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Abstract. This article is a translation of a paper by Theodor Förster originally published in Naturwissenschaften, Vol. 33(6), pp. 166–175 (1946), entitled "Energiewanderung und Fluoreszenz." Translated and published with permission from Springer Science+Business Media. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.1.011002]

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1 Posing the Problem

Recently, so-called *energy migration* has frequently been discussed in photobiological events.[26] It appears that in various biological systems, a quantum of energy absorbed by one molecule causes a change not in that molecule, but in a second, spatially separated molecule. In this case, the energy is transferred over distances which are large compared to the contact distance of the molecules and large compared to the distance over which chemical valence and secondary valence forces are effective.

Some observations regarding *carbon dioxide-assimilation*[*] in plants can be interpreted by such an energy migration. According to measurements by Emerson and Arnold,[6] short-term irradiation with high light intensities (sparks) produces saturation effects in the algae *chlorella* such that the assimilation yield does not exceed a certain threshold even at arbitrarily high light intensities. This limit is reached between 2 and 4 light quanta[†] per 1000 chlorophyll molecules, the number of light quanta required for the reduction of one CO_2 molecule. Gaffron and Wohl[10,45] conclude from this that this number of molecules contribute their absorbed energy for the reduction of a single CO_2 molecule. As this has to be assumed to take place in a distinct location, this interpretation requires energy migration from the chlorophyll molecule to the location where the reduction takes place. At the same time, this interpretation accounts for the magnitude of the saturation for continuous illumination as well as for a lack of an induction period at low intensities, which would be required if a single chlorophyll molecule had to acquire the necessary number of quanta for the reaction. All these observations can be easily interpreted by assuming an assimilation unit,[‡] and result in quantitatively correct numbers for its size. A detailed discussion regarding the experimental results from this point of view by the aforementioned authors can be found in this journal.[§10]

However, it has to be pointed out that the existence of an assimilation unit, and therefore energy migration in this assimilation unit, is very controversial. Franck and Herzfeld[8] have outlined a reaction scheme, according to which light saturation is supposed to occur through a chain reaction directed against the assimilation, which starts at high light intensities. In addition, the lack of an induction period can also be explained by this scheme. So at least it is clear that the interpretation according to Gaffron and Wohl—despite its indisputable elegance—is not the only one possible.

Therefore, it is important that properties of other biological systems can readily be interpreted by the assumption of energy migration. Let us just mention the mutation and damage of the genes contained in the chromosomes in the cell nucleus[‖] by corpuscular radiation or ultraviolet light. The required radiation dose allows the calculation of the size of the regions in which the ionization or absorption processes are effective. These regions are significantly larger than the molecules, or parts of molecules, on which the finally occurring change is to be assumed. This problem has been addressed in detail in this journal[§] in the context of an interpretation of different alternative energy migration mechanisms by Riehl, Timofeeff-Ressovsky, and Zimmer[30a], as well as Möglich, Rompe, and Timofeeff-Ressovsky.[26¶]

To understand these phenomena, it is desirable to consider effects in nonbiological matter. We know of many chemical reactions in which one molecule absorbs a quantum of light and thereby causes a change in other molecules. These are the so-called *sensitized* photochemical reactions. The investigation of such reactions has shown that the sensitizer molecule either is permanently in proximity to the reacting system (e.g., the sensitization of AgBr crystals in photographic layers) or moves toward it and transfers the energy upon collision. An atom or electron is often liberated from the sensitizer molecule which in turn causes the change elsewhere. In all of these cases, energy transfer, if it occurs over large distances at all, is inextricably linked to a material carrier. However, the assumption of such energy transfer mechanisms in the aforementioned biological systems leads to difficulties. Except for closely interacting molecules, a transfer of liberated atoms is possible only in the

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[*] Translator's note: i.e., photosynthesis.

[†] Translator's note: i.e., photons.

[‡] Translator's note: i.e., photosynthetic apparatus.

[§] Translator's note: i.e., Naturwissenschaften.

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[‖] Translator's note: Note that the structure of DNA was not discovered until 1953, seven years after the publication of this paper.

