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Flavin Nucleotides and Flavoproteins[1]

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Due to their bright yellow color and powerful yellow-green fluorescence, the flavins were among the earliest substances to be recognized in various tissues as participants in enzymic processes. Following its isolation from various biological tissues[3], the structure of riboflavin[4], or Vitamin B_2 as it later became known, was established unequivocally as 6,7-dimethyl-9-(D-1'-ribityl) isoalloxazine (*a* in Fig. 1) through its total synthesis in 1935 independently by the laboratories of KUHN[5] and KARRER[6]. Evidence was soon produced, however, that the nucleoside, Rb, existed principally as a precursor or a breakdown product of naturally occurring flavin nucleotides. From the highly purified "old yellow enzyme", discovered earlier by WARBURG and CHRISTIAN[7], THEORELL[8] isolated and characterized the prosthetic group as FMN (*b* in Fig. 1). Following this discovery of the first flavin nucleotide, WARBURG and CHRISTIAN in 1938[9], continuing their monumental study of flavins and flavoproteins, isolated and characterized the dinucleotide, FAD (*c* in Fig. 1), and showed its participation as the coenzyme of the D-amino acid oxidase.

Thus, the stage was set with a knowledge of the nucleotide forms of flavin, as well as the vitamin precursor, and the photodegradation products, lumiflavin (6,7,9-trimethyl isoalloxazine) and lumichrome (6,7-dimethyl alloxazine). It was not surprising, therefore, that the ensuing years should witness the isolation and characterization of a variety of flavin-containing enzymes. These past developments have been reviewed comprehensively by THEORELL[10] and by SINGER and KEARNEY[11], and it is the purpose of the present paper to summarize briefly only the more recent extensions in the rapidly expanding field of flavin nucleotides and flavoproteins, particularly those areas with which our laboratory has been concerned.

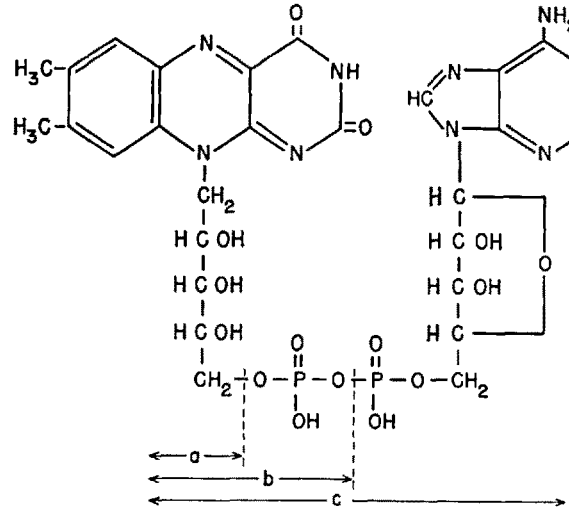

Fig. 1.–Structure of Flavin Nucleotides.

Isolation and Assay of Flavin Nucleotides.–In order to isolate and characterize various flavins, it has been necessary for investigators to devise specific analytical methods for these compounds, which must be sufficiently sensitive to detect a given flavin among large quantities of other nucleotides or other flavins.

The standard microbiological assay for Rb with *L. casei* first introduced by SNELL and STRONG[12] has continued to be the most specific and reliable assay for flavins derived from Rb. Specific enzymatic assays,

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[2] Department of Biochemistry, University of Washington, Seattle, Washington.

[3] R. KUHN, P. GYÖRGY, and T. WAGNER-JAUREGG, Ber. dtsch. chem Ges. *66*, 317 (1933). – P. ELLINGER and W. KOSCHARA, Ber. dtsch. chem. Ges. *66*, 315 (1933).

[4] The following abbreviations will be used: Rb, riboflavin; FMN, riboflavin-5'-phosphate or flavin mononucleotide; FAD, flavin-adenine-dinucleotide; FAD-X, a cyclic form of FAD; AMP, ADP, and ATP, adenosine mono-, di-, and triphosphates; PP, pyrophosphate; CoA, Coenzyme A.

