*A THEORY OF ENERGY TRANSFER IN THE PHOTOSYNTHETIC UNIT**

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1. *Introduction.*—Recent developments have encouraged us to propose a theory of energy transfer in the photosynthetic unit. The PSU[1] was first postulated as a physiological unit by Emerson and Arnold in 1932.[2] This concept, based on flashing-light studies, now seems firmly established.[3] More recently, attempts have been made to identify the PSU as a definite cellular substructure. Park and Pon have isolated particulate fragments,[4] called quantasomes, from the lamellae of spinach chloroplasts, which can efficiently perform the light reactions of photosynthesis. It has been proposed that quantasomes may be the PSU's of algae and higher green plants.

The role of the PSU, as a unit for transferring energy absorbed anywhere in an aggregate of pigment molecules to a single "active" site, has been discussed by many authors.[5] Following Franck and Teller's initial discussion[6] of physical mechanisms for energy transfer in the PSU, two schools of thought emerged. Förster[7] proposed that energy is transferred between adjacent pigment molecules via weak (dipole-dipole) resonance interactions. His proposal was criticized by Franck and Livingston,[8] but the idea of resonance transfer was revived by Duysens,[9] who studied sensitized fluorescence *in vivo*.

Various strong interaction mechanisms have also been proposed. These include conduction bands, free excitons, and electron or hole diffusion.[5] All of these mechanisms require either electron-orbital overlap or electromagnetic coupling frequencies higher than molecular relaxation rates. Rabinowitch has pointed out that spectroscopic evidence is against the existence of such crystalline or quasi-crystalline states of chlorophyll *in vivo*.[10] We consider it unlikely, therefore, that strong coupling mechanisms play an important role in energy transfer in the PSU. We will show that a theory based on the incoherent limit[11] of weak coupling leads to a working model of the PSU.

Our interaction strengths are taken from Förster's weak coupling case.[12] However, as noted by Rabinowitch[10] and by Lumry and Spikes,[13] a simple pairwise interaction model is inadequate to deal with the PSU, because the "jumping time" (making extreme use of the localized picture) depends on interactions with many neighbors. The transfer rate increases with the number of nearest-neighbor pathways of energy transfer,[11] or branchings, B. Neglecting interactions with nonnearest neighbors, the relation between actual transfer time (reciprocal of rate) t_B and pairwise transfer time t_1 is

$$t_B \doteq t_1/B. \tag{1}$$

Since B increases in general with D, the number of dimensions in which the molecules are arrayed, the transfer rate is greatest, for given t_1, when $D = 3$. The concept that Chl is arrayed in monomolecular layers ($D = 2$), derived from elec-

tron microscope observations of chloroplast lamellae,[14] need not be retained in light of the quantasomes. We consider both $D = 3$ and $D = 2$ cases.

2. *Theory.*—We view the PSU as an aggregate of pigment molecules and an active center, the trap, bound in a lipid-protein environment.[13] We consider only algae and higher green plants in which Chl-a is the most abundant pigment. Recent evidence suggests that there may be two pigment systems absorbing quanta for two light reactions *in vivo*.[15] However, investigators affirm that energy absorbed by any pigment is only usefully trapped for photosynthesis through excited states of Chl-a.[15, 26] Thus, we construct a theory of energy transfer through the bulk Chl-a (of either pigment system) to the trap, which is independent of whether the Chl-a is excited by accessory pigments or directly by the radiation field. In addition, we assume that each PSU transfers energy only to a uniquely associated trap, as is indicated by experiments of Park and Pon[4] and of Butler and Baker.[16]

The apparent red-shift[17, 18] of the trap absorption band with respect to that of the bulk Chl-a justifies the assumption that energy transfer to the trap be highly irreversible. For simplicity, we treat the trap as a perfect energy sink, when the trap is open (see *Discussion*). If the bulk Chl-a molecules have equal probabilities for initial excitation and a uniform spatial distribution (at least for PSU-ensemble averages), the trap is most efficiently located with regard to energy transfer at the geometrical center of the bulk Chl array. However, if the trap is located near an extremity of the array, the mean trapping time is only increased by about a factor of two. Results are given for a central trap.