[¶] Note added in proof: Recent results of carbonoxide myoglobin photolysis obtained by Bücher and Kaspers [Naturw. 33, 93 (1946)] also point to an energy migration.

gas phase, and a transfer of electrons appears impossible as the material has no electrical conductivity whatsoever.

For these reasons, it is now mostly assumed that in biological processes the energy migrates *without being linked to a material carrier* of any kind.[16,34,44]

There are several *fluorescence* phenomena in dye solutions that have been known for some time, which suggests energy migration should be defined in these terms. These are *depolarization* and *quenching* of fluorescence, which, since 1924, J. and F. Perrin[28,29] have interpreted by intermolecular energy migration. Even if today we have to slightly modify this interpretation, the conclusion of a transfer and migration of energy remains unchanged. It occurs in solution of moderate concentration, in which the overwhelming fraction of the molecules is present independently—i.e., they are not associated.[**]

Later, using *polymers* of dye molecules that he had discovered in a certain class of dye molecules, Scheibe[34] showed energy migration between individual polymer molecules. Since then, discussion about the biological problems mentioned above tends to focus on analogies with these polymers, while observations of fluorescent monomers[††] are less of a focus. It thus appears desirable to treat both phenomena together and clarify the fundamental mechanisms. This not only contributes to the understanding of these incompletely understood phenomena, but, at the same time, also provides a broad foundation for the subsequent discussion about the biological problems already mentioned.

2 Concentration Depolarization

In 1920, Weigert[43] discovered that dyes like fluorescein and eosin, dissolved in gelatine or a viscous solution, emit polarized light when irradiated by polarized light. This can be explained as follows: in dye molecules, the electrons which participate in the absorption and fluorescence are bound *anisotropically.* Thus every molecule possesses a preferred orientation in which it is excited with a high probability by the electric vector of the incident light wave. In an *isotropic* distribution of the molecules in solution, these will be excited with varying probability, depending on their orientation with respect to that of the polarization. The distribution of the *excited* molecules is thus *anisotropic*. In solvents of low viscosity (water, alcohol), the time elapsed between absorption and subsequent emission, around 10^{-8} sec, is sufficient to establish, by Brownian rotational motion, an *isotropic* distribution even among the *excited* molecules. The fluorescence emission is therefore *unpolarized.* In rigid or sufficiently viscous solvents, however, the molecules cannot significantly change their orientation in the given time period. Thus the orientational distribution of the emitting molecules is *also anisotropic*, and the fluorescence is, under appropriate direction of observation— e.g. in the direction of the illuminating beam— partially *polarized.* The maximum degree of polarization under these conditions is 50%. However, even in solvents of sufficient viscosity, this value is only approximately reached, and only in very dilute solution. In 1924, Gaviola and Pringsheim[12] established that with increasing concentration the degree of polarization drops to very small values. The course of this so-called *concentration depolarization* is shown in Fig. 1 on a logarithmic concentration scale according to recent measurements for fluorescein in glycerol by Pheofilov and Sveshnikov.[30] The maximum polarization value is only observed up to concentrations of around 10^{-4} mol/l. From here on, the polarization decreases, with half the maximum value reached at 3×10^{-3} mol/l. The most probable distance between two neighboring molecules in a solution at this concentration is around 50 Å, i.e., very large compared to molecular dimensions. The decrease of the degree of polarization is not accompanied by any other changes in the absorption or fluorescence of the solution. Absorption and fluorescence spectra, quantum yield, and fluorescence lifetime are the same as in the most dilute solutions.

Since in the more concentrated solution the molecules are without doubt as rigidly oriented as in dilute solution, the concentration depolarization can only be explained by assuming that the fluorescence originates from a molecule which is oriented differently from the absorbing one. Thus a *transfer* of excitation energy must occur between absorption and fluorescence. A trivial possibility here would be the reabsorption of the primary fluorescence and a subsequent *secondary fluorescence*. However, it was already noted by Gaviola and Pringsheim[12] that the secondary fluorescence is not sufficient to explain the observed depolarization. Compared to the absorption spectra, the fluorescence spectra of dyes are shifted to longer wavelengths according to Stokes' rule to such an extent that only a few percent of the fluorescence is reabsorbed. As the depolarization caused by this cannot be larger either, even higher order fluorescence (i.e., multiple reabsorption) cannot explain the measured degree of depolarization (Fig. 1).

