1 for $X > 0$ and 0 for $X \leq 0$. The solution is $U = {}^1/_2[1 + \mathrm{erf}\,[X/2(Dt)^{1/2}]]$ (Margenau & Murphy, 1956). That is, we can watch how the concentration, U, changes at a point X units away from the boundary $X = 0$. Obviously, equilibrium in this model requires an infinite amount of time, but we can see how long it takes to reach 90 or 99% of the final value for U. For X, we use the size of a domain in the solid phase, 300 nm (Hui & Parsons, 1975). The self-diffusion coefficient, D, has been measured for phospholipids in the low-temperature phase to be 10^{-10} cm^2/s (Fahey & Webb, 1978). Using these values, we find that it takes several minutes to reach 90% of the equilibrium value and several hours to attain 99% of it. We therefore feel that in waiting 24 h in the low-temperature phase we are allowing enough time for solid-phase immiscibility to appear.

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Electronic Transitions in the Isoalloxazine Ring and Orientation of Flavins in Model Membranes Studied by Polarized Light Spectroscopy[†]

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ABSTRACT: The orientation of flavin mononucleotide (FMN) in model membranes and the directions of the transition moments of the first three bands in the electronic absorption spectrum of the oxidized form of the isoalloxazine ring have been determined by means of linear dichroism and polarized fluorescence spectroscopy. Measured counterclockwise relative to the axis connecting the two nitrogens in the central ring (considered positive when going in the direction from –CN< to ⩾N), these angles are 58 ± 4° (450-nm band), 97 ± 3° (350-nm band), and 119 ± 2° (260-nm band).

Biological membranes, being well organized structures, owe many, if not all, of their functions to the spatial arrangement of their constituents. Information about molecular orientation is therefore necessary for unravelling the functional mechanisms of a membrane. This pertains in particular to the enzymes (flavoproteins and cytochromes) in the respiratory chain. Here we have studied the flavin mononucleotide (FMN), which is a prosthetic group of many flavoproteins.

A technique that has long been used for studying the orientation of chromophores in biological membranes is linear dichroism (LD) in which one studies the absorption of plane-polarized light by an ensemble of oriented chromophores (Schmidt, 1938; Johansson et al., 1978). Given the knowledge of the orientation, the directions of the transition moments of the different electronic transitions giving rise to various absorption bands can be determined; conversely, if the directions of the transition moments are already known, information regarding the orientation of the chromophore can be extracted. For highly symmetric molecules, the directions of the transition moments are relatively easy to ascertain; unfortunately, flavins, like most other biological chromophores, do not fall in this convenient category, which makes the determination of the directions of the transition moments a demanding task, but a task that must nonetheless be undertaken if the orientation of FMN is to be studied.

In the absorption spectrum of an oxidized flavin, the first three bands, to be denoted here as I, II, and III, occur at 450, 375–330 (depending on the solvent polarity), and 270 nm, respectively; all three have been assigned to $\pi^* \leftarrow \pi$ transitions (Weber, 1966; Palmer & Massey, 1968; Sun et al., 1972; Yu et al., 1976). Though a great deal of attention has been paid to the spectroscopic aspects of the flavins, no previous work has grappled with the problem which is considered here, viz., the location, in the molecular framework, of all three transition moments. To reach this goal, we have studied the fluorescence polarization of lumiflavin, riboflavin, and FMN and the electric linear dichroism (ELD) of lumiflavin. The results of this study have allowed us to grasp, and describe, the orientation of FMN in model membranes.