[5] R. KUHN, K. REINEMUND, H. KALTSCHMITT, R. STRÖBELE, and H. TRISCHMANN, Naturwissenschaften *23*, 260 (1935).

[6] P. KARRER, K. SCHÖPP, and F. BENZ, Helv. chim. Acta *18*, 426 (1935).

[7] O. WARBURG and W. CHRISTIAN, Biochem. Z. *254*, 438 (1932); *257*, 492 (1933).

[8] H. THEORELL, Biochem. Z. *272*, 155 (1934); *278*, 263 (1935); *290*, 293 (1937).

[9] O. WARBURG and W. CHRISTIAN, Biochem. Z. *295*, 261 (1938); *296*, 294 (1938); *297*, 417 (1938); *298*, 150 (1938).

[10] H. THEORELL in *Methoden der Fermentforschung*. Vol. III, ed. by E. BAMANN and K. MYRBÄCK, Photo Offset Reproduction (Academic Press, Inc., New York, 1945, pp. 2361–84. – H. THEORELL in *The Enzymes*, Vol. II, Part 1, ed by J. B. SUMNER and K. MYRBÄCK (Academic Press, Inc., New York, 1951), pp. 335–56.

[11] T. P. SINGER and E. B. KEARNEY in *The Proteins*, Vol. II, Part 2 (in press), ed. by H. NEURATH and K. BAILEY (Academic Press, Inc., New York, 1951).

[12] E. E. SNELL and F. M. STRONG, Ind. Eng. Chem. Anal. Ed. *11*, 346 (1950).

using dissociated flavoproteins, are available for certain of the flavin nucleotides[13]. Thus, FAD and FMN may be assayed accurately in minute quantities ($< 1\ \mu g$) using the apoenzymes of the D-amino acid oxidase[14] or the TPNH-cytochrome *c* reductase[15], respectively.

As with many other nucleotides, the difficulty in obtaining the pure flavins in crystalline form has delayed the establishment of accurate physical constants for these substances. However, the characteristic absorption spectrum (λ_{max} at 260, 375 and 450 mμ) of the flavins has recently taken on new significance as an analytical tool, since WHITBY[16] has provided precise values for the molecular extinction coefficients of pure Rb, FMN and FAD at these wave lengths. The intense yellow-green fluorescence has been used chiefly for the *qualitative* detection of flavins, although BESSEY *et al.*[17] have devised a *quantitative* procedure for the fluorometric estimation of Rb, FMN and FAD. In this connection, WEBER[18] has carried out a definitive study of the quenching effect of various substance supon the fluorescence of the isoalloxazine structure including the very interesting phenomenon of self-quenching by the adenine moiety in FAD (*cf.* also[17]).

Finally, the fluorescence of the flavins also enables extremely small quantities to be located on paper chromatograms. In this laboratory and elsewhere[19] the method of paper chromatography has emerged as one of the most powerful tools for the separation and identification of individual flavins. Furthermore, we have been able to utilize information gained from paper chromatograms to devise systems for the large-scale separation of particular flavins. Thus, a partition column employing phenol-butanol-water with Celite as the supporting medium has been used to separate FAD-X from FAD[20] (see also[16] for the separation of flavins by partition chromatography), while adsorption columns of Celite with phosphate buffer as the developing solvent will effect a separation of Rb, FMN and FAD[21]. It should be added that the separation of the above three classes of flavins may be accomplished also by adsorbing the mixture from a mildly alkaline solution on an ion-exchange resin (IRA-400) and eluting in order with water (Rb), 0·1 N acetic acid-acetate buffer (FMN) and 1·0 N buffer (FAD)[21].

Vitamin Analogues.–Several years ago WHITBY observed that riboflavin, incubated with liver homogenates, gave rise to a new and unidentified flavin, as judged by paper chromatography[22]. Further studies on the purified compound disclosed that it was, in fact, a glucoside of Rb. At present, it is not known whether this novel enzymatic reaction involves a nonspecific formation of a glycoside, with Rb serving conveniently as a chromophoric substrate, or whether the condensation is actually of specific importance in the metabolism of either Rb or glucose.

