a substantial subfraction (1%) of the immunoglobulin (IgG) in normal human plasma has been demonstrated (176). These IgG's and other plasma proteins may function when albumin is not available, for example, in hypoanalbuminemia, in which there is a compensatory increase in other plasma proteins. In addition, the binding of riboflavin to albumin may be due to contaminants or subspecies of albumin, since it has been reported that riboflavin is associated mainly with the cathodic edge of the albumin electrophoretic band (32).

Other riboflavin-binding immunoglobulins have been found, including a monoclonal IgA produced by mouse plasmacytoma MOPC-315 (51) and a monoclonal IgG produced by a patient with multiple myeloma (53). Interestingly, this patient did not show signs of riboflavin deficiency, despite the very tight binding of riboflavin by IgG (53, 204).

Laying hens have two specialized riboflavin-binding proteins, which apparently have the same polypeptides but differ in carbohydrate and phosphate composition (179, 199, 211). One is produced by the oviduct and is incorporated into egg white (54); the other is synthesized in the liver in a precursor form (62), and transported via blood (16, 62, 174, 175) to the ovary for incorporation into the egg yolk (199). The biosynthesis of both proteins is induced by estrogens (188). These riboflavin-binding proteins are required for storage of riboflavin in the egg for use by the developing embryo (276). Avian riboflavinuria, in which laying hens lose riboflavin by glomerular filtration and produce eggs that do not mature beyond 10–14 days of incubation, has been shown to be caused by a single genetic defect that yields nonfunctional riboflavin-binding proteins (276).

Riboflavin-binding proteins have also been implicated in mammalian reproduction. The plasma of pregnant cows contains a riboflavin-binding protein absent in plasma from nonpregnant animals (172). This protein, purified to homogeneity, had a low molecular weight and bound riboflavin very tightly. A similar protein has been more recently identified and purified from pregnant rats (186). Available evidence indicates that such high-affinity, riboflavin-binding proteins are also found in human fetal (cord) blood (176) and may explain the riboflavin found in the uterine secretions of pigs (187) during portions of the estrus cycle. A factor in the plasma of pregnant rats was found to cross-react with antibodies prepared against

avian riboflavin-binding proteins (185). Injection of these antibodies into pregnant rats resulted in fetal resorption. If this cross-reacting factor is the mammalian riboflavin-binding protein, these results strongly suggest that such carrier proteins are crucial for mammalian, as well as avian, reproduction. Riboflavin nutriture is of special importance during pregnancy. Inadequate consumption of this vitamin by pregnant laboratory animals yields offspring with a variety of congenital malformations (265). Although these effects have not been confirmed for humans, the frequent occurrence of riboflavin deficiencies among certain pregnant women (38, 221) warrants consideration. It is also possible that a number of drugs that influence riboflavin status, including phenothiazine derivatives, boric acid, and possibly oral contraceptives, pose dangers during pregnancy (216).

Conversion to Coenzymatic Forms

The first step in the utilization of riboflavin by tissues is its conversion to the coenzyme FMN by flavokinase, or riboflavin kinase (ATP:riboflavin 5'-phosphotransferase, EC 2.7.1.26). Flavokinase is found in the cytosol of numerous organisms (164), with especially high levels in liver and intestine of animals (171). Microbial, plant, and partially purified mammalian flavokinases have been extensively studied (for a review see Ref. 164).

Flavokinase has recently (177) been purified to apparent homogeneity from an animal source (rat liver) by using flavin affinity chromatography (175), and its kinetic mechanism has been studied (170). The purified enzyme has a nearly absolute specificity for ATP or dATP; a divalent cation is required for maximal activity. Flavokinase interacts with a large number of riboflavin analogues. Modifications at positions 3, 5, 6, or 7 of the 7,8-dimethylisoalloxazine ring decrease binding and/or activity with flavokinase. Some substitutions at position 8 are readily accommodated; for example, roseoflavin is comparable to riboflavin as substrate. The 8α-(amino acid) riboflavins are poor substrates and inhibitors. Riboflavin analogues that lack the ribityl sidechain [e.g. lumiflavin, lumichrome, and 10-(ω-hydroxyalkyl)-7,8-dimethylisoalloxazines] are good inhibitors, whereas isomers of the ribityl sidechain are generally poor substrates or inhibitors.

Several factors influence the activity of flavokinase (216). In riboflavin deficiency, the tissue levels of flavokinase decrease by 11–45% for the rat (171, 218); upon refeeding riboflavin, the activities in liver and intestine recover rapidly, reaching normal levels within 6–8 h (171). This recovery precedes the dramatic weight gain and general improvement of deficient animals. Evidence for hormonal regulation of flavokinase activity has been complicated by interfering levels of phosphatases, which mask activity by hydrolyzing FMN (159). Nonetheless, it appears that ACTH administra-

tion increases the rate of in vivo synthesis of FMN from riboflavin in liver and kidney, presumably by increasing flavokinase (56, 214). Co-administration of aldosterone and riboflavin analogues [7,8-dimethyl-10-formylmethyl isoalloxazine or 7,8-dimethyl-10-(2'-hydroxyethyl) isoalloxazine] decreased the formation of renal FMN while increasing the urinary output of Na^+, compared to aldosterone alone (253); hence, it was concluded that stimulation of FMN (and FAD) synthesis by aldosterone may be a causative factor in the increased reabsorption of Na^+. Thyroid hormones also change the FAD content in liver; this may be due to regulation of flavokinase activity (214). The increase in flavokinase activity induced by thyroxine is not blocked by actinomycin D (214, 217). This finding, plus the observation that an unidentified small molecule (possibly riboflavin) in liver extracts from hyperthyroid animals apparently stabilizes the enzyme, suggests that thyroxin increases activity by modifying flavokinase degradation.

The enzyme that catalyzes the synthesis of FAD from FMN, FAD synthetase (ATP : FMN adenylyltransferase, EC 2.7.7.2), has not yet been purified to homogeneity. It is widely distributed in tissues (164) and is mostly located in the cytosol (45). Studies of the partially purified enzyme from rat liver have demonstrated an apparently absolute specificity for ATP (162) (a divalent cation is also required). A variety of flavin 5'-phosphates are substrates (162), including FMN analogues with modifications in the flavin ring or sidechain. Interestingly, isoriboflavin inhibits activity with FMN, which may explain its antagonistic effect in vivo (161, 164). The FAD synthetase activity of various tissues has been found to increase in riboflavin deficiency (55), in hyperthyroidism (214), and, perhaps, in cancer (215).

The biosynthesis of proteins that have covalently bound flavin has been studied on a molecular level only with a microbial system. The holoenzyme of D-6-hydroxynicotine oxidase can be formed in a cell-free translation system by using polysomes from nicotine-induced cells of *Arthrobacter oxidans* (74, 75). Because [^{3}H]adenine-labeled FAD was incorporated into the nascent polypeptide, it appears that covalent attachment of the coenzyme occurs co-translationally. It is likely that FMN and FAD are also utilized in the biosynthesis of the mammalian flavoproteins, since the 8α-(amino acid) riboflavins are not substrates for flavokinase (177). At physiological levels, neither 8α-S-(cysteinyl)riboflavin nor 8α-N^3-(histidyl)-riboflavin adequately replaces or antagonizes riboflavin in rats (34). The covalent incorporation of flavins into proteins of rat liver outer mitochondrial membranes is inhibited by cycloheximide (144), but only after a lag of approximately 2 h. Hence, for some proteins, biosynthesis of the apoenzyme appears complete prior to covalent attachment of the flavin. Thyroid hormones may be involved in regulating the formation of covalently bound

flavins; administration increased the level of incorporation of radiolabeled riboflavin into covalently bound forms in rat liver and brain (205).

The enzymes involved in the hydrolyses of FMN and FAD have been characterized in impure forms. The FMN phosphatases are widespread (166), have both acidic and basic pH optima (164), and, at least for the acidic phosphatase of liver, show a strong substrate preference for FMN (166). Some catalyze the transphosphorylation of riboflavin (279), although this reaction is probably of little physiological importance compared to that catalyzed by flavokinase. The FMN phosphatase activities of rat liver and intestine did not change significantly in riboflavin deficiency (171), but the activity in liver was increased by administration of thyroid hormones. Enzymes that catalyze FAD hydrolysis (FAD pyrophosphatase, EC 3.6.1.18) are also found in most tissues (164). The FAD hydrolytic activity is elevated in the serum of patients with liver disease and in some rat liver tumors (282).

COENZYME BINDING BY APOFLAVOPROTEINS

The reversible resolution of the flavin coenzymes from their respective apoenzymes has been the focus of studies in many laboratories since the demonstration by Theorell (252) of the resolution and reconstitution of the FMN coenzyme from yeast NADPH-oxidoreductase (Old Yellow Enzyme). Many FAD- and FMN-containing enzymes have been investigated; values have been determined for dissociation constants ranging from 10^{-10} to 10^{-5} M. In most cases, the flavoenzyme under investigation becomes amenable to reversible resolution when conditions are found to stabilize the resultant apoenzyme. A recent review (91) outlines the methods used successfully. Binding of flavin analogues to various apoenzymes has been employed as a probe of noncovalent flavin-protein interactions. To date, the major number of such studies have dealt with the binding of riboflavin analogues to the riboflavin-binding protein from egg white (35, 189, 263) and the binding of FMN analogues to flavodoxins (11, 49, 64, 156), NADPH-cytochrome *c* reductase (5), NADPH diaphorase (222), Old Yellow Enzyme (1, 255), and liver pyridoxamine phosphate oxidase (115, 116, 173).

Studies on the FAD-requiring apoenzymes are not as numerous since FAD analogues are more difficult to synthesize chemically, though both flavin-derivatized (33) and adenosyl-replaced (165) analogues have been tested with porcine D-amino acid oxidase and the former with adrenodoxin reductase (138). The development of the widely specific flavokinase/FAD-synthetase complex from *Brevibacterium ammoniagenes* (240) promises to rectify this situation and provides the means to prepare a large number of FAD analogues with ease.

One important result of the studies completed thus far is the finding that apparently the redox potential differences found among various flavin analogues in solution also exist when they are bound to their coenzyme binding site on the apoenzyme. The first demonstration of this was the finding (1) that the energy of the long wavelength transition of the charge transfer complex of phenolic compounds with Old Yellow Enzyme correlated directly with the oxidation-reduction potential of a number of FMN analogues that had been reconstituted with the apoenzyme. Similarly, a correlation of maximal catalytic activity with oxidation-reduction potential of the reconstituting flavin analogue is known for the enzymes pyridoxamine phosphate oxidase (173) and adrenodoxin reductase (138). It should be pointed out that the validity of this correlation is for enzyme systems where either reduction or reoxidation constitutes the rate-limiting step in catalysis. For example, the replacement of FAD by 7,8-dichloro-FAD in D-amino acid oxidase (which differ in redox potential by 80 mV) does not affect the maximal velocity since the rate-limiting step is product dissociation (148).

Flavin-binding studies that use analogues in which the sidechain has been modified show the binding process to be more sensitive to alterations in the sidechain, in general, than to ring modifications. An example of this is the relatively high specificity exhibited by apoenzymes for their respective flavin nucleotide (FMN or FAD). Lumiflavin, in which the ribityl sidechain is replaced by a 10-methyl substituent, has a low affinity for practically all apoflavoproteins tested. The stereochemistry of the ribityl chain has been shown in a number of instances to be critical for holoenzyme reconstitution. An early report (113) showed that synthetic L-riboflavin exhibited antivitamin activity in vivo. Binding of flavin analogues varying in the sidechain showed a marked dependence on chain length (5, 33, 64). The presence of a 2'-hydroxyl group is important in the restoration of catalytic activity of apo-D-amino acid oxidase (33), even though analogues deficient in this group are tightly bound.

To date, our best insight into the nature of flavin-protein interactions comes from the X-ray crystallographic studies (142, 267, 268) on the flavodoxins (low potential FMN-containing electron carriers), on glutathione reductase (an FAD-containing enzyme) (227), and on *p*-hydroxybenzoate hydroxylase (an FAD-containing enzyme) (274). The FMN moiety in flavodoxin is in an extended conformation with the ribityl phosphate sidechain penetrating into the protein molecule (142, 267, 268). Numerous hydrogen bonds are apparent in the interactions of the phosphate oxygens and of the ribityl hydroxyl groups with the protein, though no electrostatic interactions are apparent between the phosphate and any basic amino acid groups. The isoalloxazine ring is largely shielded from the solvent, with only the dimethylbenzenoid portion exposed to the solvent milieu. Thus, flavin ana-

logues with bulky substituents at position 8 can be tightly bound (192, 235). Additional binding studies show the same situation exists for Old Yellow Enzyme (1) and for pyridoxamine-5'-phosphate oxidase (173); however, apparently the opposite is true for the riboflavin-binding protein from egg white (35, 263).

