A CONNECTED MODEL OF THE PHOTOSYNTHETIC UNIT

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ABSTRACT The concept of photosynthetic unit (PSU) is reviewed in the light of the authors' results in the fields of fluorescence and luminescence (delayed light). Models of PSU are mainly distinguished by the amount of exciton exchange which is allowed between units. The "separate" model, with its "first-order" character, is not consistent with fluorescence kinetic data. The sigmoidal rise of fluorescence under actinic light is best explained by "nonseparate" models; however, most of these models assume a delocalization of excitons or centers. The "connected" model introduced here is not subject to this criticism. It discloses a new effect (the "*îlot*" effect): a nonrandom grouping of fluorescent units the consequences of which are discussed. It is noted that a "two-quantum" model for the photochemical reaction gives results very similar to those of the connected model. A relation between luminescence intensity and fluorescence yield is seen as a necessary consequence of the PSU concept. Its meaning is different in separate and nonseparate models. This relation is discussed in connection with the true system II fluorescence emission.

I. INTRODUCTION

Few ideas have been as fruitful, at least in the field of photophysics and photochemistry of photosynthesis, as that of the PSU. It was one of the outstanding contributions of Emerson, in collaboration with Arnold, to demonstrate in 1932 that chlorophyll acted in a cooperative fashion to collect and trap the light quanta (Emerson and Arnold, 1932 *a*, *b*). In modern terms, we understand that to each photochemical center (system I or system II) there belongs an assembly of about 300 chlorophyll molecules which serves as an antenna for light collection and as a conducting medium for exciton migration during the brief life of electromagnetic energy which precedes photochemical conversion. To those physicochemists and biologists who entered the realm of photosynthesis in the 1950s, it was mainly through the lucid presentation in Rabinowitch's celebrated treatise that the pleasure of this discovery was conveyed (Rabinowitch, 1945). We will attempt to illustrate the concept of PSU (of system II type) and its elaboration in detail mainly through our own contributions in the fields of fluorescence and luminescence (or delayed light).

Early in the history of PSU, the question was raised whether the unit should be conceived as a morphological, separate entity (the "puddle" model) or rather as a "statistical," structureless entity (the "lake" model). The following features inherent in the concept of PSU may be remarked:

(*a*) The center is a fixed and permanent locus within the assembly of collector molecules; kinetically, this is to be contrasted with the greater or lesser degree of freedom of motion of the exciton (following property *b*).

(*b*) As far as exciton migration is concerned, the units may be either disconnected or partially or totally connected; in this sense we may speak respectively of separate, connected, or statistical models. (In "random walk" terminology, we may say that the chlorophyll territory belonging to each unit is separated from neighboring units by reflecting or partly reflecting boundaries, or that the boundaries are virtual.)

(*c*) In the over-all competition between photochemistry, fluorescence, or any other kind of energy dissipation, the centers may act as "absolute" or "relative" traps, in the sense that the probability of trapping may be equal to or less than one.

Before further consideration of these properties and of the models resulting from their combinations, a few general remarks are in order. Property *b* has an immediate consequence from the standpoint of kinetics: only in the separate model are the units truly independent, in the sense that the probability for a photochemical center to trap a light quantum does not depend on the state of the other units of the system; this is not true for the connected or the statistical models. In terms of kinetics, the behavior of the separate model is expected to be first order, whereas that of the two other models will rather be "second order." Property *c* should at once be qualified. In any reasonable model of the PSU, it is common sense to require that the centers in the photoactive or "open" state should be considered as absolute traps (or nearly so); however, the question of where the exciton is confined can be raised for the nonphotoactive or "closed" state of the centers. We will use the term "fluorescent center" in this context to mean that the centers, even in the closed state, "trap" the excitons with high probability and, subsequently, emit light. In units with fluorescent centers, the variable fluorescence is that of the centers, whereas in units with ordinary centers it is to be attributed to the whole unit.

II. FLUORESCENCE

1. *The Separate Units*

All conclusions drawn from fluorescence kinetics of in vivo chlorophyll rest upon a general law that can hardly be overstated. It may be expressed by

$$\Phi_{PS} + \Phi_F/\alpha = 1, \tag{1}$$

which is a conservative linear relation between the photochemical yield Φ_{PS} and the fluorescence yield Φ_F. This law rests upon the well documented assumption that

photochemical conversion (system II) is competing with the natural decay of the first excited state of chlorophyll. It is also commonly assumed that fluorescence is a constant proportion (α) of all nonphotochemical losses. We call this law the "fundamental alternative."

One straightforward application of the law is first given in the case of the photochemical rise of fluorescence yield at the beginning of the "induction" period (Kautsky and Franck, 1943), that is, in response to a moderate-to-strong light intensity after a long time in darkness. The fluorescence induction may be characterized by three consecutive states *O*, *P*, and *S*, of alternating low, high, and medium (to low) fluorescence yield. This pattern is characteristic of an organism like *Chlorella* (Fig. 1 A) and, in general, at least the initial *O* to *P* rise is seen, even with isolated chloroplasts or system II particles prepared from them.