Materials and Methods

The absorption spectra were recorded on a Cary 118C spectrometer. Measurements of LD and ELD (Davidsson &

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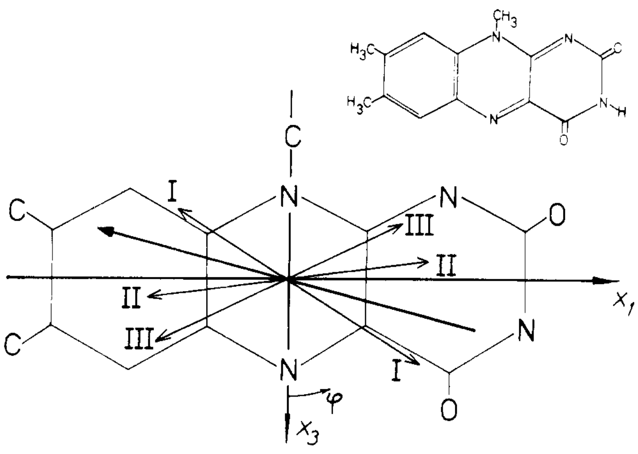

FIGURE 1: Skeleton of the isoalloxazine ring with the $x_1x_2x_3$ coordinate system. The transition moments I, II, and III and the angle ϕ are shown. The molecular structure of lumiflavin is shown in the upper right of the figure.

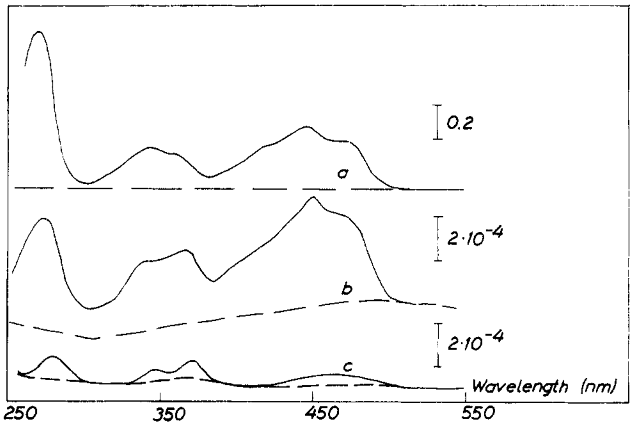

FIGURE 2: (a) Absorption spectrum of lumiflavin in a mixture of $CHCl_3$–CCl_4. (b) ELD for the case when the linear polarized light is parallel to the applied electric field. $E_{app} \simeq 10^6$ V/m. (c) Same as in part b, but at the angle 54.7° between the polarized light and the applied field.

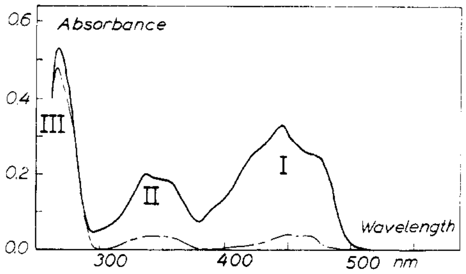

FIGURE 3: Absorbance parallel (—) ($A_{\parallel\mu}$) and perpendicular (- - -) ($A_{\perp\mu}$) to the electric dipole moment.

Norden, 1977; Davidsson, 1978) were performed with a modified Jasco J-40 CD spectrometer. Polarized fluorescence spectra were recorded on a purpose-built instrument of standard design; the band-pass of the excitation monochromator and the analyzing monochromator was limited to 10 nm. The degree of polarization, p, of the fluorescence (monitored at 520 nm) was determined in the usual manner (Albrecht, 1961), and the measured values of p were converted to "intrinsic" values, denoted by p_i, by introducing a randomization factor in the manner described by Albrecht (1961).

Lumiflavin and flavin mononucleotide (FMN; 7,8,10-trimethylisoalloxazine) were purchased from Sigma, and riboflavin was from Koch-Light. Monooctanoin was synthesized at "Syntestjänst", Chemical Center, Lund.

The refractive indexes at 23 °C (the temperature at which all spectra were recorded) of the monooctanoin samples (containing 70% monooctanoin and 30% water) were 1.43 at 589 nm and 1.50 at 250 nm. The solvent used in the ELD measurements was a 1:1 mixture by volume of CCl_4 and $CHCl_3$. The dielectric constant of this was measured with a WTW dipolmeter DMOI to be 3.30 ± 0.05.