Although the X-ray data on glutathione reductase is not yet at the level of that for the flavodoxins, conclusions regarding the binding of FAD can be made. One end (presumably dimethyl benzenoid) is in a hydrophobic milieu, whereas the other end "forms an electron-dense bridge to the backbone of the other subunit" (227). The geometry of the FAD-binding site is similar to that of other nucleotide-binding proteins: The adenine ring is "stretched" away from the isoalloxazine ring while the linking pyrophosphate moiety interacts strongly with the polypeptide backbone. For *p*-hydroxybenzoate hydroxylase (274), the dimethylbenzene ring also appears to point toward the solvent. Furthermore, positions 2 and 3 of the ureido-ring are hydrogen bonded to protein groups. The N-5 edge of the flavin ring is oriented toward the substrate *p*-hydroxybenzoate. It appears almost certain from studies of model compounds and flavoprotein structures that interactions such as hydrogen bonding at the pyrimidine ring and stacking of the 7,8-dimethylisoalloxazine ring and aromatic amino acids are responsible for the alterations in redox chemistry obtained upon binding the flavin to an apo-flavoprotein.

INFLUENCE OF NUTRITIONAL STATUS ON FLAVOPROTEINS

The available literature on the influence of nutritional status on flavoproteins is so vast that a complete review cannot be attempted here due to space limitations. The physiological responses to inadequate dietary intake of riboflavin are numerous and, depending on the extent and duration of the deprivation, severe (69, 213). Typically, growth is stunted and a variety of lesions appear, including changes in skin, degeneration of tissues, and teratogenic effects that may result from effects on multiple metabolic pathways.

However, remarkable differences occur in the relative depletion of particular flavoproteins and in the results for different tissues (25). Lipid metabolism is altered dramatically in ariboflavinosis and there are corresponding alterations in cell structure and function (85). Because flavoproteins are involved in the metabolism of other vitamins, synergistic effects are expected upon consumption of suboptimal levels of several vitamins. Riboflavin nutriture also influences the microsomal drug-metabolizing enzyme

systems (28). Reports are also becoming more common on the treatment of metabolic diseases, such as recessive congenital methemoglobinaemia (112, 234) and glutaric aciduria (18), by administration of riboflavin.

A number of toxic compounds found in dietary foodstuffs inhibit flavoenzyme activities. The component responsible for the animal toxicity of *Indigofera endecaphylla* Jacq. was identified in 1954 as 3-nitropropionic acid (182), which subsequently was found to be widely distributed in nature (3). In its dianionic form, 3-nitropropionate is isoelectronic with succinic acid and was found to be an irreversible inhibitor of succinate dehydrogenase (3). Later studies (40) showed that inhibition was due to the formation of 3-nitroacrylate (the product of the succinate dehydrogenase-catalyzed oxidation of 3-nitro-propionate), which inactivates the enzyme by reacting with a thiol group at the substrate binding site. Thus, 3-nitropropionate irreversibly inhibits mitochondrial respiration by functioning as a "suicide" substrate of the flavoenzyme succinate dehydrogenase.

Two other naturally occurring inhibitors of flavin enzymes have been found that interfere with essential metabolic pathways. The amino acid analogue hypoglycin A is present in the unripe arillus of the aekee fruit (*Blighia sapida*), which when ripe serves as a dietary staple in Jamaica. Ingestion of the unripe fruit leads to the often fatal Jamaican vomiting sickness (251). Hypoglycin drastically reduces the flavoenzyme acylcoenzyme-A (CoA) dehydrogenase activity, consistent with the notion that the compound interferes with fatty acid β-oxidation (89). The active agent is methylenecyclopropylacetyl-CoA, a hypoglycin metabolite (118).

The naturally occurring compound Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is a derivative of Sennosides, a name applied to certain glycosides that occur in plants of the genus *Cassia* (117). This compound has been found to be a competitive inhibitor (K_i=2 μM) of mitochondrial NADH dehydrogenase (119) and other enzymes such as mitochondrial transhydrogenase, DT-diaphorase, and lactate and malate dehydrogenases.

Antagonistic action between drug therapy and nutritional intake can occur. The development of drugs based on the site-specific irreversible inhibition of target enzymes is an active area of research. A flavoenzyme that is the object of such activity is monoamine oxidase, which functions to regulate the level of biogenic amines in vivo. Antidepressant drugs that irreversibly inhibit monoamine oxidase were found to have a serious side reaction known as the "cheese effect" (14). The inhibition of the enzyme led to an interference with the metabolism of ingested amines; some patients under treatment with antidepressant drugs who ate tyramine-containing foods such as cheese showed a marked rise in blood pressure, which sometimes proved fatal. The development of deprenyl, an irreversible monoamine oxidase inhibitor without the adverse "cheese effect" side reaction

(129), alleviated the antagonistic reaction between this drug therapy and nutritional intake by a mechanism currently under investigation.

FLAVOPROTEIN REACTIONS AND MECHANISMS

Survey of Flavoenzymes

Flavoproteins are remarkable in the diversity of chemical reactions they catalyze. As shown in Table 2, their functions include 1-electron transfers, dehydrogenation reactions (both pyridine nucleotide dependent and independent), reactions with sulfur-containing compounds, hydroxylations, oxidative decarboxylations, dioxygenations, and reduction of O_2 to hydrogen peroxide. Each reaction is considered below. Table 2 catalogs most known mammalian flavoenzymes. While concentrating on the mammalian enzymes, we also consider a variety of bacterial enzymes whenever their mechanisms provide a groundwork for understanding the mammalian enzymes.

The goals of research on flavoproteins can be considered as threefold. First, the types of reactions that free flavins are capable of carrying out and their chemical mechanisms must be delineated. A large number of studies have been published along these lines, often using model flavin compounds, and the reader is referred to recent review articles and symposia for details beyond the scope of this review (21, 22, 80, 83, 109, 202, 230, 262, 280). A second goal, and the one to which this section is largely devoted, is to determine which reactions occur biologically and what the chemical and kinetic mechanism is for each physiologic reaction. Considerable interest during the past 10 years has focused on covalent flavin-substrate or flavin-oxygen adducts as intermediates in the electron transfer process. A third goal (and one as yet largely unrealized) is to determine how an apoprotein stabilizes one reaction pathway for a particular flavoenzyme. This function of apoprotein is illustrated by the 10,000-fold increase in reactivity of oxygen with reduced flavin when the coenzyme is bound in the enzyme *p*-hydroxybenzoate hydroxylase. Progress in describing the immediate protein environment of bound flavin is being made by using several techniques, including electron paramagnetic resonance (EPR) as well as visible absorption spectroscopy (see below), X-ray crystallography (142, 227, 267, 268, 274), chemical and photochemical derivatization of the active site (257), ^{13}C-labeled NMR (139, 184), and chemically modified flavins to probe the steric and charge requirements of the flavin binding site (173). Recent reviews and symposia have considered specific aspects of flavoprotein reaction mechanisms, including flavoprotein dehydrogenases (275), monoxygenase (58, 147), oxidases (20), complex flavoenzymes (i.e. those containing one or more redox-active groups in addition to the flavin) (78),

Table 2 Redox-active mammalian and selected bacterial flavoproteins classed according to reaction type

Reaction type/enzyme	Example electron donor	Example electron acceptor	Comments	Ref.
1-electron transferase				
Bacterial flavodoxins	—	—	—	157
FMN of NADPH-cytochrome P-450 reductase[a]	FAD of same enzyme	Cytochrome P-450	Microsomal	259, 275
ETF	Fatty acyl-CoA dehydrogenase	ETF-ubiquinone oxido-reductase	Mitochondrial	72, 157
ETF-ubiquinone reductase[a]	ETF	Coenzyme Q, respiratory chain	Iron-sulfur flavo-protein, mito-chondrial	223
Dehydrogenases				
Pyridine nucleotide dehydrogenases or reductases				
Cytochrome b_5 reductase	NADH	Cytochrome b_5	Microsomal	141, 244, 275
Methemoglobin reductase	NADH	Methemoglobin	Erythrocyte	141, 275, 285
FAD of NADPH-cytochrome P-450 reductase[a]	NADPH	FMN, same enzyme	Microsomal	95, 259, 275
Methylene tetrahydrofolate reductase	NAD(P)H	N^5,N^{10}-Methylene tetra-hydrofolate	—	23, 46
Adrenodoxin reductase	NADPH	Fe_2S_2* of adrenodoxin	Mitochondrial	92, 93, 127, 133
NADH dehydrogenase[a]	NADH	Iron sulfur center, same enzyme	Mitochondrial iron-sulfur flavoprotein	78
Menadione reductase	NADPH	Menadione	—	130
Vitamin K-epoxide reductase	NADH	Vitamin K 2,3-epoxide	Microsomal	228, 273
Xanthine dehydrogenase/oxidase[a]	Xanthine	NAD^+/O_2	Contains iron-sulfur, molybdenum	9, 19, 276
Iodotyrosine deiodinase	NADPH	Mono- and diiodotyrosine	Bovine thyroid	220
Superoxide-forming oxidase	NADPH	O_2	Human neutrophils	43
Azoreductase	NAD(P)H	Some azo dyes	Liver cytosol	88

Table 2 *(Continued)*

Reaction type enzyme	Example electron donor	Example electron acceptor	Comments	Ref.
Nitrate reductase	NAD(P)H	Nitrate	—	196
NADH-FMN oxidoreductase	NADH	FMN, as substrate	—	98
Non-pyridine nucleotide dehydrogenases				
Succinate dehydrogenase[a]	Succinate	Fe_2S_2*, same enzyme	Mitochondrial	78
Fatty acyl-CoA dehydrogenase (3 types)-	Fatty acyl-CoA's	ETF	Mitochondrial, 3 acyl chain length-dependent enzymes	72
L-Glycerol phosphate dehydrogenase	L-Glycerol phosphate	ETF	Mitochondrial	78
Dihydroorotate dehydrogenase	Dihydroorotate	Coenzyme Q	Mitochondrial[b]	59, 122, 180
Choline dehydrogenase (2 forms)				
mitochondrial	Choline	Coenzyme Q	—	78, 229
cytosolic	Choline	NAD^+	—	78, 229
Dimethyl glycine dehydrogenase	Dimethylglycine	—	Mitochondrial	61
Sarcosine dehydrogenase	Sarcosine	Electron transport chain	Mitochondrial	61
Trimethylamine dehydrogenase	Trimethylamine	Fe_4S_4*, same enzyme	Bacterial	242
Pyridine nucleotide-disulfide oxide reductases[a]				
α-Ketoacid dehydrogenase	—	—	—	203
Lipoamide dehydrogenase	NADPH	Enzyme disulfide	Mitochondrial	149, 275
Glutathione reductase	NADPH	Enzyme disulfide	—	31, 197
Thioredoxin reductase	NADPH	Enzyme disulfide	—	135, 275
Dehydrogenase-oxygen reductase reactions				
Flavoprotein oxidases [$O_2 \rightarrow H_2O_2$]				
D-Amino acid oxidase	Glycine	O_2	Peroxisomes	79, 97, 120, 169
L-Amino acid oxidase	L-Tryptophan	O_2	Peroxisomes, mitochondria	44, 79, 169
Glucose oxidase	D-Glucose	O_2	—	281

Glycollate oxidase	Glycollate	O_2	—	99, 158, 236
Aldehyde oxidase[a]	R-CHO	O_2	Contains iron and molybdenum	19
Monoamine oxidase[a]	Serotonin	O_2	Outer mitochondrial membrane	42, 284
Polyamine oxidase	Spermidine	O_2	—	84
L-Gulono-γ-lactone oxidase	L-Gulono-γ-lactone	O_2	Rat liver microsomes	123
Pyridoxamine 5′-phosphate oxidase	Pyridoxamine 5′-phosphate	O_2	—	116
Sulfhydryl oxidase	2 RSH	O_2	Seminal vesicle secretions	198
Flavoprotein monoxygenases [$\frac{1}{2}O_2 \rightarrow H_2O$]				
Internal—$R\text{-}\underset{\displaystyle OH}{\underset{|}{C}}H\text{-}COO^- \rightarrow R\text{-}CO_2 + CO_2$				
Lactate monoxygenase	Lactate	O_2	Bacterial	58, 147
External—(R–H → R-OH)				
Bacterial hydroxylases (at least 8 types)	NADPH	O_2	—	58, 147
Microsomal FAD-containing monoxygenase	NADPH	O_2	Microsomal	
Bacterial luciferase	$FMNH_2$	O_2	Light is a reaction product, bacterial	77
Kynurenine 3-hydroxylase	NADPH	O_2	Outer mitochondrial membrane	147, 190

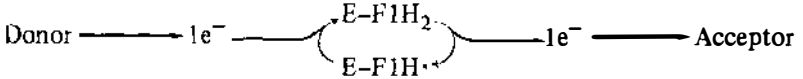
[a] These flavoenzymes are complex flavoenzymes, defined here as any flavoprotein containing one or more additional redox-active groups. When possible, the redox-active group that acts as a direct electron acceptor/donor from the flavin is specified in the adjacent columns. For example, although the succinate dehydrogenase complex catalyzes reduction of coenzyme Q, the direct electron acceptor from flavin is a 2 iron, 2 labile-sulfur center (Fe_2S_2*); thus Fe_2S_2 rather than coenzyme Q is specified as the electron acceptor to more closely define the role of the flavin. When the direct acceptor is not known with certainty, the entire enzyme complex is specified. NADPH-cytochrome P-450 reductase is classified under two headings to describe the roles of each of its two flavins. ETF-ubiquinone reductase is tentatively classed under 1-electron transferase, but may be subject to reclassification should further studies demonstrate other functions. Among the enzymes that might be flavoproteins, but that were not listed, are O-acylthiamin oxidase (108), histaminase (111), "demethylase" (181), N-alleyl-lysinase (201), and 5-hydroxytryptophan pyrrolase (256).