Instead, one has to assume that energy transfer occurs between molecules in close proximity, according to J. and F. Perrin, which is more rapid and complete than reabsorption. Energy transfer taking place, before emission, between the originally excited molecule and a second molecule would, in the most favorable case, occur with equal probability with either molecule. Every conceivable mechanism which allows a transfer between like molecules inevitably yields a reverse transfer, such that, at best, an equalization of the emission probability between the participating molecules occurs. For a transfer to *n* molecules

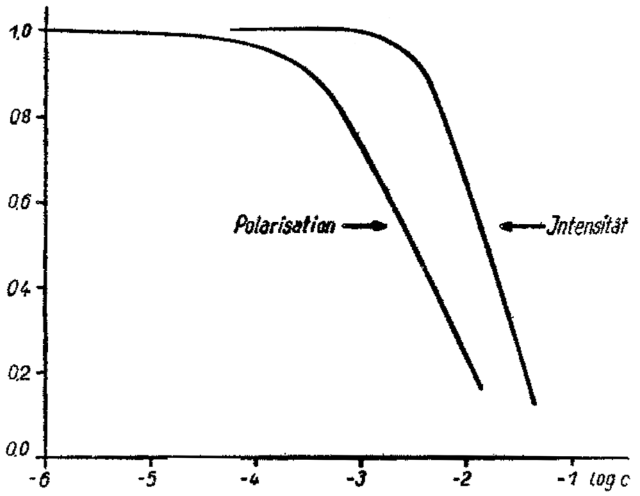

Fig. 1 Polarization and intensity of fluorescein fluorescence in glycerol according to Pheofilov and Svesnikov.[30] Concentration in moles per liter. The values of polarization and intensity at infinite dilution are set to one.

[**] Translator's note: i.e., aggregated.

[††] Translator's note: Förster uses the term "Einzelmolekül" which literally translates as "single molecule" or "individual molecule" but also as "monomer", which is what he means in this context.

during the emission period, each of those has the same emission probability. Only the primary molecule produces a maximum of polarized fluorescence. The other molecules will also yield a little polarized fluorescence if the transfer mechanism, as discussed in Sec. 4, preferentially selects molecules of a similar orientation. If we make the approximately correct assumption that the overall degree of polarization is the sum of the degree of polarization of the participating molecules' emission processes, this results in $1/n$ of the emission emanating from the primary molecule, with a degree of polarization of at least $1/n$ of the maximum value. A degree of polarization of 20% of the maximum, as obtained for fluorescein in glycerol in a 10^{-2} molar solution, thus corresponds to a distribution of the excitation probability over at least five molecules within the lifetime of the excited state.

While the fluorescence intensity stays constant with the onset of the depolarization, it too drops as the concentration increases further. The corresponding curve for fluorescein in glycerol is also shown in Fig. 1, according to measurements by the same authors.[30] This so-called *concentration quenching* complicates the observation of concentration depolarization at higher concentrations than those shown. Moreover, it has to be assumed that both processes are linked, i.e., that energy migration which manifests itself in concentration depolarization is also affected by quenching. The results obtained from depolarization at high concentrations thus relate to a case perturbed by quenching.

So in the 10^{-2} molar solution considered above, the fluorescence intensity is only half of that in dilute solution as indicated in Fig. 1. According to the theory of concentration quenching to be outlined in Sec. 3 below, the other half has been transferred from the primary molecule to its neighbors such that, if the energy propagation had not been perturbed by quenching, a distribution of the excitation probability over 10 molecules would be obtained. The possibility of realization of such a scenario will be discussed further below.