Results

Fluorescence Measurements. In the following, we will be interested in the polarization excitation spectrum, i.e., in the variation in p_i with λ_e, the wavelength of excitation. No significant difference was found in the polarization spectra of lumiflavin, riboflavin, and FMN; for our purpose, therefore, the three molecules may be adjudged to be spectroscopically akin, all three faithfully representing the spectral properties of the parent molecule, the oxidized form of the isoalloxazine ring. The results of our study, which are in excellent agreement with the data reported by Chen & Bowman (1965) and by Gordon-Walker et al. (1970) can be briefly stated: $p_i(450) = 0.5$, $p_i(375) = 0.345 \pm 0.01$, and $p_i(260) = 0.100 \pm 0.04$, where λ_e is indicated in the parentheses in nanometers.

The angle, θ, between the absorption dipole and the emission dipole, calculated by using the relation shown in eq 1 and

$$p_i = (3\cos^2\theta - 1)/(3 + \cos^2\theta) \qquad (1)$$

inserting the above figures for $p_i(375)$ and $p_i(260)$, comes out to be 29 ± 1° and 48 ± 3°, respectively. The quantities we seek are $\theta_{I,II}$ and $\theta_{I,III}$, where the former is the angle between the transition moments for bands I and II and the latter is the corresponding angle for bands I and III; $\theta_{I,II}$ and $\theta_{I,III}$ can be equated to 29 and 48°, respectively, only if we assume that, at 375 and 260 nm, absorption arises solely from one transition. A simple calculation shows that even if 1% (1.5%) of the total absorption at 375 nm can be traced to the tail of band I, $\theta_{I,II}$ increases to 31° (33°). We will return to the question of overlapping transitions in the concluding section; we state here only the main outcome of our fluorescence study, for it will serve, so to speak, as the peg on which the next section is to be hung: $\theta_{I,III} > \theta_{I,II} > 30°$.

ELD Measurements (*Figures 2 and 3*). The experiment consists of measuring ELD(0°) and ELD(54.7°), which are defined in eq 2, where $A(\theta')$ denotes the absorbance of the

$$\mathrm{ELD}(0°) = A(0°) - A_r \qquad (2a)$$

$$\mathrm{ELD}(54.7°) = A(54.7°) - A_r \qquad (2b)$$

sample when the applied electric field makes an angle θ' to the electric vector of the linearly polarized measuring light and A_r is the absorbance in the absence of the electric field (i.e., when the molecules are distributed randomly). According to Labhart (1961), the relations shown in eq 3 enable us to

$$[\mathrm{ELD}(0°) - {}^9\!/_5\mathrm{ELD}(54.7°)]\frac{45k^2T^2}{2\mu^2E^2} = \Delta \qquad (3a)$$

$$A_{\parallel\mu} = A_r + (2/3)\Delta \qquad (3b)$$

$$A_{\perp\mu} = A_r - (1/3)\Delta \qquad (3c)$$

evaluate $A_{\parallel\mu}$ and $A_{\perp\mu}$, absorption parallel and perpendicular to the permanent electric dipole moment μ; in the relations shown in eq 3, k stands for the Boltzmann constant, T is the temperature, and E denotes the electric field at the molecule.

The angle, Ω, between the direction of the transition moment and the dipole moment can be calculated in accordance with the relation shown in eq 4, provided that μ and E are known;

$$\Omega = \pm\arctan\,(A_{\perp\mu}/A_{\parallel\mu})^{1/2} \qquad (4)$$

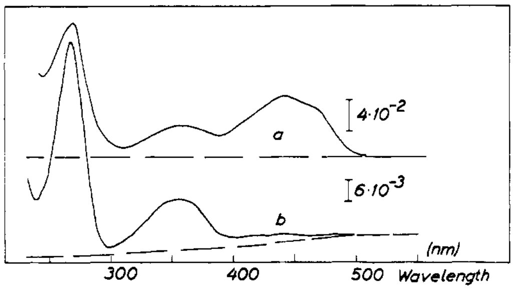

FIGURE 4: (a) Absorption spectrum for FMN solubilized in a lamellar liquid crystal (system A, see text). (b) Corresponding LD for the aligned lamellar mesophase.