The fluorescence induction clearly exhibits a constant component, the *O* level, and a variable one of maximum amplitude $P - O$. The variable fluorescence obviously is associated with the setting up of photochemistry. In fact, it may be demonstrated that the initial rise, almost to the *P* level, is photochemical (Delosme, 1967). In contrast, the *P* to *S* phase is not directly photochemical; its meaning is very controversial and we will no longer be concerned with it here.

The presence of two fluorescence components may in general be handled taking into account two types of effects (Lavorel, 1962). The first is an intrinsic variation of yield as a result of a change of photochemical state of the units in accordance with equation 1 ("two-yields" hypothesis); the second type would consist of two separate fluorescence emissions arising from different parts of the system; in particular the constant emission might well be a system I fluorescence ("two-emissions" hypothesis). With the inclusion of this second effect, some heterogeneity is expected in the fluorescence spectrum. Such a heterogeneity has been found, although not a large one; the comparison of the emission spectra discloses some difference in the 720 nm range where a fluorescent component is more prominent

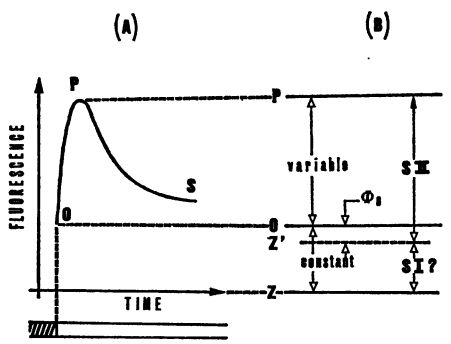

FIGURE 1 (A) Scheme of the normal induction curve of fluorescence (photochemical rise from *O* to *P*, nonphotochemical decay from *P* to *S*). (B) Location of *O* and *P* levels with respect to the hypothetical *Z'* zero level of system II fluorescence.

for the constant than for the variable part. This finding is a strong argument in favor of the origin of the variable component lying somewhere between zero and the O level (at Z' of Fig. 1 B), that is, a combination of the two-yields and the two-emissions hypotheses. An entirely homogeneous emission would mean dropping the two-emissions hypothesis with this origin at zero (Z' at Z). Another not less strong argument is that locating Z' at Z would put one in serious trouble with the energy balance: the O level is typically at 0.3 of the P level (*Chlorella*) and the maximum elementary quantum yield would have the value of 0.7 at most, in consequence of the fundamental alternative, unless the assumption of constant proportion of fluorescence (α in equation 1) in the various decays were dropped, which would be difficult to justify. A high photochemical quantum yield thus implies that Z' lies slightly below the O level. Finally, the variability of position of the O level with respect to the P level encountered in various organisms (and various samples of the same organism) is also an argument against the homogeneous hypothesis. In what follows, it is to be understood that equation 1 is strictly applied to the fluorescence component with the origin at Z'.

Let us for the moment concentrate on the variable fluorescence as a consequence of the first effect and on its relation to the concept of PSU. A low initial yield of fluorescence is expected if all units have their photochemical centers in the active state. In the separate model, assuming a quantum yield of nearly one and an overall electron transfer of negligible rate as compared with the photochemical rate, each unit will change its fluorescent state as soon as it has received a photon. Ultimately, all units will have received at least one quantum, with the result that all centers will have changed from the open to the closed state; that is, all units will have changed from the "non- (or weakly) fluorescent" O state to the "fluorescent" P state.

This process may be simply expressed by:

$$O \xrightarrow{h\nu} P. \tag{2}$$

By virtue of the kinetic independence of the units in this model, the O to P rise should be exponential, giving an exact measure of the increasing number of units in the closed state as a function of time. Alternatively this result can follow from:

(*a*) the fundamental alternative (equation 1) in the form

$$\mathrm{d}[Q^-]/\mathrm{d}t = k^*(1 - \Phi_F), \tag{3}$$

where $[Q^-]$ is the concentration of closed units and k^* is a photochemical constant;

(*b*) the simple relation between Φ_F and $[Q^-]$ in the separate model

$$\Phi_F = \varphi[Q^-]/[Q]_0\,, \tag{4}$$

φ, a constant, being the fluorescence yield of the closed unit.

The O to P rise was actually first seen as an exponential curve (Lavorel, 1959; Kautsky et al., 1960). A priori this behavior is not remarkable, except for the numerical value of k^* in equation 3. For isolated molecules, this first-order photochemical constant is computed as:

$$k^* = 10^3 \epsilon I, \tag{5}$$

where ϵ is the molar extinction coefficient (1/mole centimeter), and I is the quantum intensity (einsteins per second per square centimeter). In our separate unit, the collector of the unit plays the role of the isolated molecule with, however, a photon cross section (ϵ) n times larger. In effect, comparison of k^* calculated for an isolated chlorophyll molecule and of k^* experimentally measured in *Chlorella* yields a value of n of the order of 100, which is what one may expect for the PSU.