3 Concentration Quenching

Although *concentration quenching* perturbs the observation and data analysis of concentration depolarization at high concentrations, it offers an independent possibility of establishing energy migration which is, moreover, not tied to viscous solvents. The explanation originally proposed by J. Perrin[29] was based on the assumption that the energy drawn by the neighboring molecules from the excited molecule is annihilated. This is incompatible with the later interpretation of concentration depolarization because the latter is due to the emission of the transferred energy. The core of Perrin's theory can, however, stand, if one makes an initial, apparently forced assumption that, apart from fluorescent molecules, there are others which annihilate the excitation energy if it reaches them during its migration. During rapid energy transfer, the existence of a small fraction of such molecules suffices to obtain complete quenching. However, such an assumption appears credible only if it can be supported in another way.

Another interpretation of concentration quenching has already made the assumption of a nonfluorescing type of molecule. In 1889, Walter[41] already explained quenching by formation of nonfluorescent *associates*, which enabled him to interpret, apart from concentration dependence, the decrease in quenching upon temperature increase as a decrease in association. He found a confirmation of the assumed association in deviations in the absorption coefficient from Beer's law, which were later confirmed by different authors[20,31,37] using a range of fluorescent dyes. In the short-wavelength range of their absorption spectra there appears, in addition to the low concentration band, a second band which can only be assigned to association. This is particularly evident in aqueous dye solutions of Rhodamine 6G and methylene blue (Lewschin[23a] and Holst[13]).

In 10^{-3} molar solutions of those dyes, the short-wavelength band of the associates is already stronger than that of a monomer. The entire spectrum as a function of concentration can be explained by the assumption of an exclusive formation of bimolecular associations (dimers).[‡‡] According to Rabinowitsch and Epstein,[33] the equilibrium constant of the association of methylene blue is 2.8×10^{-4} mol/l (27°C). In the fluorescence spectra of these dyes there is only one band which corresponds to monomers which decreases in intensity at high concentrations as the monomer concentration decreases. This demonstrates that the dimers are indeed nonfluorescent.

Evidently, Walter's interpretation of concentration quenching due to vanishing fluorescent monomers during association is correct. However, the absorption spectra show only a fraction of the degree of association expected, according to this model, from the interpretation of the quenching data. This is the case for most fluorescent dyes in this range of quenching concentration. This is true, for example, for fluorescein and eosin in aqueous solution. In alcohols and other organic solvents, there is no trace of association in the absorption spectra in this range of quenching concentration.

However, a small fraction of nonfluorescent dimers is sufficient to explain quenching if *energy migration* takes place in solution and if the energy absorbed by available monomers is passed on to dimers and then annihilated in the same way as if they had been illuminated directly.[§§]

In favor of this amalgam of Walter's and Perrin's theory of concentration quenching, proposed by Duschinsky[4] in an apparently ignored publication, are, for example, the following observations: according to Szymanowski,[40] the fluorescence decay of fluorescein, eosin, and related dyes shortens with increasing quenching. This can be understood according to the view considered here, because only those absorption processes lead to fluorescence where the emission occurs before the energy reaches one of the quenching dimers. Thus the emission processes which occur with different speeds favor the faster ones. There is no contribution of molecular diffusion in concentration quenching; this can be concluded from the fact it also occurs in the same way in the most viscous solutions (e.g., sugar-glycerol mixtures, according to Banow[1]).

For aromatic hydrocarbons like anthracene, chrysene, etc., various observations (cf, for example, Pringsheim[32] as well as Kortum and Finck[21]) indicate that the concentration quenching here does not occur via energy migration to already existing dimers but by collisions between excited and ground-state monomers to form a nonfluorescent bimolecular unit.[‖‖] Neither does energy migration participate in quenching of dyes like methylene blue. Here, the association is so strong that monomers are replaced by dimers even at concentrations at which no energy migration takes place. However, for the majority

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[‡‡] The electrical conductivity as a function of concentration also shows this association.[22]

[§§] Translator's note: Even in the original German text, this paragraph is a single sentence over 6 lines.

[‖‖] Translator's note: Förster is already talking about excimers here!

of fluorescent dyes, energy migration appears to contribute significantly to fluorescence quenching.

For all these cases of concentration quenching, the decisive property of the *associates* is to *annihilate* the energy radiated or passed on to the associates—this appears practically certain from experimental observations but should also be accessible to a theoretical justification. There will be an opportunity to discuss this further below, after first dealing with the theoretical foundations of energy migration in such systems.