unfortunately, this proviso cannot be met for lumiflavin, the molecule subjected to ELD measurement. However, since the angle $\theta_{I,II}$ between the transition moments for the bands I and II is approximately known, it is possible to analyze our data by treating the product μ^2E^2 ($=\xi$, say) as a variable parameter whose value is to be optimized until agreement with the fluorescence polarization study is obtained, bearing in mind that neither $A_{\|\mu}$ nor $A_{\perp\mu}$ can be negative. We found, to our initial dismay, that for $\theta_{I,II} < 39 \pm 4°$ the criterion $A_{\|\mu}, A_{\perp\mu} \geq 0$ could not be fulfilled; the value of ξ corresponding to $\theta_{I,II} = 39°$ was therefore considered to be the optimum and led to the results $\Omega_I = \pm 17°$, $\Omega_{II} = \pm 22°$, and $\Omega_{III} = \pm 44°$.

We need now the direction of the dipole moment in the isoalloxazine ring; at present the only course open to us is to use the result of the semiempirical theoretical calculations of Grabe (1972), who found the angle α between the dipole moment and the x_3 axis in Figure 1 to be 75°; later, independent evidence will be supplied in support of this choice for α. Using her result, we converted the angles Ω into the angles ϕ (measured with respect to the x_3 axis) and arrived at the following values: $\phi_I = 92 \pm 4°$ or $58 \pm 4°$, $\phi_{II} = 97 \pm 3°$ or $53 \pm 3°$, and $\phi_{III} = 119 \pm 2°$ or $31 \pm 2°$. Of the eight possible choices, six can be discounted by invoking the conclusion reached earlier, viz., $\theta_{I,III} > \theta_{I,II} > 30°$, the surviving two sets being $\{\phi_I = 92°, \phi_{II} = 53°, \phi_{III} = 31°\}$ and $\{\phi_I = 58°, \phi_{II} = 97°, \phi_{III} = 119°\}$. We will see shortly that the first set of angles is incompatible with our LD results together with previous data obtained by Eaton et al. (1975). We now state the main conclusion of our study: $\phi_I = 58 \pm 4°$, $\phi_{II} = 97 \pm 3°$, and $\phi_{III} = 119 \pm 2°$.

If we assume that no more than 10% of the absorption of the II and III bands is due to $\pi^* \leftarrow$ n transitions, this would correspond to molar absorptivity contributions of ≤ 800 and ≤ 3000 M^{-1} cm^{-1}, respectively. However, this contribution leads only to very small changes ($\sim 2°$) in the angles ϕ_{II} and ϕ_{III}. Thus, we can conclude that the effect of possible $\pi^* \leftarrow$ n transition will not exceed the experimental error of the method.

LD Studies of FMN Solubilized in Macroscopically Aligned Lamellar Liquid Crystals of Monooctanoin and Water (Figure 4). An almost perfect alignment of some lamellar liquid crystals can be obtained by pressing the sample to a thin layer between quartz plates (Johansson et al., 1978). The order parameter of solubilized chromophores in the lamellae can be determined by measuring the LD at an inclined incidence of light relative to the normal of the plates. The order parameter, S, which is a measure of the degree of orientation of the electronic transition moment relative to the director (i.e., the normal of the lamellae), can be obtained from the relation (Johansson et al., 1978) shown in eq 5, where n

$$(\mathrm{LD}/A_r)_\omega = 3Sn^{-2}[1 - (n^{-2}\cos^2\omega)]^{-1/2}\cos^2\omega \quad (5)$$

is the refractive index of the lamellar phase and ω is the angle between the incident light and the plane of the quartz plates.