[b] The flavin content of mammalian enzymes has not been firmly established.

and flavodoxins (157). This section provides a general overview of flavoprotein mechanisms, emphasizes recent advances, and, where possible, relates flavoenzyme function with structure.

One-Electron Transferases

Flavins can exist in three oxidation-reduction states: oxidized, semiquinone (1-electron reduced), and fully reduced (2-electron reduced). Perhaps the simplest reaction catalyzed by flavoenzymes is a 1-electron transfer, which utilizes only two oxidation-reduction states. Catalysis of a 1-electron transfer by a flavoprotein is exemplified by the bacterial flavodoxins that accept an electron from a low redox potential center and donate an electron to a higher potential 1-electron acceptor (157). These FMN-containing enzymes utilize the fully reduced and neutral semiquinone, but not the oxidized form in their cycle of electron transfers:



These systems operate at negative midpoint potentials, ranging from –335 to –450 mV for the $FlH\cdot/FlH_2$ couple (157). The protein, in addition to dictating donor/acceptor specificities, modulates the properties of the bound flavin: Binding of the flavin stabilizes the semiquinone form by 6–8 kcal (10), or stated in another way, the protein raises the oxidation-reduction potential of the Fl/FlH· couple. The protein exerts this effect by preferentially binding the semiquinone state; the binding site provides a better geometric and ionic "fit" for the flavin semiquinone than for oxidized or fully reduced flavin. In this way, the apoprotein modulates the potential of the isoalloxazine moiety and can apparently stabilize either 1- or 2-electron transfer pathways. In addition, protein-bound FMN semiquinone, because of the increase in midpoint potential (Fl/FlH•), is poorly reactive with oxygen, whereas free flavin is rapidly autooxidizable (157).

Although no flavodoxins have been discovered in mammalian systems, the FMN portion of the NADPH-cytochrome P-450 reductase (95, 258, 259) appears to bear a striking functional similarity to flavodoxins. This microsomal enzyme contains both FAD and FMN and transfers electrons from NADPH to microsomal cytochrome(s) P-450, which function in a variety of drug and steroid oxidations (41). As in flavodoxin, the FMN

portion of this enzyme has a thermodynamically stable neutral semiquinone (96, 150, 260), which is poorly reactive with oxygen (95, 150) and probably cycles between the semiquinone and fully reduced forms.

Recent studies (73) have suggested a different mechanism for 1-electron transfers by the FAD-containing electron transferring flavoprotein (ETF): This enzyme is involved in the transfer of electrons from the FAD-protein fatty acyl-CoA dehydrogenase to the mitochondrial electron transport chain via the recently discovered membrane-bound flavoprotein ETF-ubiquinone oxidoreductase (223). The anionic (red) flavin semiquinone of ETF was shown to function catalytically (73), and cycling between the oxidized and semiquinone states was suggested. The iron-sulfur flavoprotein ETF-ubiquinone oxidoreductase (223), the electron acceptor from ETF, is also tentatively classified in Table 2 as a 1-electron transferase, though definitive classification will require further studies.

Dehydrogenases

Dehydrogenation or net removal of proton(s) plus electrons is a common flavoenzyme reaction. Data have not provided a unified flavoenzyme-mediated dehydrogenation mechanism and at least two classes of mechanisms must be considered; thus enzymes are cataloged under pyridine nucleotide-dependent and non-pyridine nucleotide-dependent dehydrogenases. In addition, some enzymes in both categories (see footnote to Table 2) are classed as complex flavoenzymes or flavoproteins that contain one or more additional redox-active groups (hemes, second flavins, or iron-sulfur centers). A special category of complex flavoproteins is also listed separately: pyridine nucleotide-disulfide oxidoreductases. This class of enzymes has a redox-active disulfide and is involved in reactions with sulfur-containing compounds.

PYRIDINE NUCLEOTIDE-DEPENDENT DEHYDROGENASES Pyridine nucleotide-dependent dehydrogenases catalyze 2-electron transfer reactions, carrying out the oxidation of NADH or NADPH to form $NAD(P)^+$ by a net hydride transfer mechanism (two electrons plus one proton). Indirect evidence for hydride transfer from reduced pyridine nucleotides has been provided by model reactions (245, 277). Direct hydrogen transfer to the 5 position of a flavin analogue, 5-deazaflavin, has been observed in at least seven different flavoenzymes when the deazaflavin coenzyme was bound in place of FMN or FAD (57, 100). Whereas hydrogens at N-1 or N-5 exchange rapidly, the deazaflavins "trap" transferred hydrogen in a stable configuration (191, 240); hence, these findings provide strong evidence that flavin can be bound in such a geometry as to allow

direct hydrogen transfer from pyridine nucleotides and other substrates (possibly as hydride ion). Nevertheless, it has been argued (81) that 5-deazaflavin bears more of a chemical resemblance to pyridine nucleotide than to isoalloxazine and therefore is activated for hydride transfers at the C-5 position. Results therefore may not reflect the mechanism that occurs with the native isoalloxazine ring system.

An intermediate in the reduction of flavin by pyridine nucleotide has been observed in model systems (15, 207) and in various enzymes (1, 145, 149, 207) and is characterized by long wavelength absorbance. The chemical nature of such a species has been proposed to be either a charge transfer complex or a biradical complex. One such long wavelength-absorbing complex has been recently demonstrated by Porter & Bright (207) to be a precursor of hydrogen transfer from pyridine nucleotide. Although such species have been observed for a large number of flavoprotein-substrate complexes (145), the rigorous demonstration of charge transfer has been accomplished in only one enzyme system (1) and it is not clear whether this species is involved catalytically. Thus, the precise chemical mechanism for 2-electron transfer from pyridine nucleotides to enzyme flavins remains uncertain, but appears to differ in many respects from dehydrogenation of other non-pyridine nucleotide substrates (see below).

NON-PYRIDINE NUCLEOTIDE-DEPENDENT DEHYDROGENASES

Non-pyridine nucleotide-dependent hydrogenases (Table 2) are involved in the removal of hydrogens from substrates, often creating double bonds, as in the dehydrogenation of succinate to form fumarate or of fatty acyl-CoA's to form α-β unsaturated fatty acyl derivatives. A growing body of evidence indicates that at least some flavin-catalyzed dehydrogenations occur via a mechanism that involves proton abstraction from substrate with consequent carbanion formation and subsequent or simultaneous formation of a covalent adduct of substrate with the N-5 position of the flavin. Evidence for proton abstraction and a carbanion intermediate has been reviewed (262). The use of β-chloroalanine as a substrate for D-amino acid oxidase[1] results in loss of chloride ion under anaerobic conditions. Such a result is expected if the enzyme abstracts a proton to form a substrate α-carbanion, which subsequently rearranges in the absence of oxidant to eliminate the β-halo substituent (264). Studies on lactate oxidase showed that when a transition state analogue that does not contain an abstractable proton reacts with the

[1]The enzymes classified in Table 2 under oxygen reductases have dehydrogenase activities discussed here.

enzyme, a proton is rapidly taken up from solution (66). These authors propose that when a normal substrate is bound, a basic residue abstracts a proton from substrate to form a substrate carbanion. Additional evidence that suggests carbanion intermediates comes from studies that use "suicide substrates," or substrates that when acted upon by an enzyme produce reactive intermediates that inactivate the enzyme. Production of these reactive species by various flavoenzymes would require abstraction of a substrate proton. Strong evidence was provided for a covalent flavin-substrate intermediate during the oxidation of nitroethane by D-amino acid oxidase (206); using cyanide to chemically "trap" an N-5 covalently bound species, the authors deduced the existence of a covalent N-5 flavin-substrate intermediate. Glycollate oxidation by lactate oxidase results in an N-5 covalent fluorescent adduct of substrate and flavin, the formation of which is dependent upon hydrogen abstraction, and the kinetics of which are consistent with it being on the normal reaction pathway (66, 67, 146). These studies suggest the picture of flavoprotein dehydrogenation shown in Figure 2. An enzyme base abstracts a proton to form a substrate carbanion that then forms an N-5 covalent adduct with flavin. Subsequent breakdown of the adduct produces reduced flavin and oxidized substrate. A histidyl residue has been implicated as the functional base in pyridoxamine (pyridoxine) 5'-phosphate oxidase (86).

COMPLEX FLAVOPROTEINS Flavins and flavoproteins have long been known to combine both their 2-electron dehydrogenase and 1-electron transferase activities to yield a 2 → 1 electron "stepdown" activity in which an electron pair from substrate is split and donated to two 1-electron acceptors (76, 178). The existence of three oxidation states in flavin allows such a transformation to occur. The simplest case utilizes only the flavin group, which is fully reduced by substrate and which becomes oxidized in two successive 1-electron transfers to 1-electron acceptors. Such appears to be the case for NADH reduction of cytochrome b_5 by the microsomal

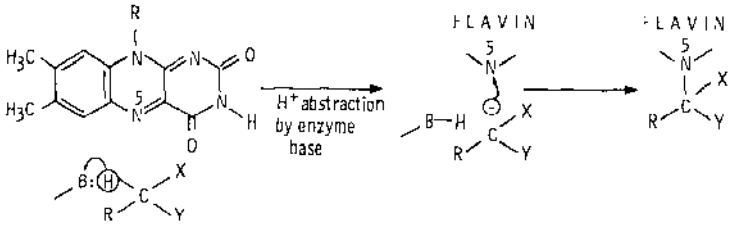


Figure 2 Flavoprotein-catalyzed dehydrogenation.

flavoprotein NADH-cytochrome b_5 reductase (244). However, a large number of flavoprotein dehydrogenases also contain one or more additional 1-electron-carrying redox-active groups (see Ref 110 for a recent review), including iron-sulfur centers and hemes. The fully reduced flavin transfers one electron at a time to the second redox group on the enzyme prior to reduction of the ultimate electron acceptor. Such a pattern is utilized frequently (see Table 2) in the oxidation of various substrates (including NADH and succinate) by the mitochondrial electron transport chain and is also used in the oxidation of NADPH by the microsomal NADPH-cytochrome P-450 reductase (see above). Kinetic mechanisms for flavin-flavin (283), flavin-iron-sulfur (133), and flavin-heme (29, 30) electron transfers have been reported, but little is known of the chemistry of these flavin to second group electron transfers. Model complexes between flavin and metals have been reported (136), but their relation to physiologic systems is unclear. Flavin-iron-sulfur interactions in mitochondrial membrane-associated succinate dehydrogenase have been investigated (194, 225). EPR spectroscopy was used to ascertain that the flavin resides near the surface of the enzyme, iron-sulfur centers I and II near the flavin, and iron-sulfur center III deeply buried in the mitochondrial membrane and in contact with a bound coenzyme Q. In trimethylamine dehydrogenase, flavin and iron-sulfur centers are very close (probably <10 Å) (233). The adrenal cortex enzymes NADPH-adrenodoxin reductase (an FAD protein) and adrenodoxin (an Fe_2S_2 protein) form a tightly associated 1:1 complex (37, 134), which may provide a model for more complex iron-sulfur flavoenzymes such as succinate dehydrogenase. Electron transfer from flavin to iron-sulfur in this system has been shown to be rate limiting in NADPH-supported cytochrome *c* reduction (132, 133). When the midpoint redox potential of either center is varied (using flavin derivatives or pH), the rate of cytochrome *c* reduction is correlated with the difference between redox potentials of flavin and iron-sulfur (133, 138).