2. *The Connected Units and the Statistical Units*

As opposed to the separate model, these two nonseparate models belong to a different family. One might further distinguish them with respect to the minimum degree of built-in "structure" which must be assumed for each one. The statistical model, as an n-dimensional array (presumably $n = 2$) of collector chlorophyll molecules with a uniform distribution of discrete centers throughout, is the simplest, most homogeneous, structureless model. In the connected model, one may think of discrete units with zones of contact or channels through which the exciton may "leak" to visit any neighboring unit. From the kinetic standpoint, however, as noted above, the behavior is in both cases nonindependent and may become identical in the sense that an "extremely connected" model cannot be distinguished from a "quasi-statistical" model.

This class of models was advocated upon reexamination of the O to P rise (Joliot and Joliot, 1964; Morin, 1964; Delosme, 1967). When the curve was recorded in strong light with a fast opening device, it turned out to be actually sigmoidal rather than exponential (see Fig. 3 of Delosme, 1967).

We notice first that one may stay in the frame of the separate model to explain the sigmoidal O to P rise provided one adds the hypothesis that the O to P transition is a two-quantum (instead of a one-quantum) process (Morin, 1964). Specifically, one has to postulate an intermediate fluorescent state X in addition to the previous ones. The process is summarized by the following equation:

$$O \xrightarrow{h\nu} X \xrightarrow{h\nu} P. \tag{6}$$

According to the fundamental alternative if X is more fluorescent than O the first photochemical constant k_{OX} is larger than the second one k_{XP}. It is found that the O to P rise is also sigmoidal if $k_{XP} > 0.5\, k_{OX}$.

Since no other facts have been ascertained in support of this "photochemical"

model, however, another interpretation has been given in terms of the nonseparate PSU. This step is quite natural since one can show qualitatively that the sigmoidal shape is a simple consequence of the properties of these models. The differential equation for the photochemical fluorescence rise equivalent to but more general than equations 3 and 4 is:

$$d\Phi_F/dt = (\partial\Phi_F/\partial[Q^-])k^*(1 - \Phi_F). \tag{7}$$

The slope of the $\Phi_F = f(t)$ curve cannot increase initially (e.g., the curve cannot be sigmoidal) unless $\partial^2\Phi_F/\partial[Q^-]^2$ is initially greater than 0. This obviously does not hold for the separate model (see equation 4). On the other hand, with the nonseparate model, as more units go to the closed state more chlorophyll territories become open for migration of excitons, with corresponding increased exciton lifetime and increased change of fluorescence. Initially, the fluorescence per unit territory should be increased inasmuch as unit territories merge into larger ones (see below concerning the *îlot* effect) and this is the physical meaning of a positive $\partial^2\Phi_F/\partial[Q^-]^2$; however, this qualitative argument is not so convincing. The merging of individual territories is also tantamount to an increased photic cross section for the centers, which would be expected to oppose increased fluorescence yield. Tumerman and Sorokin (1967) and Briantais et al. (1971) have concluded from in vivo parallel increases of fluorescence lifetime and yield that the statistical model was verified (see also Müller et al., 1969); however, the separate model together with the two-yields hypothesis could also produce a concomitant variation of lifetime and yield.

Joliot and Joliot (1964) have treated the problem in the frame of the nonseparate models by assuming that a geometric law can describe a chain of n transfers ending with the fluorescence decay of the exciton. The relative variable fluorescence yield is given by:

$$\Phi_F = [(1 - p)(1 - Q)]/[1 - p(1 - Q)], \tag{8}$$

where Q is the concentration of open centers and p, the probability of interunit transfer. Equation 3 is integrated with the help of equation 8 to give:

$$-p + (1 - p) \ln Q + pQ = -k^*t. \tag{9}$$

From equations 8 and 9 a relation may be found between Φ_F and t. The result is a sigmoidal curve.

From the fundamental alternative and equation 8 the trapping yield is:

$$\Phi_{PS} = Q/[1 - p(1 - Q)], \tag{10}$$

which is clearly not first order. The rate of oxygen evolution V_{O_2} as a function of the concentration of active system II centers Q actually follows such a law (See Fig. 2 of Joliot and Joliot, 1964). The best fit to these two different experimental curves gives for *Chlorella* a value of p of the order of 0.5–0.6.

This theory has, however, been criticized on the grounds that an exact meaning can be attributed to the probability factors p and $(1 - Q)$ only with assumptions that are not warranted for the PSU (Lavorel, 1967). Thus either

(*a*) — (1) p is the probability of transfer to any chlorophyll molecule [and $p(1 - Q)$ is correctly the probability of transfer to a collector chlorophyll molecule]. Then p cannot be a constant except when one is considering with Robinson (1966) an extremely fast mechanism of migration with the consequence that exciton trapping practically always needs several "hits" with a center. Dropping for the moment this assumption, clearly p, as we now define it, should be a function of the configuration of the whole system, that is, of the distribution of open and closed centers (Fig. 2). This is another aspect of the remark we made earlier, that the photic cross section per center increases from O to P for any nonseparate model. As for the hypothesis of a very fast migration mechanism, it is unlikely to hold both for chlorophyll to chlorophyll transfers within the unit and for unit to unit transfers which are called for in a nonseparate model. In such a case, the over-all transfers are likely to become less fast in view either of the inherent slowing down of diffusive motion with distance, or of the assumption of partly permeating barriers between the units.