Table I[a]

sample composition monooctanoin–H_2O (w/w)	$(\mathrm{LD}/A_r)_I$	$(\mathrm{LD}/A_r)_{II}$	$(\mathrm{LD}/A_r)_{III}$
(A) 90:10	0.016	0.33	0.35
(B) 70:30	−0.016	0.25	0.30
(C) 50:50	−0.021	0.08	0.09

[a] $(\mathrm{LD}/A_r)_{\omega=45°}$ for bands I, II, and III at three different compositions (A, B, C) of monooctanoin–water lamellar liquid crystals, temperature 23 °C.

For a nonsymmetric molecule like the isoalloxazine ring, the measured order parameter, S, can be related to the order parameters of an arbitrarily chosen coordinate system in the molecule (see Figure 1). Thus, $S = \langle D^{(2)}_{00}(\Omega_{DM})\rangle$ can be transformed to a new molecular frame (M′) as follows. Here $D^{(2)}_{00}$ is a Wigner rotation matrix element and Ω_{DM} specifies the Euler angles (α, β, γ) between the molecular (M) and the director frame (D).

For the matrix elements one gets (Brink & Satchler, 1962)

$$\begin{gathered}\langle D^{(2)}_{00}(\Omega_{DM})\rangle = \sum_q \langle D^{(2)}_{0q}(\Omega_{DM'})\rangle\langle D^{(2)}_{q0}(\Omega_{M'M})\rangle = \\ (3/8)^{1/2}\sin^2\phi[\langle D^{(2)}_{0-2}(\Omega_{DM'})\rangle + \langle D^{(2)}_{02}(\Omega_{DM'})\rangle] + \\ (1/2)(3\cos^2\phi - 1)\langle D^{(2)}_{00}(\Omega_{DM'})\rangle + \\ (3/2)^{1/2}(\sin\phi\cos\phi)[\langle D^{(2)}_{0-1}(\Omega_{DM'})\rangle - \langle D^{(2)}_{01}(\Omega_{DM'})\rangle] = \\ S_{11}\sin^2\phi + S_{33}\cos^2\phi + 2S_{13}\sin\phi\cos\phi \quad (6)\end{gathered}$$

Here ϕ is the angle between the transition moment and the x_3 axis of the molecular coordinate system $x_1x_2x_3$ of a planar molecule. The order parameters S_{11}, S_{33}, and S_{13} are related to the Wigner matrix elements and

$$S_{33} = \langle D^{(2)}_{00}\rangle = \langle(3\cos^2\beta - 1)/2\rangle$$

$$S_{11} - S_{22} = (3/2)^{1/2}(\langle D^{(2)}_{02}\rangle + \langle D^{(2)}_{0-2}\rangle) = (3/2)\langle\sin^2\beta\cos 2\gamma\rangle$$

$$S_{13} = (3/8)^{1/2}(\langle D^{(2)}_{0-1}\rangle - \langle D^{(2)}_{01}\rangle) = -(3/2)\langle\sin\beta\cos\beta\cos\gamma\rangle$$

Further

$$S_{11} + S_{22} + S_{33} = 0$$

Thus, if there are three different transitions polarized in the molecular plane and the directions of the moments are known (i.e., the angle ϕ), then the order of parameters S_{11}, S_{33}, and S_{13} can be determined. On the other hand, if the transition moments are unknown, the range of possible angles ϕ can be estimated since the order parameters are subjected to certain inequalities: $-^1/_2 \leq S_{11}, S_{33} \leq 1$, and $-^3/_4 \leq S_{13} \leq {}^3/_4$. This particular property of the order parameters has been used in our work to distinguish between the different values of the angle obtained from our ELD results (or from other studies as well).

The following lamellar systems, with solubilized FMN, have been studied: (A) 90% w/w monooctanoin, (B) 70% w/w monooctanoin, and (C) 50% w/w monooctanoin.