PYRIDINE NUCLEOTIDE-DISULFIDE OXIDOREDUCTASES A 2-electron transfer reaction catalyzed by glutathione reductase, lipoamide dehydrogenase, and thioredoxin reductase is the reversible reduction of dithiols ($R\text{-}S\text{-}S\text{-}R + NADPH/H^+ \leftrightarrows 2RSH + NADP^+$) (275). Each of these enzymes contains an oxidation-reduction-active disulfide. This group is located near the flavin, based upon the observation of apparent charge-transfer between a proposed thiolate anion of the reduced disulfide and the oxidized flavin (149). This thiolate anion is stabilized by a basic amino acid residue, and a C-4a flavin adduct with a cystinyl sulfur is proposed as an intermediate in the electron transfer process (154). Model studies (21)

support the existence of such an adduct. X-ray data of glutathione reductase (227) show a cystinyl sulfur next to the flavin 4a position and a nearby histidine, which could act as the active site base. The crystal structure also demonstrates that reaction with pyridine nucleotide and cystinyl sulfur must occur on opposite faces of the isoalloxazine ring system. Sulfur-containing substrates react at the redox-active disulfide and do not appear to react directly with the flavin.

Dehydrogenase/Oxygen Reductase Enzymes

In a large number of flavoenzymes with dehydrogenase activities, the reduced flavin reacts with oxygen to reduce oxygen to one of several forms. Many of the dehydrogenase-electron transferases discussed above can reduce oxygen to superoxide anion (O_2^-) in a 1-electron reaction. Flavoprotein oxidases catalyze the 2-electron reduction of O_2 to hydrogen peroxide with both electrons derived from substrate, whereas flavin monoxygenases catalyze the reduction of oxygen to water with the concomitant hydroxylation of substrate (one oxygen atom ends up in H_2O and one ends up in substrate). Flavin monoxygenases can be further classified into "internal" and "external" types. For internal monoxygenases all electrons for the reaction are derived from the hydroxylated substrate. Flavoprotein internal monoxygenases catalyze the oxidative decarboxylation of a substrate ($R\text{-}CHOH\text{-}CO_2^- + O_2 \rightarrow R\text{-}CO_2 + CO_2 + H_2O$). As pointed out in recent reviews (58, 147), this reaction may be a variant of the flavoprotein oxidase reaction in which hydrogen peroxide produced at the active site then reacts with the bound dehydrogenated substrate. External flavin monoxygenases obtain electrons for hydroxylation from NAD(P)H. In flavoprotein dioxygenases, both oxygen atoms end up in substrate. Examples of each reaction type are provided in Table 2.

A catalytic role for a covalent adduct of oxygen and flavin has been proposed based upon several lines of evidence: An oxygen-containing species of flavin has been observed in several bacterial external monoxygenases (52, 209, 226, 239), bacterial luciferase (77), and mammalian microsomal FAD-containing monoxygenase (12, 208). In the luciferase system (a flavin-dependent system, of which one reaction product is light), this enzyme-bound species can be produced enzymatically from reduced FMN plus O_2 and can be isolated from reactants at low temperature (77). Upon warming, the species yields oxidized FMN plus H_2O_2, thus suggesting the species is a flavin hydroperoxide. Various structures for such a species have been proposed based on model studies and theoretical considerations (82, 143, 183, 184). A stable C-4a hydroperoxyflavin derivative has been synthesized (121) with spectral properties similar to this species. In addition, flavin

derivatives carrying an oxygen substituent at C-4a produce a spectrum virtually identical to the catalytic oxygenated species when the derivative is bound to a variety of hydroxylase and oxidase apoproteins (65). Convincing evidence for a C-4a oxygenated intermediate also comes from ^{13}C-labeled NMR studies (139, 184), and recent X-ray data of *p*-hydroxybenzoate hydroxylase (274) show the C-4a position (but not other proposed positions) near the bound substrate to be hydroxylated. Thus, the proposed oxygenated flavin intermediate involved in enzymatic monoxygenations is as shown in Figure 3.

Such an intermediate is involved catalytically and could be the active oxygen donor to substrate. Studies that use salicylate hydroxylase to metabolize various substituted salicylates (209), as well as similar studies in other hydroxylases (90) suggest that the hydroxylating species of oxygen is electron deficient (oxenoid) and that the transition state involves the aromatic substrate. Transfer of oxenoid oxygen from the C-4a hydroperoxyflavin has been suggested to result in an open-ring flavin compound where the C-4a-N-5 bond is broken. A kinetic intermediate with unusual spectral properties has been reported (52) upon reoxidation of reduced *p*-hydroxybenzoate hydroxylase-substrate complex by oxygen. Its formation follows the formation of the C-4a hydroperoxy intermediate, and the disappearance of the 4a-peroxy intermediate appeared to parallel substrate hydroxylation. These authors suggest that the second intermediate represents a transient open-ring form of flavin. Thus, a C-4a hydroperoxyflavin appears to be a catalytic intermediate in substrate hydroxylations by external flavoprotein monoxygenases (12, 208), including the mammalian microsomal FAD-containing monoxygenase.

ACKNOWLEDGMENTS

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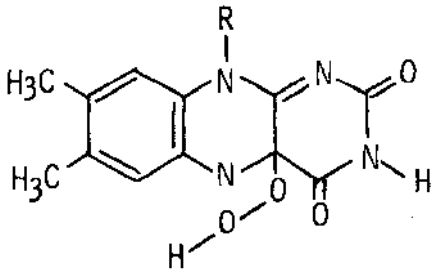

C - 4a peroxyflavin

Figure 3 Structure of C-4a peroxyflavin.

Literature Cited

1. Abramovitz, A., Massey, V. 1976. Interaction of phenols with old yellow enzyme: physical evidence for charge-transfer complexes. *J. Biol. Chem.* 251:5327–36
2. Addison, R., McCormick, D. B. 1978. Biogenesis of flavoprotein and cytochrome components in hepatic mitochondria from riboflavin-deficient rats. *Biochem. Biophys. Res. Commun.* 81: 135–38
3. Alston, T. A., Mela, L., Bright, H. J. 1977. 3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase. *Proc. Natl. Acad. Sci. USA* 74:3764–71
4. Araya, M., Selenk, S. J., Nobile, S., Walker-Smith, J. A. 1975. Blood vitamin levels in children with gastroenteritis. *Aust. NZ J. Med.* 5:239–50
5. Arsenis, C., McCormick, D. B. 1964. Coenzyme specificity of nicotinamide-adenine dinucleotide phosphate cytochrome *c* reductase for flavin phosphates. *Biochem. Biophys. Acta* 92: 440–45
6. Bacher, A., Mailander, B. 1978. Biosynthesis of riboflavin in *Bacillus subtilus:* function and genetic control of the riboflavin synthase complex. *J. Bacteriol.* 134:476–82
7. Baker, H., Frank, O. 1975. Analysis of riboflavin and its derivatives in biologic fluids and tissues. In *Riboflavin,* ed. R. S. Rivlin, pp. 49–79. New York: Plenum
8. Baker, H., Thind, I. S., Frank, O., De Angelia, B., Caterini, H., Louria, D. B. 1977. Vitamin levels in low-birthweight newborn infants and their mothers. *Am. J. Obstet. Gynecol.* 129:521–24
9. Barber, M. J., Bray, R. C., Cammack, R., Coughlan, M. P. 1977. Oxidation reduction potentials of turkey liver xanthine dehydrogenase and the origins of oxidase and dehydrogenase behavior in molybdenum-containing hydroxylases. *Biochem. J.* 163:279–89
10. Barman, B. G., Tollin, G. 1972. Flavin-protein interactions in flavoenzymes: Thermodynamics and kinetics of reduction of *Azotobacter* flavodoxin. *Biochemistry* 12:4755–59
11. Barman, B. G., Tollin, G. 1972. Flavin-protein interactions in flavoenzymes: Temperature-jump and stopped-flow studies of flavin analog binding to the apoprotein of *Azotobacter* flavodoxin. *Biochemistry* 11:4746–54
12. Beaty, N. B., Ballou, D. P. 1980. Transient kinetic study of liver microsomal FAD-containing monoxygenase. *J. Biol. Chem.* 255:3817–19
13. Bessey, O. A., Lowry, O. H., Love, R. H. 1949. The fluorometric measurement of riboflavin and their concentration in tissues. *J. Biol. Chem.* 180: 755–69
14. Blackwell, B., Marley, E., Price, J., Taylor, D. 1967. Hypertensive interactions between monoamine oxidase inhibitors and foodstuffs. *Br. J. Psychiatr.* 113:349–65
15. Blankenhorn, G. 1975. Intermolecular complexes between N-methyl-1,4-dihydronicotinamide and flavins. *Biochemistry* 14:3172–76
16. Blum, J. C., Gaudin-Harding, F. 1966. Les flavoprotéines de l'oeuf et du sérum sanguin de la poule pondeuse. *C. R. Acad. Sci. D* 262:1299–301
17. Brady, M. E., Hayton, W. L. 1977. Gastrointestinal absorption in rats exposed to cobalt-60 gamma radiation. Part 1. Extent of absorption. *J. Pharmacol. Sci.* 66:361–65
18. Brandt, N. J., Gregersen, N., Christensen, E., Gron, I. H., Rasmussen, K. 1979. Treatment of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria). Experience with diet, riboflavin, and GABA analog. *J. Pediatr.* 94:669–73
19. Bray, R. C. 1975. Molybdenum iron-sulfur flavin hydroxylases and related enzymes. *Enzymes* 12:299–419 3rd ed.
20. Bright, H. J., Porter, D. J. T. 1975. Flavoprotein oxidases. *Enzymes* 12: 421–505
21. Bruice, T. C. 1976. Models and flavin catalysis. In *Progress in Bioorganic Chemistry,* ed. E. T. Kaiser, F. J. Kezdy, 4:1–87. New York: Wiley. 288 pp.
22. Bruice, T. C. 1976. Some pertinent aspects of mechanism as determined with small molecules. *Ann. Rev. Biochem.* 45:331–73
23. Buchanan, J. M. 1971. Methionine biosynthesis (Hog liver). *Methods Enzymol.,* 17B:371–78
24. Burch, H. B., Bessey, O. A., Lowry, O. H. 1948. Fluorometric measurements of riboflavin and its natural derivatives in small quantities of blood serum and cells. *J. Biol. Chem.* 175:457–70
25. Burch, H. B., Lowry, O. H., Padilla, A. M., Combs, A. M. 1956. Effects of riboflavin deficiency and realimentation on flavin enzymes of tissues. *J. Biol. Chem.* 223:29–45
26. Cairns, W. L., Metzler, D. E. 1971. Photochemical degradation of flavins.