4 Mechanisms of Energy Migration

To begin with, we consider the transfer of excited state energy between two molecules. In the sense of the original theory by J. Perrin,[29] we preliminarily consider the electronic system of such a molecule as a classical mechanical oscillator. On the one hand, the oscillating electric charge causes a coupling with the radiation field and thus emission of energy into the surrounding space. On the other hand, the mutual electrostatic forces yield a coupling of the oscillators of adjacent molecules. At the same frequencies, this leads to an energy transfer quite similar to the well-known example of two mechanically coupled pendulums of the same frequency (resonance pendulum), where the pendulum set in motion transfers its energy to the second one. If in our case the coupling of the two oscillators is significantly weaker than the coupling with the radiation field, emission occurs only from the primary excited oscillator. In the opposite case, multiple transfer of energy occurs from the first oscillator to the second one and back before emission—the emission thus occurs uniformly from both. Due to the distance dependence of the mutual coupling, the first case is realized for large oscillator distances d, the second case for small ones (schematic illustration in Fig. 2).

They merge at a critical distance d_0, where the mutual coupling is equal to the coupling with the radiation field. In the case of exact resonance, the calculation performed by Perrin yields

$$d_0 \sim c/\omega = \lambda/2\pi, \tag{1}$$

(c: speed of light, ω: angular frequency = oscillator's eigenfrequency divided by 2π, λ: corresponding wavelength).

We can confirm Perrin's result by a simple rough estimate. Emission and transfer are determined by the oscillating electric dipole moments which both oscillators possess at a certain energy. Although this can of course be arbitrary in the framework of a classical treatment, we conveniently fix it to be the quantum theoretical value $\hbar\omega$ ($\hbar$ is Planck's constant divided by 2π). If we denote the corresponding value of the dipole moment M, then the mean duration of emission τ is according to a well-known formula[¶¶]

$$\tau = \hbar c^3/M^2\omega^3. \tag{2}$$

On the other hand, the interaction energy of both oscillators at a distance d is equal to the electrostatic energy of both dipoles and hence of the order of

$$U \sim M^2/d^3. \tag{3}$$

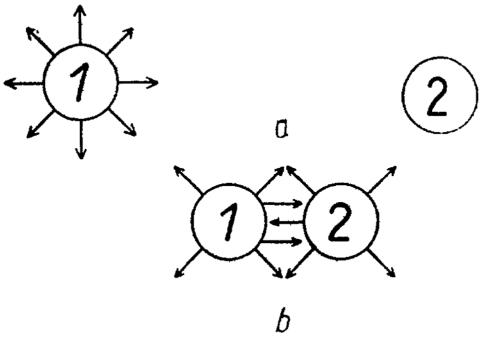

Fig. 2 Emission of two oscillators (schematic). (a) $d \gg d_0$. Emission by primary excited oscillator 1. (b) $d \gg d_0$. Equal emission probability of oscillator 1 and 2.

During one period of oscillation, or, more accurately, during a time of $1/\omega$, an energy of this order is transferred from one oscillator to the other because of the coupling. In the *case of resonance*, these contributions add up such that per unit of time an energy of $U\omega$ is transferred. The transfer of the entire energy of order $\hbar\omega$ thus occurs in a time of

$$t_0 \sim \hbar/U \sim \hbar d^3/M^2. \tag{4}$$

For the critical distance d_0 the transfer time t_0 is equal to the lifetime τ, which yields the value in Eq. (1) from Eqs. (3) and (4).

For a molecule which absorbs in the visible spectrum at around 6000 Å, a d_0 of 1000 Å is thus obtained, corresponding to a concentration of around 10^{-6} mol/l. Yet, according to the observations in concentration quenching, energy transfer actually only occurs at much higher concentrations of around 3×10^{-3} mol/l, which corresponds to a mean molecular distance of around 50 Å.

This shortcoming of J. Perrin's formula is due to the fact that the condition of *exact resonance* is not fulfilled. This is already apparent in the spectrum of a dye molecule which is not a sharp line but rather a broad band. The reason for this broad band is the coupling of the electronic motion with the atomic oscillations[***] in the molecule itself[†††] and in the surrounding solvent. Moreover, the resonance is also perturbed by the fact that the spectrum of the excited, energy-emitting molecule is different from the unexcited, energy-absorbing molecule. The former is the fluorescence, the latter the absorption spectrum of the molecule which are shifted with respect to one another according to Stokes' rule and show little overlap,[23b,28a] (See Fig. 3 further down below).