Because of the inverse sixth power dependence on distance of dipole-dipole interaction rates, regular spacing of the bulk Chl-a molecules is more efficient for energy transfer than random spacing. The high concentration of Chl-a in the PSU prevents large spacing fluctuations. In addition, Butler and Baker's recent finding that the ratios of pigment concentrations in quantasomes are the same as in intact chloroplasts[16] suggests that pigment molecules are bound in the PSU. Thus, it is likely that the spacing fluctuations are quite small. Average fluctuations on the order of 10% reduce the transfer rate only by about 15% from the regular spacing rate. For simplicity, we use a mathematical model based on regular spacing.

As noted in reference 11, the equations for resonance transfer in the incoherent limit are

$$\left(\frac{d}{dt} + \frac{1}{\tau}\right)\rho_k = \sum_{l=1}^{N} F_{kl}(\rho_l - \rho_k), \tag{2}$$

$k = 1, \ldots N$. Here, ρ_k is the average excitation amplitude squared of the k^{th} molecule, N the number of molecules in the aggregate, F_{kl} the pairwise transfer rate between molecules k and l, and τ the excitation decay time constant due to radiation and ordinary quenching processes (degradation to heat).

Since roughly 80% of the dipole-dipole interaction energy comes from nearest-neighbor interactions, we neglect all other interactions for simplicity; inclusion of the remaining terms leads to slightly higher transfer rates. Equations (2) then reduce to a single second-order difference equation, which in turn is very well approximated by a diffusion equation,[19]

$$\left(\frac{\partial}{\partial t} + \frac{1}{\tau}\right)\rho(\mathbf{r},t) = \Lambda\nabla^2\rho(\mathbf{r},t), \tag{3}$$

where ρ as a function of the continuous variable $\mathbf{r}$ is an excitation probability density, $\mathbf{r}$ is a vector defining coordinates in two or three dimensions, ∇^2 the Laplacian operator, and Λ the diffusion constant.

The probability, $P(t)$, that the bulk Chl-a of the PSU, as a whole, is excited at time t is

$$P(t) = \int_{\text{PSU}} \rho(\mathbf{r},t)dV, \tag{4}$$

where dV is an area or volume element. The mean de-excitation time of the PSU is

$$\bar{l} = \int_0^\infty t\left(\frac{dP}{dt}\right)dt \Big/ \int_0^\infty \left(\frac{dP}{dt}\right) dt. \tag{5}$$

We normalize to $P(0) = 1$; then, since $P(\infty) = 0$,

$$\bar{l} = \int_0^\infty P(t)dt. \tag{6}$$

Now,

$$\frac{dP}{dt} = -\left(\frac{dP_T}{dt} + \frac{dP_F}{dt} + \frac{dP_Q}{dt}\right), \tag{7}$$

where dP_T/dt is the rate of trapping, dP_F/dt that of fluorescence, and dP_Q/dt that of quenching. Since the latter two are much smaller than dP_T/dt (see *Discussion*), they can be neglected in (5), and thus $\bar{l} = \bar{l}_T$, the mean trapping time.

In terms of the random walk description, the mean number of jumps to reach the trap, n, is related to the mean trapping time by

$$\bar{l}_T = nt_B, \tag{8}$$

where t_B is given by (1). Thus, $\bar{l}_T$ may be calculated directly from the solution of the diffusion equation, or from (8) if n is known. We rely primarily on the former for the $D = 3$ case. For $D = 2$, recent calculations have given a value for n, which we use below.

$D = 3$ case: The diffusion equation (3) may be solved when appropriate boundary and initial conditions are prescribed. For mathematical simplicity, we approximate the ellipsoidal quantasome by a sphere of radius a, the trap by a small concentric spherical cavity of radius b. It is then most convenient to use a system of spherical coordinates, with $r = |\mathbf{r}|$, θ, and ϕ. The statement that there be no energy transfer between PSU's becomes mathematically,

$$(\partial\rho/\partial r)_{r=a} = 0; \tag{9a}$$

that the trap be a perfect sink, by analogy to the theory of heat conduction,[20]

$$[\rho(\mathbf{r},t)]_{r=b} = 0. \tag{9b}$$

Since we are only interested in average properties of ensembles of PSU's, we may treat the problem as if the excitation energy were initially uniformly delocalized over the PSU.[11] Thus, $\rho(\mathbf{r},0)$ is independent of $\mathbf{r}$, and because $P(0) = 1$, equation (4) gives

$$\rho(\mathbf{r},0) = 3/4\pi a^3(1 - \epsilon^3), \tag{10}$$

where $\epsilon = b/a$. The problem is then independent of θ and ϕ, and equation (3) simplifies to

$$\left(\frac{\partial}{\partial t} + \frac{1}{\tau}\right)\rho = \frac{\Lambda}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial \rho}{\partial r}\right). \tag{3'}$$