Or (*b*) — (2) p is the probability of transfer to a nearest neighbor; then $p(1 - Q)$ is not the right expression for the probability of transfer to a nontrapping unit. It would actually be right if one were considering the unit as rapidly fluctuating (compared with the visiting time of an exciton), so that any member of the superunit would on the average spend the fractions Q and $1 - Q$ in the trapping and nontrapping states respectively. There are no compelling reasons for such an assumption.

It is instructive to note that the idea behind the above reasoning is that of an extreme delocalization of the exciton with trapping being considered as the limiting step of the whole process. In fact a classical kinetic description may be given where

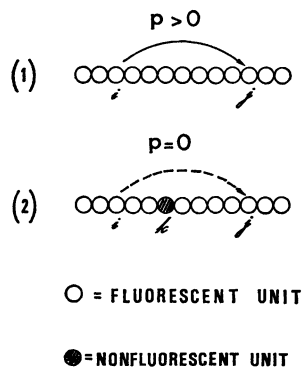

FIGURE 2 In a one-dimensional system of units with nearest neighbor transfer when in the closed fluorescent state, the over-all transfer probability p between units i and j may be nonzero if units i and j belong to an uninterrupted series of fluorescent units (1), whereas p is zero if the series is interrupted (k) by an open nonfluorescent unit (2).

trapping is considered as a simple (not diffusion-limited!) bimolecular reaction between excitons and centers (Lavergne, unpublished data):

$$dc/dt = k^* - k_F c - k_{PS} c Q, \text{ and} \qquad (11\ a)$$

$$dQ/dt = k_{PS} c Q, \qquad (11\ b)$$

where c is the exciton "concentration," k^*, k_F, and k_{PS} are the rate constants for exciton creation, decay by fluorescence (or other dissipative processes), and trapping. Solving equation 8, one finds also a sigmoidal O to P rise. This clearly shows that this property stems from the essentially bimolecular character of the exciton center interaction. Still more directly, under the mild assumption that $(dc/dt) = 0$, there results from equation (11 *a*) a classical "Stern-Volmer" formula

$$\Phi_F = k_F/[k_F + k_{PS}(Q)] \quad \text{and} \quad \Phi_{PS} = k_{PS}(Q)/[k_F + k_{PS}(Q)]. \qquad (12)$$

It is easily ascertained that the system has a sigmoidal solution from the formal identity of Φ_{PS} in equations 10 and 12. Several such kinetic derivations of the Stern-Volmer type have already been made in the literature (Teale, 1960; Duysens, 1966; Clayton, 1967).

The idea of "diffusion" (or migration) limitation, instead of trapping limitation, is best treated starting from the classical partial derivative equation for material diffusion. Properly modified to express exciton trapping (Lavorel, 1967), this equation has a solution also consistent with a sigmoidal O to P rise. Two other properties of the solution are worth noting. The fluorescence decay is no longer exponential, but rather polyphasic, and the fluorescence yield and the quenching yield, or the rate of trapping of excitons, are functions of Q which contain n, the number of dimensions, as a parameter. None of these properties have been put to use in the case of the PSU so far.

Although the diffusion approach correctly expresses the migration step as the limiting one during the progressive photochemical transformation of centers, its use is limited for reasons of mathematical awkwardness to the case of a homogeneous system, that is, the pure statistical model; and yet spectroscopic difference between the variable and constant fluorescence is, as noted above, in favor of some heterogeneity. This does not seem to be merely the addition of independent components. For instance, the 720 nm component, although prominent in the constant part, is also seen to a lesser extent in the variable part. Many spectroscopic and other properties indicate heterogeneity of the pigment apparatus, hence of the PSU; and any homogeneous model of it cannot be but an approximation, especially regarding the relation of exchange of excitons between units.

From this last point of view, at least, a clear-cut conclusion may be reached. Barring a two-quantum O to P photochemical rise, the sigmoidal behavior is in conflict with the separate model and strongly suggests some degree of exciton ex-

change between units. At this point, we may as well dispose of the case of fluorescent centers, which we considered earlier, since with such centers any type of units will behave as separate units!

3. *The Connected Units and the Îlot Effect*

We are thus led to the connected model as a safe position between two extreme models: the separate or the statistical one. Delosme (1967) had earlier hinted at such a model by allowing for a distinction between chlorophyll to chlorophyll transfers within the unit on the one hand and transfer from center to center, or from unit to unit, when they are in the closed state, on the other. Offhand, the connected model appears to be a generalization of the statistical model whereby some structure may be introduced in the system and expressed in a noncommittal way.