If there is little interaction between the molecules, the atomic oscillations, superimposed onto the electronic oscillations, occur rapidly compared to energy transfer. In this case, the frequency distribution in the spectra is determined solely by electronic and atomic oscillations within a molecule; fluorescence and absorption spectra are therefore unchanged compared to those of an isolated monomer. Within the long duration of energy transfer the occurrence of each frequency corresponds to its intensity in its fluorescence and absorption spectrum, respectively. The condition for resonance is only fulfilled during the short periods

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[¶¶] cf for example Cl. Schaefer, Einf. In die Theoretische Physik (Introduction to Theoretical Physics) W. de Gruyter, Berlin and Leipzig 1927 III, 2 p. 431, Eq. (75), where, using a different notation, the exact expression is given for the emission probability which is the inverse of the lifetime.

[***] By atomic oscillations we mean slow oscillations compared to electronic motion, which the entire atom (nuclei and electrons) carries out.

[†††] Translator's note: i.e., molecular vibrations.

when the eigenfrequencies of both molecules agree within the coupling width, i.e., a frequency range with a width of $U/\hbar$. To take this into account in the framework of our rough estimate, we consider the absorption and fluorescence spectra to be two rectangles with a width Ω, which overlap in a strip of width Ω' (Fig. 3, all frequencies are angular frequencies).

The resonance condition demands that the frequency of one oscillator is within Ω', and at the same time the frequency of the other one coincides with it within $U/\hbar$. The probability of this is

$$W = (\Omega'/\Omega)(U/\hbar\Omega).$$

As transfer only occurs in a certain fraction of time, the duration given in Eq. (4) has to be divided by this value[‡‡‡]

$$t_0 \sim \hbar^2\Omega^2/U^2\Omega' \sim \hbar^2 d^6\Omega^2/M^4\Omega'. \tag{4'}$$

Setting $t_0 = \tau$ and eliminating M using Eq. (2), we now obtain for the critical molecular separation

$$d_0 = \lambda/2\pi(\Omega'/\tau_0\Omega^2)^{1/6}. \tag{1'}$$

For dyes of the type considered here, the full width at half maximum of the spectra is around 1/20 of the frequency, which yields $\Omega \sim 1.5 \times 10^{14}\ \mathrm{sec}^{-1}$ for $\lambda = 6000$ Å. The ratio Ω'/Ω which represents the spectral overlap is around 1/10 for normal temperatures. Using these numbers and $\tau = 0.5 \times 10^{-8}$ sec[11] yields $d_0 \sim 75$ Å, i.e. the correct order of magnitude for the critical distance.

The calculation performed here only represents a rough estimate based on classical physics.[§§§] An extended calculation based on quantum mechanics, to be published elsewhere, yields essentially the same result.[‖‖‖]

The energy transfer considered here is thus not a quantum mechanical one, but a *classical physics resonance effect*. Of course, the interpretation of this effect is different in the two models. In the classical model, after the transfer time, the two oscillators have nearly identical oscillation amplitudes. In the quantum mechanical model, either one or the other oscillator is excited, both with equal probability. The observed optical consequences, for example with regards to depolarization, are the same for both models. However, for chemical effects it is of course decisive that the excitation energy is not distributed between two molecules but is available for one molecule *in its entirety*.