$(\mathrm{LD}/A_r)_{\omega=45°}$ was measured for the I, II, and III bands of FMN for the three different monooctanoin systems, and the results are summarized in Table I. By using eq 5 and 6, the order parameters have been calculated for different angles ϕ for FMN [considering also the data given by Eaton et al. (1975); see below]. These data are summarized in Table II. As can be seen from this table, the only possible combination of the angles is when $\phi_I = 58°$, $\phi_{II} = 97°$, and $\phi_{III} = 119°$,

Table II[a]

ϕ_{I} (deg)	ϕ_{II} (deg)	ϕ_{III} (deg)	A	B	C	order parameter
56	53	119	−1.2	−0.99	−0.38	S_{11}
			−3.1	−2.6	−0.90	S_{22}
			4.3	3.59	1.28	S_{33}
			−0.39	−0.35	−0.13	S_{13}
58	97	119	*0.40*	*0.28*	*0.08*	S_{11}
			−0.20	*−0.21*	*−0.04*	S_{22}
			−0.20	*−0.07*	*−0.04*	S_{33}
			−0.23	*−0.23*	*−0.08*	S_{13}
92	53	119	0.0	0.0	−0.03	S_{11}
			−1.5	−1.3	−0.42	S_{22}
			1.5	1.3	0.45	S_{33}
			−0.11	−0.13	−0.04	S_{13}
92	97	119	−0.20	−0.20	−0.1	S_{11}
			8.9	7.2	2.6	S_{22}
			−8.7	−7.0	−2.5	S_{33}
			−3.1	−2.6	−0.93	S_{13}

[a] Order parameters (S_{11}, S_{22}, S_{33}, and S_{13}, where $\Sigma_{i=1}^{3} S_{ii} = 0$) calculated from eq 5 and 6 using the transition moment directions $\{\phi_{\mathrm{I}}, \phi_{\mathrm{II}}, \phi_{\mathrm{III}}\}$ obtained from the ELD study on lumiflavin. Three different concentrations (A, B, C; see text) of monooctanoin in lamellar liquid-crystalline phase have been used. Note that the results italicized in the table are the only values of the order parameters that are permitted: $-^1/_2 \leqslant S_{11}, S_{22}$ and $S_{33} \leqslant 1$, and $-^3/_4 \leqslant S_{13} \leqslant {}^3/_4$.

since these angles are the only ones that lead to an acceptable set of order parameters.

Discussion

Three optical techniques have been brought to bear on the problem of locating the transition moments for the first three bands in the absorption spectrum of the oxidized form of the isoalloxazine ring. If we calculate relative angles, using the $\{\phi_{\mathrm{I}}, \phi_{\mathrm{II}}, \phi_{\mathrm{III}}\}$ set finally chosen in the last section, we find $\theta_{\mathrm{I,II}} = 39 \pm 4°$ and $\theta_{\mathrm{I,III}} = 61 \pm 6°$, values which are not in satisfactory agreement with those of ~30 and ~50° furnished by the fluorescence study. It is thus natural to inquire into the source(s) of this discrepancy and to assess the reliability of the set of values finally chosen by us. For this purpose, we turn to a comparison of our results with those of some previous studies.

Polarized Spectroscopy of FMN in Single Crystals of Flavodoxin. Eaton et al. (1975) have studied the polarized absorption spectra of FMN in the spectral range covered by bands I and II. They too arrived at a pair of values for each of the angles ϕ_{I} and ϕ_{II}: $\phi_{\mathrm{I}} = 75 \pm 4°$ or $57 \pm 4°$ and $\phi_{\mathrm{II}} = 95 \pm 4°$ or $37 \pm 4°$. They chose the set $\{\phi_{\mathrm{I}} = 75°, \phi_{\mathrm{II}} = 95°\}$, eliminating the other three on the basis of (1) linear dichroism studies, conducted by Lhoste (1971), on stretched PVA films impregnated with lumiflavin and (2) a value of $\theta_{\mathrm{I,II}} = 20 \pm 5°$ deduced by Sun et al. (1972) from their fluorescence polarization data. As stated in the introductory paragraph, the directions of the transition moments can be unambiguously determined only if the orientational distribution of the chromophore is known; Lhoste's conclusion, made in the absence of this information, is therefore unwarranted. Also, the data reported by Sun et al. (1972) were in the form of measured degrees of polarization rather than the spectroscopically more meaningful intrinsic values. A fresh look at the data published by Eaton et al. (1975) is therefore necessary.