VI. A new photoproduct and its use in studying the photolytic mechanism. *J. Am. Chem. Soc.* 93:2772–77
27. Cammack, R. 1975. D-2-Hydroxy acid dehydrogenase from animal tissue. *Methods Enzymol.* 41:323–29
28. Campbell, T. C., Hayes, J. R. 1974. The role of nutrition in the drug-metabolizing enzyme system. *Pharmacol. Rev.* 26:171–97
29. Capeillere-Blandin, C., Bray, R. C., Iwatsubo, M. J., Labeyrie, F. 1975. Flavocytochrome b_2: Kinetic studies by absorbance and electron-paramagnetic-resonance spectroscopy of electron distribution among prosthetic groups. *Eur. J. Biochem.* 54:549–66
30. Capeillere-Blandin, C., Iwatsubo, M., Labeyrie, F., Bray, R. C. 1976. Flavocytochrome b_2: Studies of intramolecular electron exchange between prosthetic groups. In *Flavins and Flavoproteins,* ed. T. P. Singer, pp. 621–33. Amsterdam: Elsevier. 813 pp.
31. Carlberg, I., Mannervik, B. 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J. Biol. Chem.* 250:5475–80
32. Cavalli-Sforza, L. L., Daiger, S. P., Rummel, D. P. 1977. Detection of genetic variation with radioactive ligands. I. Electrophoretic screening of plasma proteins with a selected panel of compounds. *Am J. Hum. Genetics* 29: 581–92
33. Chassey, B. M., McCormick, D. B. 1965. Coenzyme specificity of D-amino acid oxidase for the flavin moiety of FAD. *Biochim. Biophys. Acta* 110: 91–96
34. Chia, C. P., Addison, R., McCormick, D. B. 1978. Absorption, metabolism, and excretion of 8 α-(amino acid) riboflavins in the rat. *J. Nutr.* 108: 373–81
35. Choi, J. D., McCormick, D. B. 1980. The interaction of flavins with egg white riboflavin-binding protein. *Arch. Biochem. Biophys.* 204:41–51
36. Christensen, S. 1973. The biological fate of riboflavin in mammals. *Acta Pharm. Toxicol.* 32: Suppl. II, pp. 1–72
37. Chu, J.-W., Kimura, T. 1973. Studies on adrenal steroid hydroxylases: Complex formation of the hydroxylase components. *J. Biol. Chem.* 248:5183–87
38. Clarke, H. C. 1976. In Trinidad: angular stomatitis and pregnancy. *Int. J. Vitam. Nutr. Res.* 46:366–68
39. Clarke, H. C. 1977. Distribution of riboflavin in blood: in women and in prenates. *Int. J. Vitam. Nutr. Res.* 47:361–63
40. Coles, C. J., Edmondson, D. E., Singer, T. P. 1979. Studies on succinate dehydrogenase. XXXV. Inactivation of succinate dehydrogenase by 3-nitropropionate. *J. Biol. Chem.* 254:5161–67
41. Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R., O'Brien, P. J., eds. 1980. *Microsomes, Drug Oxidations, and Chemical Carcinogenesis,* Vol. 1. New York: Academic. 618 pp.
42. Costa, E., Sandler, M. 1972. Monoamine oxidases—New vistas. In *Advances in Biochemical Psychopharmacology,* Vol. 5, ed. E. Costa, P. Greengard. New York: Raven. 454 pp.
43. Gabig, T. G., Babior, B. M. 1979. The O_2-forming oxidase responsible for the respiratory burst in human neutrophils. *J. Biol. Chem.* 254:9070–74
44. Cromartie, T. H., Walsh, C. T. 1975. Rat kidney L-α-hydroxy acid oxidase: Isolation of enzyme with one flavin coenzyme per two subunits. *Biochemistry* 14:2588–96
45. Deluca, C., Kaplan, N. O. 1958. Flavin adenine dinucleotide synthesis in animal tissues. *Biochim. Biophys. Acta* 30:6–11
46. Donaldson, K. O., Keresztesy, J. C. 1962. Naturally occurring forms of folic acid. II. Enzymatic conversion of methylenetetra-hydrofolic acid to prefolic A-methyltetrahydrofolate. *J. Biol. Chem.* 237:1298–304
47. Edmondson, D. E. 1977. 2',5'-Anhydro-8α-histydyl flavins: Their formation and structural elucidation. *Biochemistry* 16:4308–11
48. Edmondson, D. E., Singer, T. P. 1976. 8α-Substituted flavins of biological importance: an updating. *FEBS Lett.* 64:255–65
49. Edmondson, D. E., Tollin, G. 1971. Chemical and physical characterization of the Shethna flavoprotein and kinetics and thermodynamics of flavin analog binding to the apoprotein. *Biochemistry* 10:124–32
50. Eirich, L. D., Vogels, G. D., Wolfe, R. S. 1978. Proposed structure for coenzyme F_{420} from *Methanobacterium. Biochemistry* 17:4583–93
51. Eisen, H. N., Michaelides, M. C., Underdown, B. J., Schulenburg, E. P., Simms, E. S. 1970. Myeloma proteins with antihapten antibody activity. *Fed. Proc.* 29:78–84
52. Entsch, B., Ballou, D. P., Massey, V. 1976. Flavin-oxygen derivatives in-

volved in hydroxylation by p-hydroxybenzoate hydroxylase. *J. Biol. Chem.* 251:2550–63

53. Farhangi, M., Osserman, E. F. 1976. Myeloma with xanthoderma due to an IgG monoclonal anti-flavin antibody. *N. Engl. J. Med.* 294:177–83
54. Farrell, H. M. Jr., Mallette, M. F., Buss, E. G., Clagett, C. O. 1969. The nature of the biochemical lesion in avian renal riboflavin uria. III. The isolation and characterization of the riboflavin-binding protein from egg albumin. *Biochim. Biophys. Acta* 194:433–42
55. Fass, S., Rivlin, R. S. 1969. Regulation of riboflavin-metabolizing enzymes in riboflavin deficiency. *Am. J. Physiol.* 217:988–91
56. Fazekas, A. G., Sandor, T. 1971. Flavin nucleotide coenzyme biosynthesis and its relation to cortico-steroidogenesis in the rat adrenal. *Endocrinology* 89:397–407
57. Fisher, J., Spencer, R., Walsh, C. 1976. Enzyme-catalyzed redox reactions with the flavin analogues 5-deazariboflavin, 5-deazariboflavin 5'-phosphate, and 5-deazariboflavin 5'-diphosphate 5'-adenosine ester. *Biochemistry* 15: 1054–64
58. Flashner, M. S., Massey, V. 1974. Flavoprotein oxygenases. In *Molecular Mechanisms of Oxygen Activation,* ed. O. Hayaishi, pp. 245–83. New York: Academic. 678 pp.
59. Forman, H. J., Kennedy, J. 1978. Mammalian dihydroorotate dehydrogenase: Physical and catalytic properties of the primary enzyme. *Arch. Biochem. Biophys.* 191:23–31
60. Frank, O., Luisada-Opper, A. V., Feingold, S., Baker, H. 1970. Vitamin binding by human and some animal plasma proteins. *Nutr. Rep. Int.* 1:161–68
61. Frisell, W. R., Mackenzie, C. G. 1962. Separation and purification of sarcosine dehydrogenase and dimethylglycine dehydrogenase. *J. Biol. Chem.* 237: 94–98
62. Froehlich, J. A., Merrill, A. H. Jr., Clagett, C. O., McCormick, D. B. 1980. Affinity chromatographic purification and comparison of riboflavin-binding proteins from laying hen liver and blood and from egg yolk. *Comp. Biochem. Physiol. B* 66:397–401
63. Funai, Y. 1956. Studies on riboflavin in milk. Report 1: On riboflavin content in breast milk. *Tokushima J. Exp. Med.* 3:194–200
64. Gast, R., Muller, F. 1978. The binding of flavins by apoflavodoxins from *Peptostreptococcus elsdenii* and *Azotobacter vinelandii* as studied by the temperature-jump technique. *Helv. Chim. Acta* 161:1353–63
65. Ghisla, S., Entsch, B., Massey, V., Husain, M. 1977. On the structure of flavin-oxygen intermediates involved in enzymatic reactions. *Eur. J. Biochem.* 76:139–48
66. Ghisla, S., Massey, V. 1977. Studies on the mechanism of action of the flavoenzyme lactate oxidase: Proton uptake and release during the binding of transition state analogs. *J. Biol. Chem.* 252:6729–35
67. Ghisla, S., Massey, V. 1980. Studies on the catalytic mechanism of lactate oxidase: Formation of enantiomeric flavin N(5)-glycollyl adducts via carbanion intermediates. *J. Biol. Chem.* 255: 5688–96
68. Ghisla, S., Mayhew, S. G. 1976. Identification and properties of 8-hydroxyflavin-adenine dinucleotide in electron-transferring flavoprotein from *Peptostreptococcus elsdenii. Eur. J. Biochem.* 63:373–90
69. Goldsmith, G. H. 1975. Riboflavin deficiency. See Ref. 7, pp. 221–44
70. Graham, D. W., Brown, J. E., Ashton, W. T., Brown, R. D., Roger, E. F. 1977. Anticoccidal riboflavine antagonists. *Experientia* 33:1274–75
71. Green, D. R., Mii, S., Kohout, P. M. 1955. Studies on the terminal electron transport system I. succinate dehydrogenase. *J. Biol. Chem.* 217:551–67
72. Hall, C. L., Heijenskjold, L., Bartfai, T., Ernster, L., Kamin, H. 1976. Acyl coenzyme A dehydrogenases and electron-transferring flavoprotein from beef heart mitochondria. *Arch. Biochem. Biophys.* 177:402–14
73. Hall, C. L., Lambeth, J. D. 1980. Studies on electron transfer from general acyl-CoA dehydrogenase to electron transfer flavoprotein. *J. Biol. Chem.* 255:3591–95
74. Hamm, H. H., Decker, K. 1978. FAD is covalently attached to peptidyl-tRNA during cell-free synthesis of 6-hydroxy-*D*-nicotine oxidase. *Eur. J. Biochem.* 92:449–54
75. Hamm, H. H., Decker, K. 1980. Cell-free synthesis of a flavoprotein containing the 8α-(N^3-histidyl)-riboflavin linkage. *Eur. J. Biochem.* 104:3981–95
76. Hass, E., Horecker, B. L., Hogness, T. R. 1940. The enzymatic reduction of cytochrome *c*: Cytochrome *c* reductase. *J. Biol. Chem.* 136:747–74

77. Hastings, J. W., Balny, C., Dozou, P. 1976. The oxygenated luciferase-flavin intermediate. See Ref. 30, pp. 53–61
78. Hatefi, Y., Stiggall, D. L. 1976. Metal-containing flavoprotein dehydrogenases. *Enzymes* 13:175–97
79. Heacock, A. M., Adams, E. 1975. Formation and excretion of pyrrole-2-carboxylic acid. Whole animal and enzyme studies in the rat. *J. Biol. Chem.* 250:2599–608
80. Hemmerich, P. 1977. Bio(in)organic views on flavin-dependent one-electron transfer. *Advan. Chem. Ser.* 162:312–20
81. Hemmerich, P., Massey, V., Fenner, H. 1977. Flavin and 5-deazaflavin: A chemical evaluation of "modified" flavoproteins with respect to the mechanisms of redox biocatalysis. *FEBS Lett.* 84:5–21
82. Hemmerich, P., Massey, V., Weber, G. 1967. Photo-induced enzyme substitution of flavine by phenylacetate: A possible model for flavoprotein catalysis. *Nature* 213:728–30
83. Hemmerich, P., Nagelschneider, G., Veeger, C. 1970. Chemistry and molecular biology of flavins and flavoproteins. *FEBS Lett.* 8:69–83
84. Holta, E. 1977. Oxidation of spermidine and spermine in rat liver: Purification and properties of polyamine oxidase. *Biochemistry* 16:91–100
85. Hoppel, C., DiMarco, J. P., Tandler, B. 1979. Riboflavin and rat hepatic cell structure and function. *J. Biol. Chem.* 254:4164–70
86. Horiike, K., Tsuge, H., McCormick, D. B. 1979. Evidence for an essential histidyl residue at the active site of pyridoxamine (pyridoxine)-5'-phosphate oxidase from rabbit liver. *J. Biol. Chem.* 254:6638–43
87. Hovi, L., Hekali, R., Siimes, M. A. 1979. Evidence for riboflavin depletion in breast-fed newborns and its further acceleration during treatment of hyperbilirubinemia by phototherapy. *Acta Paediatr. Scand.* 68:567–70
88. Huang, M.-T., Miwa, G. T., Lu, A. Y. H. 1979. Rat liver cytosolic azoreductase: purification and properties. *J. Biol. Chem.* 254:3930–34
89. Hunt, S. M. 1975. Nutritional intake of riboflavin. See Ref. 7, pp. 199–219
90. Husain, M., Entsch, B., Ballou, D. P., Massey, V., Chapman, P. J. 1980. Fluoride elimination from substrates in hydroxylation reactions catalyzed by *p*-hydroxybenzoate hydroxylase. *J. Biol. Chem.* 255:4189–97
91. Husain, M., Massey, V. 1978. Reversible resolution of flavoproteins into apoproteins and free flavins. *Methods Enzymol.* 53:429–37
92. Ichikawa, Y., Hiwatashi, A. 1977. Isolation and properties of reduced nicotinamide adenine dinucleotide phosphate-hepatoredoxin reductase of rabbit liver mitochondria. *Biochem. Biophys. Res. Commun.* 79:443–50
93. Ichikawa, Y., Hiwatashi, A., Kim, H.-J., Maruga, N., Yamano, T. 1980. Dissimilarities among NADPH-adrenodoxin reductase, NADPH-hepatoredoxin reductase, and NADPH-renoredoxin reductase of mitochondria of bovine tissues. In *Flavins and Flavoproteins* ed. K. Yagi, I. Yamano, pp. 677–91. Tokyo: Japan Scientific Societies Press. 740 pp.
94. Im, W. B., Misch, D. W., Powell, D. W., Faust, R. G. 1980. Phenolphthalein- and harmaline-induced disturbances in the transport function of isolated brush border and basolateral membrane vesicles from rat jejunum and kidney cortex. *Biochem. Pharmacol.* 29:2307–17
95. Iyanagi, T., Mason, H. S. 1973. Some properties of hepatic reduced nicotinamide adenine dinucleotide phosphate-cytochrome *c* reductase. *Biochemistry* 12:2297–308
96. Iyanagi, T., Mason, H. S. 1975. Hepatic NADPH-cytochrome *c* oxidoreductase. In *Reactivity of Flavins*, ed. K. Yagi, pp. 145–56. Tokyo: Univ. Tokyo Press. 185 pp.
97. Jaroszewicz, L. 1975. D-Aspartate oxidase in the thyroid gland. *Enzyme* 20:80–89
98. Jones, T., Spencer, R., Walsh, C. 1978. Mechanisms and kinetics of iron release from ferritin by dihydroflavins and dihydroflavin analogues. *Biochemistry* 17:4011–17
99. Jorns, M. S. 1975. Glycolic acid oxidase from pig liver. *Methods Enzymol.* 61B:337–43
100. Jorns, M. S., Hersh, L. B. 1975. N-Methylglutamate synthetase: Substrate flavin hydrogen transfer reactions probed with deazaflavin mononucleotide. *J. Biol. Chem.* 250:3620–28
101. Jusko, W. J., Khanna, N., Levy, G., Stern, L., Yaffe, S. J. 1970. Riboflavin absorption and excretion in the neonate. *Pediatrics* 45:945–94
102. Jusko, W. J., Levy, G. 1967. Absorption, metabolism, and excretion of riboflavin 5'-phosphate in man. *J. Pharmacol. Sci.* 156:58–62