Almost concurrently with WHITBY's discovery came the announcement by PALLARES and GARZA[23] that lyxoflavin[24] had been isolated from human heart tissue. The identity of the product was tentatively established by comparison with synthetic lyxoflavin prepared by the KUHN-KARRER techniques[25]. Soon thereafter, vitamin activity was ascribed to lyxoflavin on the basis of growth studies with rats[26], chicks[27] and pigs[28]. Despite the convincing nature of these nutritional findings, the natural occurrence of lyxoflavin has been disputed recently by GARDNER *et al.*[29], and by SNELL *et al.*[30], who were unable to detect by a differential microbiological assay any lyxoflavin in the various tissues, including heart, of the rat. Of great interest in SNELL's work, however, was the finding that the organism *L. lactis* is capable of utilizing lyxoflavin for growth without converting it to Rb. Experiments in this laboratory being carried out in collaboration with E. E. SNELL have disclosed that in this organism lyxoflavin is incorporated into a flavin mono- and dinucleotide, which may be abbreviated as LMN and LAD, and that these nucleotides function as flavin coenzymes in the cell. LMN and LAD have been tested for coenzyme activity with the D-amino acid apo-oxidase[14]

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[21] Unpublished observation of this Laboratory.

[22] L. G. WHITBY, Nature *166*, 479 (1950); Biochem. J. *50*, 433 (1952).

[23] E. S. PALLARES and H. M. GARZA, Arch. Biochem. *22*, 63 (1949).

[24] The analog of Rb, wherein lyxityl, the alcohol form of the rare sugar, lyxose, has replaced the ribityl group.

[25] R. KUHN, K. REINEMUND, H. KALTESCHMITT, R. STRÖBELE, and H. TRISCHMANN, Naturwissenschaften *23*, 260 (1935). – P. KARRER, K. SCHÖPP, and F. BENZ, Helv. chim. Acta *18*, 426 (1935).

[26] G. A. EMERSON and K. FOLKERS, J. Amer. Chem. Soc. *73*, *2398* (1951); *73*, 5383 (1951). – J. M. COOPERMAN, W. L. MARUSICH, J. SCHEINER, L. DREKTER, E. RITTER, and S. H. RUBIN, Proc. Soc. Exp. Biol. and Med. *81*, 57 (1952).

[27] J. M. COOPERMAN, W. L. MARUSICH, J. SCHEINER, L. DREKTER, E. RITTER, and S. H. RUBIN, Proc. Soc. Exp. Biol. and Med. *81*, 57 (1952). – H. W. BRUINS, M. L. SUNDE, W. W. CRAVENS, and E. E. E. SNELL, Proc. Soc. Exp. Biol. and Med. *78*, 535 (1951).

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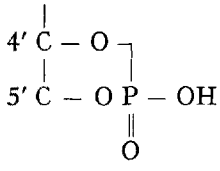
and TPNH-cytochrome *c* apo-reductase[15], respectively, and it has been found that neither is utilized quite as well (i.e., a larger value of the MICHAELIS constant is obtained) as the riboflavin analogues, FMN and FAD. The remarkable specificity of enzymes is once again demonstrated by this ability to distinguish the subtle difference between riboflavin and lyxoflavin coenzymes. In this connection it will be of interest to ascertain whether typical flavin apo-enzymes isolated from *L. lactis* will use the lyxoflavin coenzymes more preferentially than the Rb counterparts.