The connected model recently aroused renewed interest when we investigated the effect of *m*-dinitrobenzene (DNB) as an external quencher for in vivo chlorophyll fluorescence. One interesting result is that, under medium quenching conditions, the photochemical *O* to *P* rise changes from sigmoidal to exponential (Fig. 3), which suggests that the quenching effect is accompanied by an isolating effect, as if the units were somehow acquiring the independent behavior characteristic of the separate model. The isolating effect may be seen as an action of the quencher on the boundary between units preventing by capture the interunit migration of exciton ("interunit-blocking" hypothesis), or, more simply, as a consequence of the presence of additional nonphotochemical quenching "centers" transforming the corresponding units into the permanent open state, thereby hindering the freedom of exciton motion ("unit-blocking" hypothesis).

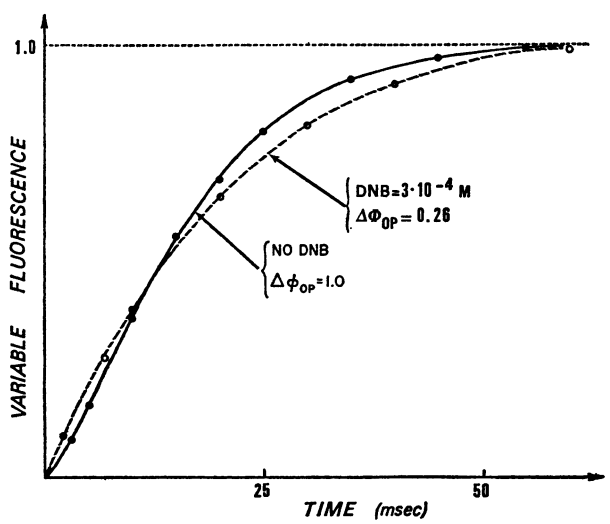

FIGURE 3 Effect of DNB on the *O* to *P* rise. *Chlorella pyrenoidosa*. Phosphate buffer, 0.05 M, pH 6.4. Temperature, 20°C. Excitation band, 440–600 nm.

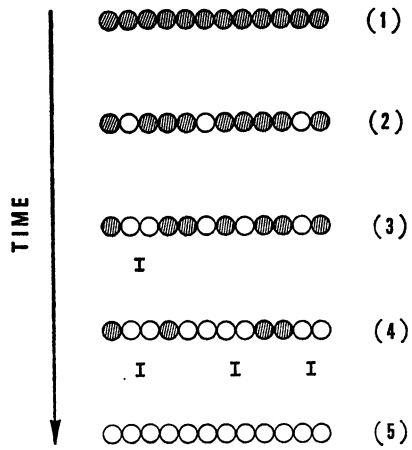

FIGURE 4 Random growth of islets in a system of separate units. *I*, islets of various orders. (Symbols, see Fig. 2).

When trying to analyze the dynamic behavior of the connected model, in fact of any nonseparate model, including the statistical one, one is faced with a characteristic and very interesting difficulty. This is best explained by considering first what occurs during the photochemical rise for a one-dimensional system of separate units. Fig. 4 shows successive phases of the process: the fluorescent units are first isolated, then they gradually merge into clusters, and these clusters grow until the whole system is filled. The picture is significantly different if some interunit transfers are allowed, in the sense that the clustering of fluorescent units will be more pronounced. Any incipient cluster randomly formed initially will have a tendency to grow at its edges; that is, the two bordering nonfluorescent units will benefit from the large photic cross section of the adjacent cluster and will have a greater probability of going to the fluorescent state. Here we find again the idea of variable, and increasing, mean photic cross section of units during the *O* to *P* rise, but with a new topological connotation (there is some similarity with the Ising model used, for instance, in magnetic phenomena). This pattern of "contagious" growth and merging of clusters or islets (*îlots*) has two consequences.

First, each islet has its own characteristic fluorescent yield depending on its size (and besides on the fluorescent and transfer parameters of the unit). This is not difficult to deduce for a one-dimensional islet of not too small size, with the help of a diffusion scheme, viz.:

$$\Phi(\varphi, \lambda; n) = 1 - \tanh[(n/\sqrt{2})(\varphi/\lambda)^{1/2}]/[(n/\sqrt{2})(\varphi/\lambda)^{1/2}], \qquad (13)$$

φ and λ being the fluorescence and transfer yields of a unit ($\varphi + \lambda = 1$), and n, the number of units of the islet. As the size of the islet grows its fluorescence yield steadily increases towards unity. In other words, its rate of edge growth steadily decreases.