103. Jusko, W. J., Levy, G. 1967. Effect of probenecid on riboflavin absorption and excretion in man. *J. Pharmacol. Sci.* 56:1145–49
104. Jusko, W. J., Levy, G. 1969. Plasma protein binding of riboflavin and riboflavin 5'-phosphate in man. *J. Pharmacol. Sci.* 58:58–62
105. Jusko, W. J., Levy, G. 1970. Pharmacokinetic evidence for saturable renal tubular reabsorption of riboflavin. *J. Pharmacol. Sci.* 59:765–72
106. Jusko, W. J., Levy, G. 1975. Absorption, protein binding and elimination of riboflavin. See Ref. 7, pp. 99–152
107. Jusko, W. J., Levy, G., Yaffee, S. J., Allen, J. E. 1971. Riboflavin absorption in children with biliary obstruction. *Am. J. Dis. Child.* 121:48–52
108. Kakie, Y. 1970. Studies on thiamine disulfide. XXXVII. Properties of O-acylthiamine oxidase. *Vitamin* 81:124–28
109. Kamin, H., ed. 1971. *Flavins and Flavoproteins,* Vol. III. Baltimore: University Park Press. 712 pp.
110. Kamin, H., Lambeth, J. D., Siegel, L. M. 1980. The role of flavins in electron transfer between two-electron donors and one-electron acceptors. See Ref. 93, pp. 341–48
111. Kapeller-Adler, R. 1949. Studies on histaminase. *Biochem. J.* 44:70–77
112. Kaplan, J. C., Chirouze, M. 1978. Therapy of recessive congenital methaemoglobinaemia by oral riboflavin. *Lancet* 2(8098):1043–44
113. Karrer, P., Solomon, H., Schopp, K., Berry, F., Becker, B. 1935. Synthesin von drei weiteren stereolsomeren des lactoflavins. *Helv. Chim. Acta* 18: 908–10
114. Kasai, S., Matsai, K. 1975. Relationship between antibacterial effects and redox potential of 8-N and 8-O flavins. *Jpn. Biochem. Met., Kinki Dist.* Transl. H. Tsuge
115. Kazarinoff, M. N., McCormick, D. B. 1973. N-(5'-Phospho-4-pyridoxal)amines as substrates for pyridoxine (pyridoxamine) 5'-phosphate oxidase. *Biochem. Biophys. Res. Commun.* 52: 440–46
116. Kazarinoff, M. N., McCormick, D. B. 1975. Rabbit liver pyridoxamine (pyridoxine) 5'-phosphate oxidase: Purification and properties. *J. Biol. Chem.* 250:3436–42
117. Kean, E. A. 1968. Rhein: An inhibitor of mitochondrial oxidations. *Arch. Biochem. Biophys.* 127:528–33
118. Kean, E. A. 1976. Selective inhibition of acyl-CoA dehydrogenases by a metabolite hypoglycin. *Biochim. Biophys. Acta* 422:8–14
119. Kean, E. A., Gutman, M., Singer, T. P. 1971. Studies on the respiratory chain-linked nicotinamide adenine dinucleotide dehydrogenase. XII. Rhein, a competitive inhibitor of the dehydrogenase. *J. Biol. Chem.* 246:2346–53
120. Keevil, T., Mason, H. S. 1978. Molecular oxygen in biological oxidations—An overview. *Methods Enzymol.* 52:3–40
121. Kemal, C., Bruice, T. C. 1979. Transfer of O_2 from a C4-OOH flavin anion to phenolate ion. A flavin catalyzed dioxygen reaction. *J. Am. Chem. Soc.* 101:1635–38
122. Kennedy, J. 1973. Distribution, subcellular localization, and product inhibition of dihydroorotate oxidation in the rat. *Arch. Biochem. Biophys.* 157: 369–73
123. Kenney, W. C., Edmondson, D. E., Singer, T. P., Nakagawa, H., Sato, R. 1976. Identification of the covalently bound flavin of gulono-γ-lactone oxidase. *Biochem. Biophys. Res. Commun.* 71:1194–200
124. Kenney, W. C., Edmondson, D. E., Singer, T. P., Nishikimi, M., Noguchi, E., Yogi, K. 1979. Identification of the covalently bound flavin of L-galactono-γ-lactone oxidase. *FEBS Lett.* 97:40–42
125. Kenney, W. C., McIntire, W., Yamanaka, T. 1977. Structure of the covalently bound flavin of *Chlorobium* cytochrome C_{553}. *Biochim. Biophys. Acta* 483:467–74
126. Kenney, W. C., Singer, T. P., Kukuyama, M., Miyake, Y. 1979. Identification of the covalently bound flavin prosthetic group of cholesterol oxidase. *J. Biol. Chem.* 254:4689–90
127. Kimura, T., Chu, J.-W., Parcells, J. 1976. Adrenodoxin reductase: Fluorescence properties and the effects of salts on its catalytic activities. See Ref. 30, pp. 637–46
128. Knobloch, E., Hodi, R., Janda, J., Herzmann, J., Houdkova, V. 1979. Spectrofluorimetric micro-method for determining riboflavin in the blood of new-born babies and their mothers. *Int. J. Vitam. Nutr. Res.* 49:144–51
129. Knoll, J. 1978. The pharmacology of selective irreversible monoamine oxidase inhibitors. In *Enzyme-Activated Irreversible Inhibitors,* ed. N. Seiler, M. J. Jung, J. Koch-Weser, pp. 253–69. Amsterdam: Elsevier-North Holland. 359 pp.

130. Koli, A. K., Yearby, C., Scott, W., Donaldson, K. O. 1969. Purification and properties of three separate menadione reductases from hog liver. *J. Biol. Chem.* 244:621–29
131. Koziol, J. 1971. Fluorometric analysis of riboflavin and its coenzymes. *Methods Enzymol.* 18:253–85. New York: Academic 857 pp.
132. Lambeth, J. D., Kamin, H. 1977. Adrenodoxin reductase and Adrenodoxin: Mechanisms of reduction of ferricyanide and cytrochrome *c*. *J. Biol. Chem.* 252:2908–17
133. Lambeth, J. D., Kamin, H. 1979. Adrenodoxin reductase-adrenodoxin complex: Flavin to iron-sulfur electron transfer as the rate-limiting step in the NADPH-cytochrome *c* reductase reaction. *J. Biol. Chem.* 254:2766–74
134. Lambeth, J. D., McCaslin, D. R., Kamin, H. 1976. Adrenodoxin reductase-adrenodoxin complex: Catalytic and thermodynamic properties. *J. Biol. Chem.* 251:7545–50
135. Larsson, A. 1973. Thioredoxin reductase from rat liver. *Eur. J. Biochem.* 35:346–49
136. Lauterwein, J., Hemmerich, P., Lhoste, J.-M. 1975. Flavoquinone-metal complexes: Structure and properties. *Inorgan. Chem.* 14:2152–60
137. Levy, G., Hewitt, R. R. 1971. Evidence in man for different specialized transport mechanisms for riboflavin and thiamin. *Am. J. Clin. Nutr.* 24:401–4
138. Light, D. R., Walsh, C. 1980. Flavin analogs as mechanistic probes of adrenodoxin reductase-dependent electron transfer to the cholesterol side chain cleavage cytochrome P-450 of the adrenal cortex. *J. Biol. Chem.* 255: 4264–77
139. Lhoste, J.-M., Favaudon, V., Ghisla, S., Hastings, J. W. 1980. NMR studies of ^{13}C-4a enriched flavins with luciferase and other flavoproteins. See Ref. 93, pp. 131–38
140. Loach, P. A. 1968. In *Handbook of Biochemistry,* ed. H. A. Sober, pp. 7–32. Cleveland: CRC Press. 256 pp.
141. Lostanlen, D., Vieira De Barros, A., Leroux, A., Kaplan, J. C. 1978. Soluble NADH-Cytochrome b_5 reductase from rabbit liver cytosol: Partial purification and characterization. *Biochim. Biophys. Acta* 526:42–51
142. Ludwig, M. L., Burnett, R. M., Darling, G. D., Jordan, S. R., Kendall, D. S., Smith, W. W. 1976. The structure of clostridium MP flavodoxin as a function of oxidation state: Some comparisons of the FMN-binding sites in oxidized, semiquinone, and reduced forms. See Ref. 30, pp. 393–404
143. Mager, H. I. S., Berends, W. 1966. The structure of transient hydroperoxides in the autooxidation of reduced flavins. *Biochim. Biophys. Acta* 118:440–41
144. Martinez, P., McCauley, R. 1977. Studies on the flavins in rat liver mitochondria outer membranes. *Biochim. Biophys. Acta* 497:437–46
145. Massey, V., Ghisla, S. 1974. Role of charge-transfer interactions in flavoprotein catalysis. *Ann. NY Acad. Sci.* 227:446–65
146. Massey, V., Ghisla, S., Kieschke, K. 1980. Studies on the reaction mechanism of lactate oxidase: Formation of two flavin-substrate adducts with glycolate. *J. Biol. Chem.* 255:2796–803
147. Massey, V., Hemmerich, P. 1975. Flavin and pteridine monooxygenases. *Enzymes* 12:191–252
148. Massey, V., Nishino, T. 1980. D-Amino acid oxidase containing 7,8-dichloro-FAD instead of FAD. See Ref. 93, pp. 1–11.
149. Massey, V., Palmer, G. 1962. Charge-transfer complexes of lipoyl dehydrogenase and free flavins. *J. Biol. Chem.* 237:2347–58
150. Masters, B.S.S., Kamin, H., Gibson, Q. H., Williams, C. H. 1965. Studies on the mechanism of microsomal triphosphopyridine nucleotide-cytochrome *c* reductase. *J. Biol. Chem.* 240:921–31
151. Masuoka, M. 1973. A nekoflavin-like substance isolated from cat liver. *Vitamins (Jpn)* 47:13–20
152. Matsui, K. 1971. *Isolation of nekoflavin. Methods Enzymol.* 18:433–37
153. Matsui, K., Juri, N., Kubo, Y., Kasai, S. 1979. *Formation of roseoflavin from guanine through riboflavin. J. Biochem.* 86:167–75
154. Matthews, R. G., Ballou, D. P., Thorpe, C., Williams, C. H. 1977. Ion pair formation in pig heart lipoamide dehydrogenase. *J. Biol. Chem.* 252: 3199–207
155. Mayersohn, M., Feldman, S., Gribaldi, M. 1969. Bile salt enhancement of riboflavin and flavin mononucleotide absorption in man. *J. Nutr.* 98:288–96
156. Mayhew, S. G. 1971. Studies on flavin binding in flavodoxins. *Biochim. Biphys. Acta* 235:289–302
157. Mayhew, S. G., Ludwig, M. 1975. Flavodoxins and electron-transferring flavoproteins. *Enzymes* 12:57–118.
158. Mayhew, S. G., Whitfield, C. D., Ghisla, S., Schuman-Jorns, M. 1974.