Equation (3') together with conditions (9) and (10) may be solved exactly by standard techniques[20] to give

$$\rho(r,t) = \frac{3\epsilon}{2\pi a^3(1-\epsilon^3)}\sum_{i=0}^{\infty}\frac{(1+\xi_i^2)\sin\left[\xi_i\left(\frac{r}{a}-\epsilon\right)\right]e^{-\beta_i t}}{\xi_i[\xi_i^2(1-\epsilon)-\epsilon]r/a}, \tag{11}$$

where $\beta_i = \tau^{-1} + (\Lambda/a^2)\xi_i^2$, and the ξ_i are all of the positive solutions to the eigenvalue equation

$$\tan[\xi_i(1-\epsilon)] = \xi_i, \tag{12}$$

ordered such that $0 < \xi_0 < \xi_1 < \ldots$.

The expression (11) for $\rho(r,t)$ is an expansion in transient diffusion modes. The function $P(t)$ derived from (4) is thus an infinite series of exponential decays. The relative coefficient of the fundamental decay mode is, however, about 95%. The theory thus predicts that the excitation decay is purely exponential, except for very small, rapidly decaying, initial transients.

For $\epsilon = 0.20$ (see *Discussion*), numerical solution of (12) gives $\xi_0^2 = 0.90$. Then, using (4), (6), and (11), and neglecting τ (see *Discussion*), the mean trapping time is

$$\bar{t}_T = 1.1\ a^2/\Lambda. \tag{13}$$

The diffusion constant is given by

$$\Lambda = \bar{R}^2/t_1, \tag{14}$$

where $\bar{R}$ is the mean separation of adjacent Chl-a molecules. For the spherical model, we have, approximately,

$$(a/\bar{R})^2 = (3N/4\pi)^{2/3}. \tag{15}$$

From the preceding, we see that

$$\bar{t}_T = 0.4N^{2/3}t_1, \tag{16}$$

and thus that,

$$n = 0.4BN^{2/3}. \tag{17}$$

D = 2 case: The solution of the random walk problem for a square lattice of N sites is given approximately by[21]

$$n = 1.3N - N^{1/2} \tag{18}$$

where it is assumed that the trap occupies one lattice site at the center of the array. We thus have immediately that

$$\bar{t}_T = (1.3N - N^{1/2})t_B. \tag{19}$$

3. *Results and Discussion.*—In order to make predictions from the theory, it is necessary to estimate values of parameters which are not well known.

Number and spacing of Chl-a molecules: Emerson and Arnold found that under conditions of saturation about 2000 Chl-a molecules are required for each CO_2 reduced in Chlorella.[2] If the quantum requirement for CO_2 reduction of each pigment system is about 4,[15] there is a total of some 500 Chl-a's available to both pigment systems, assuming the two together form a biologically operational unit. It is believed that most of the Chl-a's are associated with pigment system 1 (the long-wavelength absorbing system).[15] Thus, we take $N \simeq 400$ for system 1.

We base our estimates of $\bar{R}$ on the quantasome, described as an oblate spheroid, 100 Å by 200 Å.[4] If the sphere of the $D = 3$ model is assumed to have the volume of the quantasome, $a = 79$ Å, and hence, $\bar{R} = 17$ Å. If the square array of the $D = 2$ model is assumed to have an area equal to half[22] the surface area of the quantasome, $\bar{R} = 11$ Å.

Pairwise transfer time: If we write (for dipole-dipole interactions)

$$t_1 = \tau_0(\bar{R}/R_0)^6, \tag{20}$$

the parameter R_0 may be calculated from Förster's theory if the natural fluorescence lifetime τ_0, the overlap of absorption and fluorescence bands, the relative orientation of the two molecules, and the solvent refractive index μ are known.[23, 24] Förster gives a value of 80 Å for R_0 for Chl-a at low concentration in ethyl ether.[23] However, he assumed a value of 30 nsec for τ_0, whereas more recent calculations give $\tau_0 = 15$ nsec.[25] Based on the latter value of τ_0, $R_0 = 71$ Å for Chl-a in ethyl ether.