Obviously, this component of "active" growth must be added to the "random" growth rate (only present in the case of a system of separate units) to describe the dynamic behavior of our connected system. The kinetic formulation would go along the following steps which are reminiscent of the two-quantum hypothesis (see equation 6):

$$O \xrightarrow{h\nu} X_1 \xrightarrow{h\nu} X_2 \xrightarrow{h\nu} - - - \xrightarrow{h\nu} P, \tag{14}$$

X_i being an islet of size i. Actually, the filiation of islets incorporates two distinct processes:

(*a*) active growth of islets,

$$X_i \xrightarrow{h\nu} X_{i+1}, \quad \text{and} \tag{15}$$

(*b*) merging of islets into an "island,"

$$X_i + X_j \xrightarrow{h\nu} X_{i+j+1}. \tag{16}$$

It is seen that the analytic formulation of this problem is far from trivial since the variables are not only functions of time but also of their own spatial distribution (through equation 16). It is noteworthy that none of the previous calculations (in section II.2) could express in principle the *îlot* effect nor handle it. Thus, from this point of view, they are inherently wrong. It is also apparent that this topological difficulty results from the apparently innocuous and fairly obvious property *a* of spatial fixity of centers (see section I).

Second, the macroscopic fluorescence yield Φ_F is not an unequivocal function of Q^-, that is, of the number of fluorescent units, in that it depends on their grouping. It is evident that the actual, not intrinsic, fluorescence yield of a unit is larger the larger the islet of which it is a member. As a numerical illustration, using equation 12 with $\varphi = \lambda$, it is seen that an islet of 10 units has a fluorescence yield of 0.86, whereas that for 10 elementary islets of 1 unit is 0.5. An important practical consequence, which still remains to be quantitatively appreciated, is that a given macroscopic yield does not correlate exactly with the same value of $[Q^-]$ inasmuch as it may result from active or random islet formation. The difference between random and active islets is probably larger in some range of Φ values between O and P: Fig. 5 shows that the rate of increase of Φ is smaller after active islet formation (branch A) resulting from the photochemical O to P rise than after random islet formation, as is presumably true during dark reoxidation of Q^- starting from state P (branch B). Alternatively, there are fewer photoactive (or open) units in B than in A, hence a faster completion of the O to P phase in B than in A. (It is, however, puzzling to observe that the two-quantum photochemical model, equation 6, is also able to explain the effect.)

In view of the difficulty in solving equations 14–16, the problem has been pro-

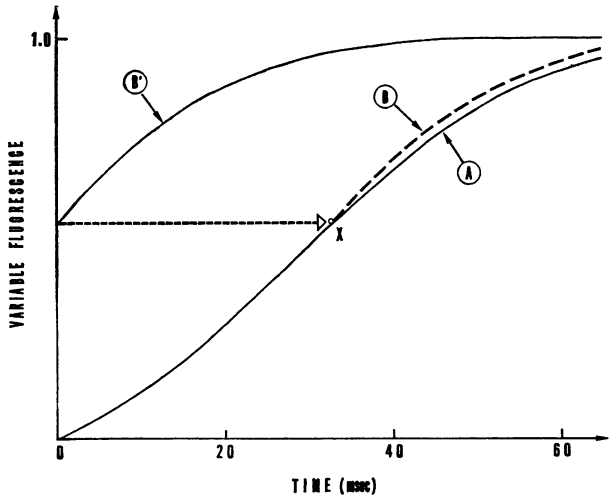

FIGURE 5 Comparison of partial with complete O to P rise. Branch A belongs to the normal, complete O to P rise; branch B' is obtained from state P after partial reoxidation of Q^-. Branch B' is shifted to B at a matching point X for comparison. *Chlorella pyrenoidosa*. Phosphate buffer, 0.05 M, pH 6.4. 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU), 10^{-6} M. Temperature, 20°C. Dark time before branch B', 200 msec. (Courtesy P. Bennoun.)

grammed for solution by the Monte Carlo method. Some of the results (to be published later) will be shown here. So far a one-dimensional system of 50 units with nearest neighbor connection has been considered. It is assumed for simplicity that trapping is 100% efficient in the open state. One parameter only, φ, the intrinsic fluorescence yield of the closed unit, needs to be specified, which consequently fixes λ, through the relation $\varphi + \lambda = 1$. The choice of this single parameter permits scanning the whole range of intermediate models between pure and separate ($\varphi = 1$), and quasi-statistical ($\varphi \ll 1$). The *îlot* effect is well substantiated upon following the kinetics of the average islet during the O to P phase for various values of φ (Fig. 6). The sigmoidal shape of the O to P fluorescence rise is the more marked the larger the ratio λ/φ. The isolating effect of DNB can also be simulated with the unit-blocking hypothesis (i.e., units associated with DNB become permanent nonphotochemical quenchers). Fig. 7 shows that quenching is attended by a shift from sigmoidal to exponential; however, comparing Fig. 7 with Fig. 3, and noting that, in the theoretical case, φ was taken to give the closest agreement with the experimental O to P rise, one sees that "exponentialization" is obtained with much more severe quenching of the total variable fluorescence for theory than for experiment. Thus, DNB seems to have in actuality a better isolating effect than according to the blocking hypothesis, in spite of the fact that an isolating mechanism is necessarily more efficient in one dimension, as computed in the model, than in two dimensions, as presumably in the PSU. The conclusion therefore would be that the interunit-blocking hypothesis appears more probable.