In 1932, a result similar to Eq. (1) had already been derived by Perrin,[28b] but he did not take into account the difference of absorption and emission spectra. He also attributed the frequency uncertainty Ω solely to collisions within the solvent. If we use $\Omega' = \Omega \sim 1/\Delta t$, where Δt is the mean time between two collisions, we obtain an equation for d which is essentially identical with Perrin's formula

$$d_0 = \lambda/2\pi(\Delta t/\tau)^{1/6}.$$

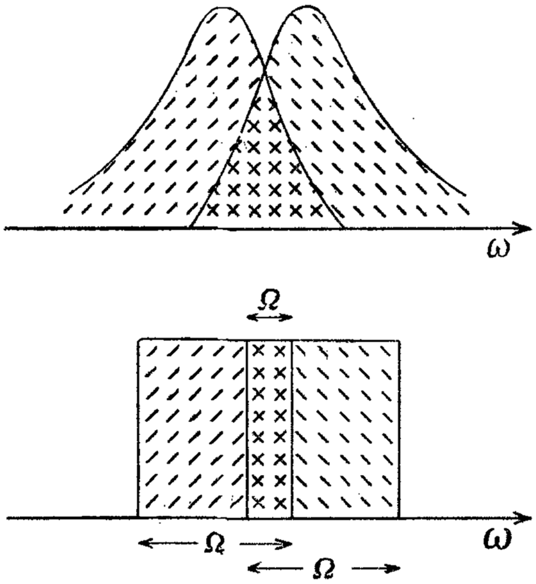

Fig. 3 Absorption and fluorescence spectrum. Top: actual intensity distribution. Bottom: schematic. Forward slash hatching ///: fluorescence; backward slash hatching \\\: absorption.

The quantity Δt is only accessible by approximate estimations; furthermore, this theory yields the opposite temperature dependence compared to ours.

The temperature dependence of energy transfer in our theory is expressed by increasingly less overlap of the absorption and fluorescence spectra with decreasing temperature, with only a small change in their FWHM.[23b,28a] Energy transfer is therefore hampered. The reason for this only becomes apparent in the context of quantum mechanics: in the transfer process, part of the electronic excitation energy is dissipated as vibrational energy. However, since the second molecule can only accept the same amount of energy the first molecule had, the difference has to be covered by addition of thermal energy.

If the distance between two molecules is significantly larger than the critical transfer distance d_0 given by Eq. (1′), then they are not in direct but indirect contact, coupled by the radiation field. Energy transfer is then only possible by emission of a photon from the excited molecule which is absorbed by the unexcited molecule. Since only those quanta in the region of the absorption spectrum can be absorbed, and absorption and fluorescence spectra only overlap a little (cf again Fig. 3), the overwhelming fraction of the emitted quanta cannot be reabsorbed. However, in the case of direct coupling of two molecules with a distance significantly less than the critical distance, complete energy transfer is actually possible.

At a low concentration of molecules, such transfer processes take place if an excited molecule is, by chance, adjacent to an unexcited molecule within the critical distance. At a higher concentration, the transfer occurs from an excited molecule to an unexcited neighbor and continues step by step like a *diffusion process*. In the classical view, the distribution of excitation to a larger number of molecules constitutes a dissipation of energy. In the quantum mechanical view, however, the excited state energy *migrates*, undiminished, like a particle undergoing Brownian motion, from one molecule to another. As such, the energy migration should be called a *quantum mechanical phenomenon*, although the fundamental energy transfer process can be understood by classical physics.

[‡‡‡] Translators note: There is a typographical error in the German original, the first $\hbar$ in Eq. (4′) should also be squared.

[§§§] The appearance of $\hbar$ in this calculation is of formal significance only, as already mentioned.

[‖‖‖] Translator's note: This work is however not published until 1948—Ann Phys 2, 55–75, 1948 (albeit "received 5 May 1947") and translated by R.S. Knox in Biological Physics, edited by E.V. Mielczarek, E. Greenbaum and R.S. Knox, New York: American Institute of Physics, pp 148–160, 1993.

According to Eq. (4′), the transfer speed between two molecules is inversely proportional to the 6th power of their distance; its mean is thus directly proportional to the square of the concentration. Thus, the number of molecules surreptitiously receiving the transferred energy until emission increases with the *square of the concentration*.[¶¶¶] This relationship can be used to extrapolate the results of depolarization measurements to concentrations higher than those that can be measured. In a 10^{-2} molar solution, these measurements yield a distribution of the excitation energy over 10 molecules, if there was no quenching. In a 0.1 molar solution (around 4%)[****] under identical conditions, *energy migration to over 1000 molecules* would result. The actual quenching inhibits such an energy migration in solutions of this concentration. As the quenching occurs via associates, the existence or formation of which is unrelated to migration, their elimination appears eminently possible. This possibility will be discussed further in Sec. 7.