Flavin Mononucleotides.–The chemical synthesis of riboflavin-5'-phosphate was reinvestigated recently by FORREST and TODD[31] who found that the classical sequence of KUHN *et al.*[32]:

Rb → 5'-Trityl-Rb → 2',3',4'-Acetyl-5'Trityl-Rb → 2',3',4'-Acetyl Rb → 2',3',4'-Acetyl-5'-Phospho Rb →
5'-Phospho Rb

can be obviated, inasmuch as Rb in dry pyridine could be phosphorylated directly with $POCl_3$ in the presence of a trace of water to initiate the reaction. It is of interest that in this simplified synthesis the primary product is riboflavin-4',5'(cyclic) phosphate, i.e.,



which can be hydrolyzed subsequently in acid to yield preferentially the 5'-phosphate, FMN. At the same time, FLEXSER and FARKAS[33] at Hoffman-La Roche developed a similar, large-scale technique with substituted phosphoryl chlorides, enabling their company to supply in commercial lots pure FMN (crystallized as the diethanolamine salt or later, as the sodium salt). Another new method for the synthesis of FMN, wherein Rb is heated directly with metaphosphoric acid, has been described by VISCONTINI *et al.*[34].

Paralleling these chemical studies, the enzymatic synthesis of FMN has been established by KEARNEY and ENGLARD[35]. From brewer's yeast they were able to purify an enzyme, *flavokinase*, which catalyzes the following reaction:

$$Rb + ATP \rightarrow Rb\text{-}5\text{-}P + ADP.$$

Other Rb analogues, such as araboflavin and 6,7-dichloroflavin were also converted to their mononucleotide forms by this enzyme[36]. The enzymatic degradation of FMN has not been explored, as yet, in a systematic way, although is has been shown by HEPPEL and HILMOE[37] that FMN is cleaved to Rb very slowly by a specific 5'-nucleotidase from bull semen.

Interest in riboflavin-5'-*pyro*phosphate has developed recently since this compound might serve as a potential intermediate in the total synthesis of FAD, and because it would be an interesting analog of FMN. The chemical synthesis of riboflavin pyrophosphate has been attempted in this laboratory by treating the silver salt of FMN with $POCl_3$ at low temperature in dimethylformamide. Another synthesis of this compound has also been reported by SERCHI and ALBERTAZZI[38] but details of the method are not yet available. The recent method of HALL and KHORANA, wherein uridine monophosphate is treated with H_3PO_4 in the presence of a carbodiimide to give a good yield of the mixed products, uridine di- and triphosphate[39], would appear to offer still another, and perhaps preferable, route to riboflavin pyrophosphate.

Flavin Dinucleotides.–In addition to the classical method of WARBURG and CHRISTIAN[40] for the isolation of FAD, several more recent methods have been developed. For routine laboratory use, FAD of 10–30% purity on a dry weight basis can be isolated almost quantitatively from suitable tissues, such as liver or yeast, by the method of DIMANT *et al.*[20]. Further purification (50–75%) of this material can be achieved by: (a) partition chromatography with phenol-butanol and water as the solvent system[20]; (b) ion-exchange chromatography on IRA-400, as mentioned above[21] or on Dowex-1; or (c) large-scale paper chromatography using t-butanol: water as the solvent system[41]. A commercial preparation of about 60% purity is now available from the Sigma Chemical Co. Another procedure for the isolation of FAD has been devised by WHITBY[16] which makes use of extraction into phenol, partition chromatography with n-butanol: n-propanol: water as the solvent system and crystallization of the final product in pure form from hot water. The obvious advantages of this latter method are offset slightly by the time required for the various steps and the resultant lower yields.

The chemical synthesis of FAD has been achieved by TODD's group[42] through the condensation of the mono-silver salt of FMN and 2',3'-isopropylidine adenosine-5'-benzyl chlorophosphonate, followed by the removal of the protective isopropylidine and benzyl groups.

[31] H. S. FORREST and A. R. TODD, J. Chem. Soc. *1950*, 3295.
[32] R. KUHN, H. RUDY, and F. WEYGAND, Ber. dtsch. chem. Ges. *69*, 1543 (1936).
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[38] G. SERCHI and G. ALBERTAZZI, *Chimica* (Milan) *8*, 54 (1953); Chem. Abst. *47*, 9555f (1953).
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[40] O. WARBURG and W. CHRISTIAN, Biochem. Z. *298*, 150 (1938).
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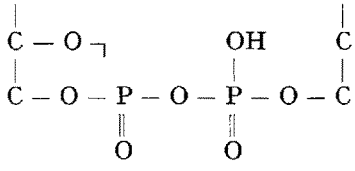
This outstanding achievement not only marks the first total synthesis of any dinucleotide, but also indicates the probable role that chemical synthesis is destined to play in the commercial supply of nucleotides[43].