It is evident that if we select, from their data, $\{\phi_{\mathrm{I}} = 57°, \phi_{\mathrm{II}} = 95°\}$ as the correct set, the choice would accord well with that made earlier on the basis of our ELD data. One of the reasons offered by Eaton et al. (1975) in support of $\theta_{\mathrm{I,II}} = 20°$ was that it led to the value of 79° for θ_{zc}, in excellent agreement with their experimental value. It is to be noted, however, that $\theta_{\mathrm{I,II}} = 38°$ also agrees with the crystallographic data, giving $\theta_{\mathrm{zc}} = 78°$.

The reader may recall, however, that our ELD data led us to state that $\phi_{\mathrm{I}} = 92$ or 58° and $\phi_{\mathrm{II}} = 97$ or 53° and wonder why, if both papers contain reliable data and their correct choices for the set $\{\phi_{\mathrm{I}}, \phi_{\mathrm{II}}\}$ are in such good agreement with each other, the discarded values of ϕ_{I} and ϕ_{II} in the two papers disagree so conspicuously. Now, for a particular transition each experiment yields two values (equal in magnitude but opposite in sign) for the angle Ω between the transition moment and a reference axis; however, the reference axis in our ELD experiment does not coincide with that used in the single-crystal experiment of Eaton et al. (1975). It follows from elementary geometry that when the angle for a given transition is referred to, say in the x_3 axis, at most one of the two values obtained in one experiment can agree with a value obtained in the other experiment. That such agreement is in fact obtained betokens, in our view, the reliability of both experiments and of Grabe's theoretically calculated value of α.

Photoselection Studies. The study of fluorescence polarization affords, to the wary worker, an exceedingly reliable method for determining the angle θ between the absorption and emission oscillators; however, in spectral characterization of the flavins, uncritical use of the data has been the rule rather than the exception, Weber (1966) being the outstanding exception in our opinion. So as not to be invidious by pointing at the pitfalls committed previously, we will discuss the necessary precautions in relation to our work.

Our first concern was the minimization of instrumental depolarization, hence the decision to dispense with the use of a cryostat and work at room temperature, using glycerol as the solvent. In glycerol, the rotational correlation time of a flavin molecule can be estimated with the aid of the Stokes–Einstein relation to be greater than 100 ns. Fortunately, the first excited state of flavins is quite short-lived, with a lifetime of ~5 ns (Gordon-Walker et al., 1970); during its lifetime, the excited molecule can therefore be assumed to be practically immobile, an assumption which will shortly be substantiated on independent grounds.

Our next concern was to see that $p_{\mathrm{i}}(450)$ can indeed be taken as 0.5, which amounts to taking as zero the angle between the emission oscillator and the transition moment for band I, an assumption that has been explicitly and implicitly questioned in the past. We draw the reader's attention to the data published by Chen & Bowman (1965); they studied fluorescein, rhodamine B, and riboflavin under conditions of negligible rotational and concentration depolarization and found that $p(\lambda_1)$ was close to 0.47, where λ_1 (=450 nm for riboflavin) denotes a wavelength in the region spanned by the first absorption band. Since the lowest energy absorption band in fluorescein and rhodamine B arises from a strong allowed electronic transition ($\epsilon > 10^5$ M^{-1} cm^{-1}) with no signs of vibronic interactions, one expects that $p_{\mathrm{i}}(\lambda_1) = 0.5$, an expectation fully borne out by experimental observation (Szalay et al., 1962, Fleming et al., 1976). One may therefore conclude that for FMN, fluorescein, and rhodamine B, in all cases $p(\lambda_1)$ was close to 0.45; for lumiflavin, $p(450)$ was 0.42. Thus, to a very good approximation, excited solute molecules may be regarded as incapable of significant Brownian rotation within their lifetimes, and the introduction of a randomization factor becomes not merely plausible but essential for a sensible analysis of the data.