We may estimate the value of R_0 *in vivo*. The greater width of the Chl-a (red) absorption band *in vivo* suggests that the spectral overlap there may be different from what it is in solution. However, it now appears that the greater width is due to two forms of Chl-a (possibly belonging to the two pigment systems).[26] Without further specific information, we assume that no correction to R_0 of the solution is required for differing spectral overlap in the plant. Since orientations *in vivo* are also unknown, we accept a random directional distribution as was used in the solution calculation. The refractive index of lipid-like materials, however, is $\sim$1.5, whereas $\mu = 1.35$ for ethyl ether. Thus, as a rough estimate, we find $R_c = 68$ Å for Chl-a *in vivo*.

With the above values of τ_0, R_0, and $\bar{R}$, we find, for system 1, the values of t_1, t_B, and Λ given in Table 1. We use $B = 6$ for $D = 3$, and $B = 4$ for $D = 2$. The diffusion constants are about 10^3 greater than those typical of diffusion of aqueous solutions into water. It appears highly unlikely, therefore, that primary energy transfer could be effected as efficiently by diffusion of high-energy intermediates[10] as by resonance transfer.

Trap parameters: When the trap is open (or active), the mean observable fluorescence lifetime, $\bar{t}_F$, is equal to $\bar{t}_T$. When the trap is closed, however, $\bar{t}_F = \tau$, which follows from (3) when the boundary condition (9b) is replaced by $(\partial\rho/\partial r)_{r=b} = 0$. Since the *primary* process of photosynthesis is thought to be more than 90 per cent efficient, it follows that $\tau \gg \bar{t}_T$. Thus, we have neglected τ in the calculations.

The diffusion model allows us to consider a range of trap sizes in the $D = 3$ case. We assume that the trap occupies a single lattice site, and thus take b to be about

15 Å, which gives $\epsilon = 0.20$. The trapping volume is then large enough to contain a number of chlorophyll molecules, and even the quantatrope of Sauer and Calvin.[27]

System 1 results: The results for n, $\bar{l}_T$, and $\varphi_F = \bar{l}_T/\tau_0$ are shown in Table 1. The $D = 2$ trapping time and fluorescence yield are about half the $D = 3$ result. However, the $D = 2$ actual transfer rate, t_B^{-1}, is greater than intramolecular relaxation rates ν_m, which are $\sim 10^{12}$ to 10^{13} sec^{-1}. Thus, the weak-coupling theory is not valid in the $D = 2$ case, and transfer rates may be even higher than calculated. As noted earlier, spectroscopic evidence rules against strong interactions in the PSU; therefore, we feel that a two-dimensional model is inappropriate for energy transfer in the PSU.

Even though t_B^{-1} for $D = 3$ is comparable to ν_m, the lack of spectroscopic evidence to the contrary indicates that the weak-coupling limit is still applicable as an approximation. The $D = 3$ results for $\bar{l}_T$ and φ_F should therefore be regarded as upper limits.

TABLE 1

SYSTEM 1 RESULTS

D	t_1 psec	t_B psec	Λ cm^2/sec	n	$\bar{l}_T$ psec	φ_F, per cent
2	0.3	0.07	0.040	500	36	0.24
3	4	0.7	0.007	130	86	0.57

System 2 (short-wavelength absorbing system): If a second pigment system, containing fewer Chl-a molecules, exists, it may be expected to make an additional contribution to the fluorescence yield. As a possibility, suppose 100 Chl-a's and a trap were arrayed within a quantasome-equivalent sphere; $\bar{l}_T$ would be about 0.4 nsec, and φ_F about 3 per cent. The *average* yield for both systems together would then be about 1 per cent. Even if both systems have identical fluorescence spectra, a fast-pulse experiment having a resolving time $\leqq 0.1$ nsec would be able to separate the two contributions.

Comparison with experiment: In addition to rapid emission by the bulk Chl-a, presumably at a wavelength around 680 mμ, there are at least two other sources of luminescence *in vivo*. These are rapid emission (supposedly by the trap itself) at around 730 mμ, with a measured lifetime of 3.1 nsec;[17] and slow emission, or delayed light, by both pigment systems predominantly at the shorter wavelength ($\sim$680 mμ).[28] It has recently been suggested that the delayed light extends to times as short as 10^{-6} sec or less, and that the integrated delayed light emission, because of its slow decay, may make a larger contribution to the integrated fluorescence yield than the fast emission components of comparable wavelength.[29]

The 1.7 nsec lifetime of Chl-a *in vivo* measured by Brody and Rabinowitch,[25] the 0.7 nsec lifetime obtained by Butler and Norris,[17] and the 2.7 per cent fluorescence yield of Latimer *et al.*[30] must be re-evaluated in light of these recent developments. Neither the Brody nor the Butler experiments discriminated between the 730 mμ and 680 mμ emissions, and may have given an average value. The Latimer experiment integrated both fast and slow emissions. Hence, the small values of φ_F and $\bar{l}_T$ calculated here are entirely compatible with available experimental evidence.