The mechanism of the biosynthesis of FAD, first recognized by KLEIN and KOHN[44], has been elucidated by SCHRECKER and KORNBERG[45] who purified an FAD-synthetase from brewer's yeast. This enzyme catalyzes the reaction:

$$FMN + ATP \rightarrow FAD + PP$$

which is seen to be analogous to the enzymatic synthesis of diphospho-pyridine nucleotide or coenzyme A. TODD[46] has called attention to the probable similarity in mechanism between enzymatic and chemical syntheses of FAD. The enzymatic degradation of FAD also has received attention, and it has been shown by KORNBERG and PRICER[47] that a general nucleotide pyrophosphatase, isolated from potato, rapidly splits FAD into FMN and AMP.

During the isolation of FAD from various tissues, DIMANT *et al.*[20] observed the presence of an unidentified flavin dinucleotide not identical with FAD. This substance, FAD-X, was later characterized as a *cyclic* form of FAD, i.e.,



since upon enzymatic hydrolysis with nucleotide pyrophosphatase, it yielded riboflavin-4',5'-cyclic-phosphate and AMP[48]. FAD-X has no coenzymatic activity with the D-amino acid apo-oxidase, but it has not yet been tested with other dissociated flavoproteins. It may well be that FAD-X is an artifact resulting from the exposure of FAD to basic solutions during the isolation procedure, since the generation of other cyclic phosphate structures (*e.g.*, adenosine-2',3'-(cyc.)-phosphate, pantothenic-2,4-(cyc.)-phosphate and glucose-1,2-(cyc.)-phosphate during hydrolysis of ribonucleic acid, coenzyme A and uridine-diphosphate-glucose, respectively) is well-established. On the other hand, the ready susceptibility of phosphorylated compounds to assume a *cyclic* phospho-diester structure under the influence of chemical reagents may suggest the occurrence of a corresponding reaction under enzymic control.

Metallo-flavoproteins.–Of more direct enzymic significance has been the discovery of "anomalous" flavins as prosthetic groups of the DPN-cytochrome *c* reductase[49] and the butyryl-CoA dehydrogenase[50]. In the former case, the highly purified enzyme contained a flavin dinucleotide, which was much more labile than either FAD or FAD-X, so that after detachment from the enzyme it is always recovered largely in its mononucleotide and vitamin forms, with only a small amount of dinucleotide remaining. The labile dinucleotide form has no activity in the D-amino acid oxidase system. Upon examining the reductase flavin by paper chromatography, it was surprising to observe that in basic solvent systems, the material exhibited only a "quenching" type spot under ultra-violet light instead of the customary yellow-green fluorescence shown by all other flavins and by this flavin in all other systems[21]. It is of interest moreover, that this "quenching" spot reacts with an aniline trichloracetate spray, indicative of free sugars or easily split glycosides. Some clarification of this problem has been provided by the recent finding[51] that 4 molecules of iron and 1 molecule of flavin are attached to a molecule of enzyme. It is possible that a metalloflavin complex would account for the above "anomalous" properties, or it may be that in addition to the metal, the reductase flavin does possess some novel structural feature.

Interest in the above problem has been stimulated further by our recent observation that the crystalline flavoprotein isolated several years ago by KUNITZ and MCDONALD[52] from baker's yeast, appears to contain the same flavin as the reductase[21]. However, this crystalline flavoprotein, whose catalytic function is still unknown, is definitely not a DPNH or TPNH cytochrome *c* reductase or diaphorase[21] and has, in addition, a second prosthetic group, whose properties have been described by BALL[53].

The butyryl-CoA dehydrogenase isolated in homogeneous form by GREEN and his colleagues[54] as one of a series of enzymes catalyzing the sequence of fatty acid oxidation is likewise an "anomalous" flavoprotein of emerald green color. In this case, the enzyme is also a metallo-flavoprotein with copper as the other prosthetic group[50]. The flavin moiety appears to be identical with FAD, since it responds quantitatively as FAD in the D-amino acid apo-oxidase test system.