Gordon-Walker et al. (1970) determined $\theta_{\mathrm{I,II}}$ by two photoselection methods: fluorescence polarization and dichroic

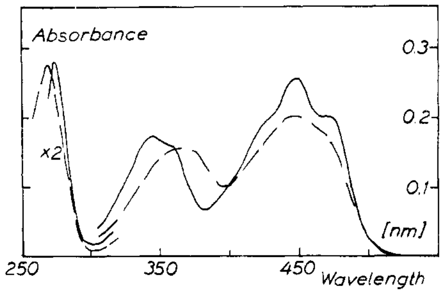

FIGURE 5: Absorption spectra of FMN in glycerol (- - -) and of lumiflavin in a 1:1 mixture (by volume) of chloroform and carbon tetrachloride (—). The absorption band at 270 nm is diminished by a factor of 2.

bleaching, a method introduced first by Lewis & Bigeleisen (1943). Their fluorescence result, i.e., $\theta_{\mathrm{I,II}} = 29 \pm 1°$, is in perfect agreement with ours; more interesting for our purpose is the result of the latter study according to which $\theta_{\mathrm{I,II}} = 38 \pm 5°$, in very satisfactory agreement with our ELD results.

The discrepancy between $\theta_{\mathrm{I,II}}$ determined from fluorescence studies ($\geq 30°$) and that from dichroic bleaching or ELD studies (38°) is most probably due to solvent shifts. As pointed out by Harbury et al. (1959), the wavelength maximum of band II is strongly dependent on the polarity of the solvent, shifting from ~370 nm in water to ~330 nm in carbon tetrachloride. Figure 5 displays the absorption spectra of FMN in glycerol, the solvent used in the fluorescence experiment, and of lumiflavin in a 1:1 mixture of chloroform and carbon tetrachloride, the solvent employed for ELD studies; it can be seen that bands I and II overlap to a much larger extent in glycerol than in the mixed solvent. If one makes the not unreasonable assumption that, in glycerol, 7% of the total absorption at 375 nm is contributed by band I, a value of $\theta_{\mathrm{I,II}} \approx 38°$ is obtained; further support for this is provided by the observation that the degree of polarization decreases as λ is scanned toward wavelengths shorter than 375 nm. The discrepancy between the two values of $\theta_{\mathrm{I,III}}$, 48° deduced from the fluorescence experiment and 61° from the ELD work, is a little more disconcerting and will persist even if allowance is made for the existence of a $\pi^* \leftarrow n$ transition of absorptivity $\leq 3000\ \mathrm{M^{-1}\ cm^{-1}}$. At present we have no satisfactory explanation, but we conjecture that the overlap between band III and the band at 220 nm (band IV) may be responsible for the discrepancy, provided that the latter band is polarized nearly parallel to band I; unfortunately, the position of band IV in the mixed solvent cannot be located due to absorption by the solvent for wavelengths shorter than 260 nm.

Linear Dichroism Studies. The orientation of FMN solubilized in the lamellar liquid-crystalline phase of monooctanoin and different water concentrations has been studied with LD spectroscopy on macroscopically aligned samples. From the magnitude of the order parameters (see Table II) it can be inferred that for the samples called A and B one set of angles ϕ can be unambiguously determined. For the system C, where the water concentration is very high, it is not possible to distinguish between the different sets of angles ϕ by using the same argument as for A and B, since all of the order parameters are very small and the inequalities $-{}^1/_2 \leq S_{ii} \leq 1$ and $-{}^3/_4 \leq S_{ij} \leq {}^3/_4$ can in such cases be satisfied for several sets of the angle. However, one has to demand that for the correct set of angles the order parameters must be acceptable for all three systems. Thus, there is only one choice of the set of angles that can be made, as can be seen in Table II.

Finally, it is interesting to note that the order parameters of FMN in the model membrane are remarkably large. Furthermore, as expected, the orientation of the water-soluble flavin molecule decreases rapidly as the water content in the system increases.

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