Summary.—A mathematical model of energy transfer in the photosynthetic unit, based on weak interactions, is developed. Predictions of mean trapping time are

derived from the estimated (dipole-dipole) interaction strength. A diffusion equation, derived directly from the delocalized picture,[11] is used in the calculations.

The trapping times calculated here are surprisingly short compared to previous estimates based on a random-walk description. It appears that two points were overlooked in the latter. First, the number of "jumps" depends sensitively on the number of dimensions. Second, a simple pairwise interaction is inadequate because the transfer rate is proportional to the number of nearest neighbors. These points are accounted for automatically by the diffusion treatment.

Estimates of transfer times, based on the dimensions of the quantasome, are given. Using these, explicit results for fluorescence lifetime and yield are calculated for the long-wavelength absorbing pigment system. The fluorescence lifetime of the short-wavelength system may be somewhat ($\sim$4 times) longer. The compatibility of the short calculated lifetimes ($\sim 10^{-10}$ sec) with known experimental results is demonstrated.

Both two- and three-dimensional arrays of chlorophyll-a molecules are considered. Experimental evidence favors the latter.

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[1] Abbreviations: PSU = photosynthetic unit; Chl = chlorophyll; nsec = nanosecond (10^{-9} sec); psec = picosecond (10^{-12} sec); a bar ($^-$) is used to denote averages.

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POLYRIBOSOME FORMATION AND HEMOGLOBIN SYNTHESIS

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Polyribosomes have been shown to be the major site of protein synthesis in reticulocytes,[1-3] liver,[4] HeLa cells,[5] and *E. coli*.[6] It seems likely that polyribosomes are clusters of ribosomes which are held together by messenger RNA.[1] This has led to the hypothesis that, during protein synthesis, single ribosomes attach to one end of the polyribosome and start the synthesis of a peptide chain. Chain growth proceeds as the ribosome moves along the messenger RNA, and finally completion and release of the polypeptide chain occurs when the ribosome detaches from the cluster at the end of the messenger.[3, 6-8] Earlier evidence for such a mechanism has been indirect. The formation of polyribosomes in the presence of various types of natural and synthetic messenger polynucleotides has been demonstrated.[5, 6, 9, 10] The breakdown of polyribosomes during protein synthesis in cell-free systems yielding single ribosomes with labeled nascent protein is consistent with such a mechanism.[6, 8] We have reported that 80S ribosomes were active in a cell-free system and joined the polyribosome clusters during hemoglobin synthesis and also that polyribosomes broke down during protein synthesis.[11] More detailed studies showed the strict dependence of polyribosome breakdown on protein synthesis and the orderly disappearance of the various sizes of polyribosomes expected for the mechanism postulated above.[12] The formation of polyribosomes using H^3-labeled ribosomes from HeLa cells has been reported.[13] Here, we demonstrate the attachment of P^{32}-labeled 80S ribosomes to polyribosomes correlated with initiation of polypeptide chain synthesis, and detachment of 80S ribosomes correlated with polypeptide chain completion and release. These results provide strong support for the hypothesis described above.

Materials and Methods.—The methods and enzyme preparations used were those described previously unless otherwise noted.[12] To prepare P^{32}-labeled ribosomes, rabbits were given the usual four daily injections of phenylhydrazine at a dosage of 0.9 ml of 2.5% phenylhydrazine per 5 lb. On the evening of the fifth day and the morning and evening of the sixth (36, 24, and 12 hr before bleeding), each rabbit received 1 mC of carrier-free P^{32} by intravenous injection (3 mC total). Carrier-free P^{32} was obtained from the Oak Ridge National Laboratories and was diluted to contain 2 mC/ml at pH 7.4 in 0.01 *N* Tris, 0.9% in NaCl. The rabbits were bled by heart puncture on the morning of the seventh day, and 1X ribosomes (pelleted once) were prepared