Identification and properties of new flavins in electron-transferring flavoprotein from *Peptostreptococcus elsdenii* and pig-liver glycolate oxidase. *Eur. J. Biochem.* 44:579–91

159. McCormick, D. B. 1961. Flavokinase activity of rat tissues and masking effect of phosphatases. *Proc. Soc. Exp. Biol. Med.* 107:784–86
160. McCormick, D. B. 1962. The intracellular localization, partial purification, and properties of flavokinase from rat liver. *J. Biol. Chem.* 237:959–62
161. McCormick, D. B. 1964. Inhibition of flavin adenine dinucleotide pyrophosphorylase by isoriboflavin. *Nature* 201:925–26
162. McCormick, D. B. 1964 Specificity of flavin adenine dinucleotide phosphorylase for flavin phosphates and nucleoside triphosphates. *Biochem. Biophys. Res. Commun.* 14:493–97
163. McCormick, D. B. 1972. The fate of riboflavin in the mammal. *Nutr. Rev.* 30:75–79
164. McCormick, D. B. 1975. Metabolism of riboflavin. See Ref. 7, pp. 153–98
165. McCormick, D. B., Chassey, B. M., Tsibris, J. C. M. 1964. Coenzyme specificity of D-amino acid oxidase for the adenylate moiety of FAD. *Biochim. Biophys. Acta* 89:447–52
166. McCormick, D. B., Russell, M. 1962. Hydrolysis of flavin mononucleotide by acid phosphatases from animal tissues. *Comp. Bichem. Physiol.* 5:113–21
167. McIntire, W., Edmondson, D. E., Singer, T. P. 1980. 8α-O-Tyrosyl FAD: a new form of covalently bound flavin from p-cresol methylhydroxylase. *J. Biol. Chem.* 255:6553–55
168. Meinen, M., Aeppli, R., Rehner, G. 1977. Studies on the absorption of thiamine, riboflavin, and pyridoxine *in vitro*. *Nutr. Metab.* 21:264–66
169. Meister, A., Wellner, D. 1963. Flavoprotein amino acid oxidases. *Enzymes,* 7:609–48
170. Merrill, A. H. Jr. 1979. *Studies on the formation of riboflavin 5'-phosphate by flavokinase and its utilization by pyridoxamine-(pyridoxine)5'-phosphate oxidase.* PhD thesis. Cornell Univ., Ithaca, NY. 183 pp.
171. Merrill, A. H. Jr., Addison, R., McCormick, D. B. 1978. Induction of hepatic and intestinal flavokinase after oral administration of riboflavin to riboflavin-deficient rats. *Proc. Soc. Exp. Biol. Med.* 158:572–74
172. Merrill, A. H. Jr., Froehlich, J. A., McCormick, D. B. 1979. Purification of riboflavin-binding proteins from bovine plasma and discovery of a pregnancy-specific riboflavin-binding protein. *J. Biol. Chem.* 254:9362–64
173. Merrill, A. H. Jr., Kasai, S., Matsui, K., Tsuge, H., McCormick, D. B. 1979. Spectroscopic studies of pyridoxamine (pyridoxine)5'-phosphate oxidase. Equilibrium dissociation constants and spectra for riboflavin 5'-phosphate and analogues. *Biochemistry* 18:3635–41
174. Merrill, A. H. Jr., McCormick, D. B. 1978. Flavin affinity chromatography: General methods for purification of proteins that bind riboflavin. *Anal. Biochem.* 89:87–102
175. Merrill, A. H. Jr., McCormick, D. B. 1980. Flavin affinity chromatography. In *Methods Enzymol.* 66:338–44
176. Merrill, A. H. Jr., McCormick, D. B. 1980. Purification of riboflavin-binding imunoglobins (IgG) from human plasma. *Fed. Proc.* 39:493 (Abstr.)
177. Merrill, A. H. Jr., Froehlich, J. A., McCormick, D. B. 1981. Isolation and identification of alternative riboflavin-binding proteins from human plasma. *Biochem. Med.* In press
178. Michaelis, L., Schubert, M. P., Smythe, C. V. 1936. The semiquinone of the flavine dyes, including vitamin B_2. *Science* 84:138–39
179. Miller, M. S. 1976. *Function of the carbohydrates of avian riboflavin-binding protein.* PhD dissertation. Penn. State Univ.
180. Miller, R. W., Kerr, C. T., Curry, J. R. 1968. Mammalian dihydroorotate-ubiquinone reductase complex. *Can. J. Biochem.* 46:1099–106
181. Moritani, M., Tung, T., Fujii, S., Mito, H., Izumiya, N., Kenmochi, K., Hirohata, R. 1954. Specificity of rabbit kidney demethylase. *J. Biol. Chem.* 209:485–92
182. Morris, M. P., Pagan, C., Warmke, H. E. 1954. Hiptogenic acid, atoxic component of *Indigofera* endecaphylla. *Science* 119:322–23
183. Muller, F. 1971. On the reaction of flavins with alcohols. See Ref. 109, pp. 363–73
184. Muller, F., Schagen, C. G. V., Van Berkel, W. J. H. 1980. Nuclear magnetic resonance study on flavins and flavoproteins. See Ref. 93, pp. 359–71
185. Muniyappa, K., Adiga, P. R. 1980. Occurrence and functional importance of a riboflavin-carrier protein in the pregnant rat. *FEBS Lett.* 110:209–12
186. Muniyappa, K., Adiga, P. R. 1980. Isolation and characterization of ribofla-

vin-binding protein from pregnant-rat serum. *Biochem. J.* 186:537–40
187. Murray, F. A., Moffatt, R. J., Grifo, A. P. Jr. 1980. Secretion of riboflavin by the porcine uterus. *J. Anim. Sci.* 50:926–29
188. Murthy, U. S., Adiga, P. R. 1978. Estrogen-induced synthesis of riboflavin-binding protein in immature chicks. *Biochim. Biophys. Acta* 538:364–75
189. Nishikimi, M., Kyoguku, Y. 1973. Flavin-protein interaction in egg white flavoprotein. *J. Biochem.* 73:1233–42
190. Nishimoto, Y., Takeuchi, F., Shibata, Y. 1977. Molecular properties of L-kynurenine 3-hydroxylase from rat liver mitochondria. *J. Biochem.* 81:1413–25
191. O'Brien, D. E., Weinstock, L. T., Cheng, C. C. 1970. Synthesis of 10-deazariboflavin and related 2,4-dioxypyrimidoquinolines *J. Heterocycl. Chem.* 7:99–107
192. Oestricher, G., Edmondson, D. E., Singer, T. P. 1976. Binding of 8α-substituted flavins and flavin peptides to *Azotobacter* flavodoxin. See Ref. 30, pp. 447–52
193. Ohishi, N., Yagi, K. 1979. Covalently bound flavin as the prosthetic group of choline oxidase. *Biochem. Biophys. Res. Commun.* 86:1084–88
194. Ohnishi, T., Salerno, J. C., Maida, T., Yu, C. A., Nagaoka, S., King, T. E. 1978. Thermodynamic and EPR properties of hydrogen carriers in the succinate-cytochrome *c* reductase segment of the respiratory chain. In *Frontiers of Biological Energetics,* ed. L. Dutton, J. Leigh, A. Scarpa, 1:165–173. New York: Academic
195. Okuda, J., Nagamine, J., Okumura, M., Yagi, K. 1978. Metabolism of injected flavins studied by using double-labeled [^{14}C] flavin adenine dinucleotide and [^{14}C, ^{32}P] flavin mononucleotide. *J. Nutr. Sci. Vitaminol.* 24:505–10
196. Omura, H. 1959. Intracellular behavior of the nitrate reductase of animal tissues. *Enzymologia* 20:271–90
197. Ondarza, R. N. 1976. Studies on GSSG- and CoASSG-reductases from rat liver and yeast. See Ref. 30, pp. 492–99
198. Ostrowski, M. C., Kistler, W. S. 1980. Properties of a flavoprotein sulfhydryl oxidase from rat seminal vesicle secretion. *Biochemistry* 19:2639–45
199. Ostrowski, W., Zak, Z., Krawczyk, A. 1968. Riboflavin flavoprotein from egg yolk. Analytical and biophysical data. *Acta Biochim. Pol.* 15:241–59
200. Otani, S. 1976. Studies on roseoflavin: isolation, physical, chemical, and biological properties. See Ref. 30, pp. 323–27
201. Paik, W. K., Kim, S. 1974. N-alkyllysinase: New assay method, purification, and biological significance. *Arch. Biochem. Biophys.* 165:369–78
202. Palmer, G., Massey, V. 1968. Mechanisms of flavoprotein catalysis. In *Biological Oxidations,* ed. T. P. Singer, pp. 263–300. New York: Interscience.
203. Pettit, F. H., Yeaman, S. J., Reed, L. J. 1978. Purification and characterization of branched chain α-keto acid dehydrogenase complex of bovine kidney. *Proc. Natl. Acad. Sci. USA* 75:4881–85
204. Pinto, J., Huang, Y. P., Chandhuri, R., Rivlin, R. S. 1975. Riboflavin excretion and turnover in an unusual case of multiple myeloma. *Fed. Proc.* 23:426A
205. Pinto, J., Rivlin, R. S. 1979. Regulation of formation of covalently bound flavins in liver and cerebrum by thyroid hormones. *Arch. Biochem. Biophys.* 194: 313–29
206. Porter, D., Voet, J. G., Bright, H. J. 1973. Direct evidence for carbanions and covalent N-5-flavin-carbanion adducts as catalytic intermediates in the oxidation of nitroethane by D-amino acid oxidase. *J. Biol. Chem.* 248: 4400–16
207. Porter, D. J. T., Bright, H. J. 1980. Oxidation of dihydronicotinamides by flavin in enzyme and model reactions. *J. Biol. Chem.,* 255:7362–70
208. Poulsen, L. L., Ziegler, D. M. 1979. The liver microsomal FAD-containing monoxygenase: spectral characterization and kinetic studies. *J. Biol. Chem.* 254:6449–55
209. Presswood, R. P., Kamin, H. 1976. Reactions of the reduced form of salicylate hydroxylase. See Ref. 30, pp. 145–60
210. Prosky, L., Burch, H. B., Bejrablaya, D., Lowry, O. H., Combs, A. M. 1964. The effects of galactoflavin on riboflavin enzymes and coenzymes. *J. Biol. Chem.* 239:2691–95
211. Rhodes, M. B., Bennett, N., Feeney, R. E. 1959. The flavoprotein-apoprotein system of egg white. *J. Biol. Chem.* 234:2054–60
212. Rivier, D. A. 1973. Kinetics and Na-dependence of riboflavin absorption by intestine *in vivo. Experientia* 29: 1443–46
213. Rivlin, R. S. 1970. Riboflavin metabolism. *N. Engl. J. Med.* 283:463–72
214. Rivlin, R. S. 1975. Hormonal regulation of riboflavin metabolism. See Ref. 7, pp. 393–426