[43] The chemical synthesis of FAD has been accomplished also by the direct condensation of FMN and AMP using di-p-tolyl carbodiimide as the catalyst [G. L. KILGOUR and F. M. HUENNEKENS, Fed. Proc. *14*, 236 (1955)].

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[53] E. BALL, J. Gen. Physiol. *29*, 413 (1946).

[54] D. E. GREEN, S. MII, H. R. MAHLER, and R. M. BOCK, J. Biol. Chem. *206*, 1 (1954).

Enzyme	Source	Prosthetic groups		Reference
		Flavin	Other	
Old yellow enzyme	yeast	FMN	–	1
New yellow enzyme	yeast	FAD	–	2
Diaphorase	pig heart	FAD	–	3
Yeast flavoprotein (TPNH oxidase)	brewer's yeast	FAD	400*	4 5 6
Crystalline flavoprotein	yeast	FR**	360	7 8 5
DPNH cytochrome *c* reductase	pig heart	FR	Fe^{+++}	9 10
DPNH cytochrome *c* reductase	*E. coli*	FAD	–	11
DPNH Peroxidase	*S. faecalis*	FAD	–	12
DPNH Menadione reductase	*S. faecalis*	FMN, FAD	–	13
DPNH-Nitrite reductase	*Neurospora Crassa*	FAD	–	14
DPNH, TPNH-Nitrite reductase	soy bean leaves	FAD, FMN	Mn^{++}	14
TPNH cytochrome *c* reductase	pig liver	FAD, FMN	–	15
TPNH cytochrome *c* reductase	beer yeast	FMN	–	16
TPNH-Nitrate reductase	*Neurospora Crassa*	FAD	Molybdenum	17
TPNH-Hydroxylamine reductase	*Neurospora Crassa*	FAD	–	18
Xanthine oxidase	milk	FAD	Molybdenum, 410	19 20 21
Aldehyde oxidase	pig liver	FAD	Molybdenum, Iron protoporphyrin	22 23 24
D-Amino acid oxidase	sheep kidney	FAD	–	25
L-Amino acid oxidase	snake venom	FAD	–	26
L-Amino acid oxidase	rat kidney	FMN	410	27 28
L-Amino acid oxidase	*Neurospora Crassa*	FAD	–	29
Glycine, D-Serine oxidase	pig kidney	FAD	410	30 5
D-Aspartic acid oxidase	rabbit kidney and liver	FAD	–	31
L-α-hydroxy acid oxidase	hog kidney	FMN	–	32
L-Lactic oxidase	*Mycobacterium phlei*	FAD	–	33
Glycollic acid oxidase	spinach	FMN	–	34
Glycollic acid oxidase	rat liver	FAD, FMN	–	35
Lactic oxidase	baker's yeast	FMN	cyt. b_2	36
Diamine oxidase	pig kidney	FAD	–	37
4-Amino azobenzene reductase	rat liver	FAD	–	38
Acyl CoA dehydrogenase (green)	beef liver	FAD	Cu^{++}	39
Acyl CoA dehydrogenase (yellow)	beef liver	FAD	Fe^{+++}	40
Acyl CoA dehydrogenase-cytochrome *c* reductase	beef liver	FAD	–	41
Fumaric hydrogenase	yeast	FAD	–	42
Glucose dehydrogenase	*P. Notatum*	FAD	–	43
Hydrogenase	*Clostridium pasteurianum*	FAD	Molybdenum	44 45

* Indicates absorption maximum of other group.

** Reductase flavin.

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[5] Unpublished observation of this Laboratory.
[6] D. E. GREEN (private communication).
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[8] E. BALL, J. Gen. Physiol. *29*, 413 (1946).
[9] H. R. MAHLER, N. K. SARKAR, L. P. VERNON, and R. A. ALBERTY, J. Biol. Chem. *199*, 585 (1952).
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In basic solvents, however, it gives rise to the same "quenching" spot as the reductase flavin[21].