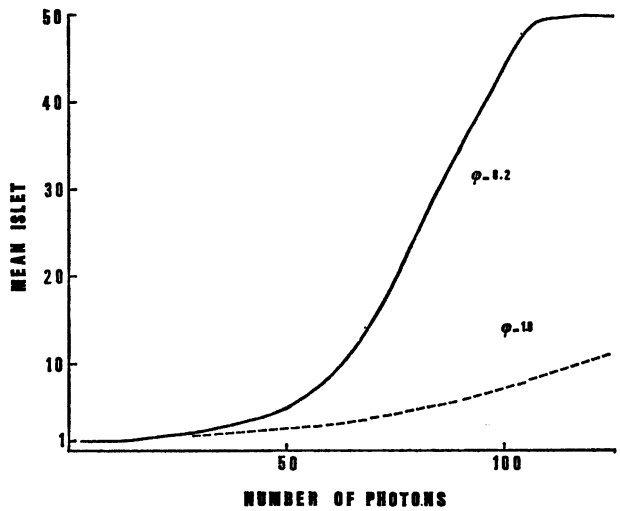

FIGURE 6 Average over islets of various sizes formed during the simulated O to P rise. Note slow growth for separate units ($\varphi = 1.0$), rapid growth for strongly connected units ($\varphi = 0.2$), and convergence of the models at the beginning, near O state.

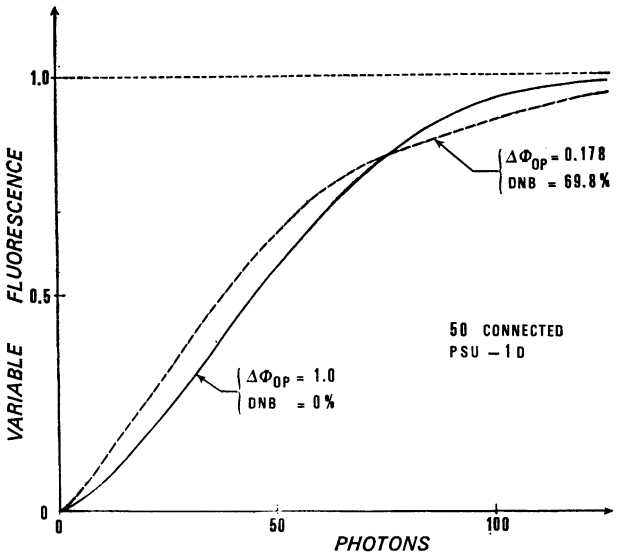

FIGURE 7 Simulated effect of DNB on the O to P rise. Compare with Fig. 3. φ was set at 0.2 to give the best fit with the $\Phi = f(Q^-)$ relation equivalent to $V_{O_2} = f(E)$. 1D, one-dimensional.

We plan, in the future, to consider a two-dimensional system and compare its behavior with that of the one-dimensional one, as well as with the actual fluorescence curve. We hope to draw from this comparison conclusions concerning the topological nature of the PSU:

III. LUMINESCENCE

1. *Fluorescence and Luminescence*

As was conclusively demonstrated by Arnold and Davidson (1954) from comparison of spectra, the luminescence of photosynthetic organisms which is evolved after light activation, but long after the "prompt" fluorescence has decayed, is actually a "delayed" fluorescence coming, as does the prompt one, from the first singlet excited state of chlorophyll. And here again we are dealing with the concept of PSU. Not only is luminescence related to fluorescence by its electronic nature, but also presumably through the details of its origin. From experimental results which have accumulated on this topic, we have every reason to believe that luminescence arises from a reaction very closely connected to the photochemical transformation of the system II centers. One of the earliest hypotheses, which we have taken into consideration, is that luminescence is simply the reversal of this transformation:

$$\text{trapping is } Z\text{Chl } Q + h\nu \rightarrow {}^{+}Z\text{Chl } Q^{-}, \quad \text{and} \tag{17}$$

$$\text{luminescence is } {}^{+}Z\text{Chl } Q^{-} \rightarrow Z\text{Chl } Q + h\nu'. \tag{18}$$

Accordingly, and following Ockham's rule, no distinction should be made between a "luminescence" exciton, once created, and an ordinary "fluorescence" exciton, and much of what we have said in connection with the PSU should also apply to luminescence. In particular, a consequence of this view is to propose the relation (Lavorel, 1968):

$$L = \Phi \cdot J, \tag{19}$$

where L is the luminescence intensity, Φ, the fluorescence yield, and J, the rate of luminescence exciton creation. The meaning of equation 19, which henceforth we will call the "L-Φ relation," can be quite different, according to the type of PSU model one is considering, that is, depending on whether the units are separate or not. Furthermore, we will show that for low levels of variable fluorescence, near the O state, the L-Φ relation takes on a simplified form essentially independent of whatever model is invoked.