215. Rivlin, R. S. 1975. Riboflavin and cancer. See Ref. 7, pp. 369–91
216. Rivlin, R. S. 1979. Hormones, drugs, and riboflavin. *Nutr. Rev.* 37:241–45
217. Rivlin, R. S., Langdon, R. G. 1966. Effects of thyroxine on biosynthesis of flavin mononucleotide and flavin adenine dinucleotide. In *Advances in Enzyme Regulation,* ed. G. Weber, 4:45–58. Oxford: Pergamon. 381 pp.
218. Rivlin, R. S., Menendez, C., Langdon, R. G. 1968. Biochemical similarities between hypothyroidism and riboflavin deficiency. *Endocrinology* 83:461–69
219. Rose, R. C. 1980. Water-soluble vitamin absorption in intestine. *Ann. Rev. Physiol.* 42:157–71
220. Rosenberg, I. N., Goswami, A. 1979. Purification and characterization of a flavoprotein from bovine thyroid with iodotyrosine diodinase activity. *J. Biol. Chem.* 254:2318–25
221. Rosenthal, S. R. 1978. Malnutrition and the developing fetus: an investigation of the effects of riboflavin, B-12, and folate deficiencies during pregnancy both on the mother and fetus. *Mt. Siani J. Med.* 45:581–90
222. Roth, J. A., Chassey, B. M., McCormick, D. B. 1966. Coenzyme activities of 2-anilino and 2-morpholine derivatives of FMN with yeast NADPH diaphorase. *Biochim. Biophys. Acta* 118:429–31
223. Ruzicka, F. J., Beinert, H., 1978. A new iron-sulfur flavoprotein of the respiratory chain. *J. Biol. Chem.* 252:8440–45
224. Salach, J., Walker, W. H., Singer, T. P., Ehrenberg, A., Hemmerich, P., Ghisla, S., Hartmann, U. 1972. Studies on succinate dehydrogenase. XVI. Site of attachment of the covalently bound flavin to the peptide chain. *Eur. J. Biochem.* 26:267–78
225. Salerno, J. C., Ohnishi, T. 1978. Electron and proton transfer in succinate-cytochrome *c* reductase segment of the respiratory chain. See Ref. 194, pp. 191–200
226. Schopfer, L. M., Massey, V. 1980. Kinetic and mechanistic studies on the oxidation of the melilotate hydroxylase. 2-OH-Cinnamate complex by molecular oxygen. *J. Biol. Chem.* 255:5355–63
227. Schulz, G. E., Schirmer, R. H., Sachsenheimer, W., Pai, E. F. 1978. The structure of the flavoenzyme glutathione reductase. *Nature* 273:120–24
228. Siegfried, C. M. 1978. Solubilization of vitamin K epoxide reductase and vitamin K-dependent carboxylase from rat liver microsomes. *Biochem. Biophys. Res. Commun.* 83:1488–95
229. Singer, T. P. 1963. Flavoprotein dehydrogenases of the electron-transport chain (Survey). *Enzymes* 7:345–81
230. Singer, T. P., ed. 1976. *Flavins and Flavoproteins,* V. Amsterdam: Elsevier. 813 pp.
231. Singer, T. P., Edmondson, D. E. 1974. 8α-Substituted flavins of biological importance. *FEBS Lett.* 42:1–14
232. Singer, T. P., Edmondson, D. E. 1980. Structures, properties, and determination of covalently bound flavins. *Methods Enzymol.* 66:253–64
233. Singer, T. P., Steenkamp, D. J., Kenney, W. C., Beinert, H. 1980. Structure and reaction mechanism of trimethylamine dehydrogenase. See Ref. 93, pp. 377–87
234. Sirivech, S., Driskell, J., Frieden, E. 1977. NADH-FMN oxidoreductase activity and iron content of organs from riboflavin-deficient rats. *J. Nutr.* 104:739–45
235. Shiga, K., Tollin, G., Falk, M. C., McCormick, D. B. 1975. Binding and oxidation-reduction of monoamine oxidase-type 8α-(S-peptidyl) flavins with *Azotobacter* (Shethna) flavodoxin. *Biochem. Biophys. Res. Commun.* 66: 227–34
236. Smith, L. H. Jr., Bauer, R. L., Craig, J. C., Chan, R. P. K., Williams, H. E. 1972. Inhibition of oxalate synthesis. In vitro studies using analogs of oxalate and glycolate. *Biochem. Med.* 6:317–32
237. Sorrell, M. F., Frank, O., Thompson, A. D., Aquino, H., Baker, H. 1971. Absorption of vitamins from the large intestine *in vivo. Nutr. Rep. Int.* 3: 143–48
238. Spector, R., Boose, B. 1979. Active transport of riboflavin by the isolated choroid plexus *in vitro. J. Biol. Chem.* 254:286–89
239. Spector, T., Massey, V. 1972. p-Hydroxybenzoate hydroxylase from *Pseudomonas fluorescens:* evidence for an oxygenated flavin intermediate. *J. Biol. Chem.* 247:5632–36
240. Spencer, R., Fisher, J., Walsh, C. 1976. Preparation, characterization, and chemical properties of the flavin coenzyme analogues 5-deazariboflavin, 5-deazariboflavin 5'-phosphate, and 5-deazariboflavin 5'-diphosphate, 5-5' adenosine ester. *Biochemistry* 15: 1043–53
241. Steenkamp, D. J., Gallup, M. 1978. The natural flavoprotein electron acceptor

of trimethylamine dehydrogenase. *J. Biol. Chem.* 253:4086–89
242. Steenkamp, D. J., Kenney, W. C., Singer, T. P. 1978. A novel type of covalently bound coenzyme in trimethylamine dehydrogenase. *J. Biol. Chem.* 253:2812–12
243. Steenkamp, D. J., McIntire, W., Kenney, W. C. 1978. Structure of the covalently bound coenzyme of trimethylamine dehydrogenase. Evidence for a 6-substituted flavin. *J. Biol. Chem.* 253:2818–24
244. Strittmatter, P., Rogers, M. J., Spatz, L. 1972. The binding of cytochrome b_5 to liver microsomes. *J. Biol. Chem.* 247:7188–94
245. Suelter, C. H., Metzler, D. E. 1960. The oxidation of a reduced pyridine nucleotide analog by flavins. *Biochim. Biophys. Acta* 44:23–33
246. Surrey, A. R. 1951. Alkaline hydrolysis of riboflavin. *J. Am. Chem. Soc.* 73:2336–38
247. Tachibana, S. 1967. The formation of new flavin phosphates by molds. *J. Vitaminol.* 13:70–79
248. Tachibana, S. 1967. Discovery of a new flavin phosphate synthetase which requires guanosine 5'-triphosphate. *J. Vitaminol.* 13:89–92
249. Tachibana, S. 1971. Isolation of riboflavin glycosides. *Methods Enzymol.* 18:413–16
250. Tachibana, S., Murakami, T. 1980. Isolation and identification of schizoflavins. *Methods Enzymol.* 66:333–38
251. Tanaka, K. 1979. Jamaican vomiting sickness. In *Handbook of Clinical Neurology*, ed. P. J. Vinken, G. W. Bruyn, 37:511–39. Amsterdam: Elsevier
252. Theorell, H. 1958. Modern trends in flavoprotein research. *Proc. 4th Int. Congr. Biochem.* 8:167–76.
253. Trachewsky, D. 1978. Aldosterone stimulation of riboflavin incorporation into rat renal flavin coenzymes and the effect of inhibition by riboflavin analogues on sodium resorption. *J. Clin. Invest.* 62:1325–33
254. Tsai, L., Stadtman, E. R. 1971. Riboflavin degradation. *Methods Enzymol.* 18:557–71
255. Tsibris, J. C., McCormick, D. B., Wright, L. D. 1966. Studies on the binding and function of flavin phosphates with flavin mononucleotide-dependent enzymes. *J. Biol. Chem.* 241:1138–43
256. Tsuda, H., Noguchi, T., Kido, R. 1972. 5-Hydroxytryptophan pyrrolase in rat brain. *J. Neurochem.* 19:887–90
257. Tu, S.-C., McCormick, D. B. 1973. Photoinactivation of porcine D-amino acid oxidase with flavin adenine dinucleotide. *J. Biol. Chem.* 248:6339–47
258. Vermilion, J. L., Coon, M. J. 1974. Highly purified detergent-solubilized NADPH-cytochrome P-450 reductase from phenobarbital-induced rat liver microsomes. *Biochem. Biophys. Res. Commun.* 60:1315–22
259. Vermilion, J. L., Coon, M. J. 1976. Properties of a highly purified detergent-solubilized NADPH-cytochrome P-450 reductase from liver microsomes. See Ref. 30, pp. 674–78
260. Vermilion, J. L., Coon, M. J. 1978. Purified liver microsomal NADPH-cytochrome P-450 reductase: spectral characterization of oxidation-reduction states. *J. Biol. Chem.* 253:2694–704
261. Walker, W. H., Singer, T. P., Ghisla, S., Hemmerich, P. 1972. Studies on succinate dehydrogenase. 8α-Histidyl-FAD as the active center of succinate dehydrogenase. *Eur. J. Biochem.* 26:279–89
262. Walsh, C. 1978. Chemical approaches to the study of enzymes catalyzing redox transformations. *Ann. Rev. Biochem.* 47:881–931
263. Walsh, C., Fisher, J., Spencer, R., Graham, D. W., Ashton, W. T., Brown, J. E., Brown, R. D., Rogers, E. F. 1978. Chemical and enzymatic properties of riboflavin analogs. *Biochemistry* 17: 1942–51
264. Walsh, C. T., Schonbrunn, A., Abeles, R. H. 1972. Studies on the mode of action of D-amino acid oxidase: Evidence for removal of α-hydrogen as proton. *J. Biol. Chem.* 246:6885–66
265. Warkany, J. 1975. Riboflavin deficiency and congenital malformations. See Ref. 7, pp. 279–302
266. Watanabe, S. 1959. Metabolism of riboflavin by animal tissues. *J. Vitaminol.* 5:254–60
267. Watenpaugh, K. D., Sieker, L. C., Jensen, L. H. 1973. The binding of riboflavin-5'-phosphate in a flavoprotein: Flavodoxin at 2.0Å resolution. *Proc. Natl. Acad. Sci. USA* 69:3185–88
268. Watenpaugh, K. D., Sieker, L. C., Jensen, L. H. 1976. A crystallographic structural study of the oxidation states of *Desulfobrio vulgaris* flavodoxin. See Ref. 30, pp. 405–10
269. Watt, G. D., Burns, A. 1975. The thermochemical characterization of sodium dithionite, FMN, FAD, and methyl and benzyl viologens as low-potential reductants for biological systems. *Biochem. J.* 152:33–37

270. Waud, W. R., Rajagopalan, K. V. 1976. Rat liver xanthine dehydrogenase: Studies on dehydrogenase to oxidase conversion. See Ref. 30, pp. 566–72
271. Weiner, J. H., Dickie, P. 1979. Fumarate reductase of *Escherichia coli:* Elucidation of the flavin component. *J. Biol. Chem.* 254:8590–93
272. Whitby, L. G. 1971. Glycosides of riboflavin. See Ref. 131, pp. 404–16
273. Whitlon, D. S., Sadowski, J. A., Suttie, J. W. 1978. Mechanism of coumarin action, significance of vitamin K epoxide reductase inhibition. *Biochemistry* 17:1371–77
274. Wierenga, R. K., de Jong, R. J., Kalk, K. H., Hol, W. G. J., Drenth, J. 1979. Crystal structure of p-hydroxybenzoate hydroxylase. *J. Mol. Biol.* 131:55–73
275. Williams, C. H. 1976. Flavin-containing dehydrogenases. See Ref. 7, pp. 89–173
276. Winter, W. P., Buss, E. G., Clagett, C. O., Boucher, R. V. 1967. The nature of the biochemical lesion in avian renal riboflavinuria. II. The inherited change of a riboflavin-binding protein from blood and eggs. *Comp. Biochem. Physiol.* 22:897–906
277. Wu, F.Y-H., MacKenzie, R. E., McCormick, D. B. 1970. Kinetics and mechanism of oxidation-reduction reactions between pyridine nucleotides and flavins. *Biochemistry* 9:2219–24
278. Yagi, K., Ohishi, N., Sato, M., Kuichi, K., Noguchi, E., Nishikimi, M. 1980. Detection of proteins containing covalently-bound flavin by polyacrylamide gel electrophoresis. See Ref. 93, pp. 245–50
279. Yagi, K., Okuda, J. 1958. Phosphorylation of riboflavin by transferase action. *Nature* 181:1663–64
280. Yagi, K., Yamano, T., eds. 1980. *Flavins and Flavoproteins,* VI. Tokyo: Japan Sci. Soc. Press. 740 pp.
281. Yamashina, S. 1971. Histochemical detection technique of glucose oxidase. *Acta Histochem. Cytochem.* 4:52–56
282. Yang, W. K., Sung, J. L. 1967. Riboflavin metabolism in liver diseases. V. Clinical studies on the enzymic splitting of flavin adenine dinucleotide in serum. *Taiwan I-Hsuch-Hui Tsa-Chih* 65:306; *Chem. Abstr.* 55:63475.
283. Yasukochi, Y., Peterson, J. A., Masters, B. S. S. 1979. NADPH-cytochrome *c* (P-450) reductase: Spectrophotometric and stopped flow kinetic studies on the formation of reduced flavoprotein intermediates. *J. Biol. Chem.* 254:7094–104
284. Youdim, M. B. H. 1976. Rat liver mitochondrial monoamine oxidase—An iron-requiring flavoprotein. See Ref. 30, pp. 593–604
285. Yubisui, T., Matsuki, T., Tanishima, K., Takeshita, M., Yoneyama, Y. 1977. NADPH-flavin reductase in human erythrocytes and the reduction of methemoglobin through flavin by the enzyme. *Biochem. Biophys. Res. Commun.* 76:174–82
286. Ziegler, D. M., Poulsen, L. L. 1978. Hepatic microsomal mixed-function amine oxidase. *Methods Enzymol.* 52:142–51.