Only brief mention will be made to the general subject of metallo-flavoproteins, since this has been reviewed in detail by MAHLER and GREEN[55]. It has been found by these investigators, and in other laboratories, that a number of flavoproteins have their electron transport scheme linked to cytochrome *c* through a metal ion, *viz.*,

$$\text{substrate} \rightarrow \text{flavin} \rightarrow \text{metal} \rightarrow \text{cytochrome } c.$$
$$\downarrow$$
$$\text{dye}$$

If the metal ion is lost during purification, then oxidation of the substrate can be coupled only with the reduction of a dye such as methylene blue or 2,6-dichlorophenol indophenol (*i.e.*, a "diaphorase" type of reaction), but not with the reduction of cytochrome *c*. At present the known metallo-flavoproteins include: (a) Xanthine oxidase[56], (b) aldehyde oxidase[57], (c) DPNH nitrate reductase[58], (d) hydrogenase[59], (e) DPNH cytochrome *c* reductase[51], (f) acyl CoA dehydrogenase (yellow enzyme)[60], and (g) acyl CoA dehydrogenase (green enzyme)[50]. Enzymes *a* through *d* contain molybdenum, *e* and *f* contain iron, while *g* contains copper.

Pseudo-flavins.–Mention should be made of a recently discovered substance, some of whose properties, and participation in processes usually mediated by flavins, give it the appearance of a "pseudo-flavin". This material, which can serve as a carrier for the oxidation of DPNH by O_2 catalyzed by a pig heart DPNH oxidase[61], is yellow-brown (λ_{max} at 400 mμ) with a weak yellow fluorescence. It is soluble in water but can be extracted into n-butanol. From a preliminary study its properties resemble somewhat those of toxoflavin, isolated many years ago by VAN VEEN and MERTENS[62] from cultures of *Pseudomonas cocovenen* (*Bacterium bongkrek*), and those of the pigment attached to the lactic oxidase isolated from *Mycobacterium smegmatis* by EDSON and COUSINS[63].

Flavoproteins.–The steady growth in knowledge of the chemical and physical properties of the flavins, and the appearance of new flavins, have been paralleled by an increase in the number of established flavoprotein enzymes and a knowledge of the mechanisms by which flavins exert their coenzyme action. The Table is a summary of the known flavoproteins indicating the nature of the flavin, and other, prosthetic groups. With the continued and widespread interest in this area of enzymology, one may look forward with assurance to the continuous expansion of this Table.

Zusammenfassung

Der vorliegende Überblick ist eine kurze Zusammenfassung der gegenwärtigen Entwicklung auf dem Gebiete der Flavinnukleotide und der Flavoproteine.

Beschrieben werden verbesserte chromatographische Methoden (Ionenaustausch, Spaltung und Adsorption) für die Isolierung von Flavinnukleotiden. Diese Substanzen können durch spezifische mikrobiologische und enzymatische Untersuchungen, Absorptions- und Fluoreszenzspektra und Papierchromatographie identifiziert und voneinander unterschieden werden.

Drei Klassen von Flavinen werden im Hinblick auf verschiedene Eigenschaften einschliesslich chemischer und enzymatischer Synthese und enzymatischen Abbaus besprochen: a) Vitamin-Analoga (Riboflavin, Riboflavinylglukosid und Lyxoflavin); b) Flavinmononukleotide (Riboflavin-5′-Phosphat, Riboflavin-4′-5′-Phosphat [Ringform] und Riboflavin-5′-Pyrophosphat); c) Flavindinukleotide (Flavin-Adenin-Dinukleotid und ein zyklisches Analogon). Ausserdem werden Metallo-Flavine und ein neuerdings entdeckter Elektronenträger, genannt Pseudoflavin, behandelt.

Die bekannten Flavoproteine sind in einer Tabelle in bezug auf die katalysierte Reaktion zusammengefasst. Für jedes Enzym werden das Flavin und andere prosthetische Gruppen angegeben.

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