The L-Φ relation was formulated on a strict analogy to the classical relation which defines the fluorescence yield Φ as a function of the fluorescence intensity F and the absorbed light intensity I:

$$F = \Phi I. \tag{20}$$

Let us first consider under which circumstances Φ in both equations 19 and 20 could be exactly the same quantity, that is, the actual system II fluorescence yield. We see that an exact identification of luminescence to fluorescence requires that early in the life of the luminescence exciton there occurs a complete "loss of mem-

ory" as to its origin. This can be accomplished in a "mental experiment" if each such exciton, once created, is withdrawn from the system and, in its place, a photon is randomly introduced into the system. Obviously we are close to this situation in the upper part of the O to P range for any nonseparate model; and, in general, we may say that the above identification will be permitted inasmuch as the state of the system of units will allow for enough delocalization of the luminescence exciton.

It is instructive to examine for a moment the separate model which provides the least amount of delocalization in the sense just explained. According to our current hypothesis, the birth of a luminescence exciton is expressed by the reversal of equation 2:

$$P \rightarrow O + \epsilon, \tag{21}$$

where ϵ stands for an exciton. In other words, the excitons always "see" units in the O state and their only chance to escape as fluorescence is that the fluorescence yield in this state Φ_o is not zero. In this model, L is simply proportional to J and actually independent of the macroscopic yield Φ. As noted above, this situation also occurs with the connected model when there are only order 1 islets (isolated fluorescent units) in the system, that is, when fluorescence is close to the O level. In fact, in this condition, all models converge to the separate model (see also legend of Fig. 6).

For the connected model, more generally, the birth of the luminescence exciton is described as:

$$X_{i+1} \rightarrow X_i + \epsilon \text{ ("edge" birth)}, \tag{22}$$

or

$$X_{i+j+1} \rightarrow X_i + X_j + \epsilon \text{ ("inside" birth)}, \tag{23}$$

(and the special case considered above is:

$$X_1 \rightarrow 0 + \epsilon).$$

Therefore, instead of equation 19, the L-Φ relation should take the following form:

$$L = \sum \Phi_i J, \tag{24}$$

where Φ_i is the fluorescence yield of an order i islet, and summation is carried over all types of islets existing in the system "just after" exciton birth. (It is also assumed that J is independent of the size of the islet.)

2. *The True Zero Level of System II Fluorescence*

The question of the nature of the constant fluorescence or, as we put it, according to the two-emissions hypothesis, the problem of locating the true zero level Z'

(see Fig. 1 B) of system II fluorescence has received little attention (see, however, Clayton, 1969). We recall that earlier (see section II.1) we argued that Z' was very likely located slightly below the O level; in other words, Φ_o was small but finite. The very existence of a slow luminescence occurring minutes after the actinic excitation has ended, the system being almost in the O state, also requires, as we have seen, a nonzero Φ_o. Although convincing, all these arguments do not help much to locate this Z' level.

Luminescence might be of some utility for quantitatively solving this problem, in spite of the complication which the connected model introduces in the L-Φ relation and of the fact that there are so far no known ways of measuring J directly; for luminescence, as a "pure" system II effect, should have its zero yield of fluorescence at Z' whereas, by optical excitation, no possibility has ever been found to completely separate the true system II fluorescence from the ZZ' background (presumably system I) fluorescence (but see Duysens and Sweers, 1963). Taking advantage of the slight spectroscopic differences between constant and variable fluorescence and comparing precisely the fluorescence and luminescence spectra, we are presently attempting to locate this Z' level.

CONCLUSIONS

Numerous discussions of experimental results have appeared in the past in attempts to decide the most correct formulation of the PSU since this germinal concept was first proposed. Among these results, it has been natural to pay particular attention to those derived in the area of photophysics and photochemistry, and especially to fluorescence quantum yield and lifetime. It appears to us, however, that kinetics of fluorescence during induction, especially the photochemical O to P rise, and connections between fluorescence and luminescence have seldom been used in this context. We believe that the last-mentioned phenomena, taken together, afford a coherent picture of the PSU in terms of the connected model.

For fluorescence, the major fact seems to be the sigmoidal shape of the O to P rise. In order to explain it, the alternative is either a two-quantum photochemical transformation of centers in separate units, or a one-quantum transformation in connected units. For lack of compelling reasons which favor the two-quantum picture, we have so far preferred to adhere to the connected model. A new effect (the *îlot* effect) has been noted as a consequence of this model, or rather it is really a consequence of the large-scale (at the level of the whole system of units), diffusion-limited character of exciton migration and, of course, of the nondiffusion of centers themselves.

It is also seen that the connection between luminescence and fluorescence depends on the nature of the PSU. As a delayed fluorescence, luminescence can be considered as a probe which allows one to "see" only the system II fluorescence. It seems impossible to look at luminescence phenomena as totally unrelated to the PSU concept without introducing very artificial *ad hoc* assumptions. Again, with

the PSU concept in mind, we may hope to get from luminescence and fluorescence studies a better knowledge of that part of the emission which belongs to system II.

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