The key properties of this type of energy migration encountered in concentration depolarization and concentration quenching in dye solutions are highlighted again: it occurs between molecules whose *spectra show no trace of interaction*. The transfer is significantly hampered by molecular vibrations; nevertheless, in high concentrations, it can lead to a migration of excitation energy over many molecules before emission occurs.

5 Associates

The theory of concentration quenching outlined in Sec. 3 lacks a theoretical justification for the ability of associated molecules to annihilate the excitation energy. This will now be addressed. Although we digress from the actual energy migration problem, the insight gained into the structure of the associates will be useful when dealing with energy migration in Scheibe's polymers.

Some years ago, Kautsky developed more specific ideas about the mechanism by which associates annihilate excitation energy. He noticed that not only concentration quenching but also a range of phosphorescence and long-lifetime fluorescence phenomena observed by him and other authors[18] could be interpreted by *associates.* One has to make the assumption that they can *undergo spontaneous transition from their primary excited state to a different metastable state of lower energy, which they can only leave radiatively with a very low probability.*[††††]

This idea will now be justified theoretically as an extension of the considerations in Sec. 4 regarding the interaction of neighboring molecules of the same type. There, it was assumed that in the case of weak coupling, the excitation energy is only transferred from one molecule to the next after several *atomic* oscillation periods. The spectrum is not affected by this coupling. A much stronger coupling is to be assumed between the individual molecules of an associate, in which, conversely, the transfer of excitation energy will be rapid with regard to the period of the molecular vibrations. Those cannot follow the rapid transfer of the excitation energy, but occur in such a way as if it were instantaneously distributed equally over both molecules.

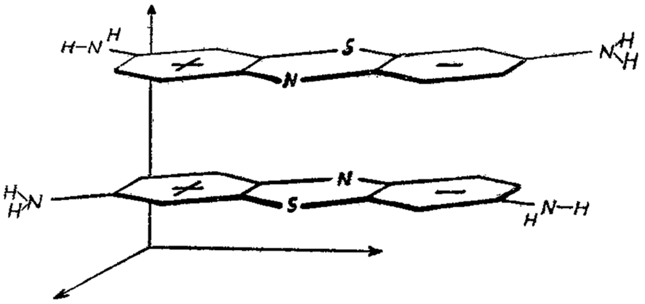

Fig. 4 Methylene blue dimer. The charges indicated (arbitrarily placed) correspond to snapshots of electronic oscillations. The stationary charges are not taken into account.

The dye associates in solutions of high concentrations can be understood in this way, since their absorption spectrum differs from that of individual molecules. Accordingly, they have their own energy levels. We can derive these from those of individual molecules by imagining them gradually merging. As in separate molecules, each molecule can be excited individually and the number of excited states does not change by coupling; a dimer has two excited states which correspond to those of a monomer. They both have a different energy, but for weak coupling, they should not differ too much from those of a monomer. It can be assumed that the energy of one is higher and of the other lower than that of the monomer, as depicted in the energy level diagram in Fig. 5.

According to this scheme, two absorption bands should be expected, while in methylene blue and other dyes only one has been found.[13,23a] The lack of the other one can be explained by a *selection rule*, which seems possible for a certain spatial *symmetry* of the dimer.

Under the assumption that the association is due to general van der Waals forces (London's dispersion forces),[‡‡‡‡] the most stable configuration of the dimer is a complete overlap of the carbon structure of both monomers lying flat on top of each other. Indeed, such a configuration, shown for methylene blue in Fig. 4, has the symmetry demanded for such a selection rule. The transition between the ground-state and the lower of the two excited states is forbidden, as indicated in Fig. 5.

The emergence of this selection rule can be understood by taking into account that the slowest electronic oscillation (absorption region of the longest wavelength) in the classical physics view is in a plane along the monomer. This can be assumed due to experimental experience[34c] and our current ideas about the absorption process.[7] The charges in Fig. 4 are supposed to be a snapshot of the charge distribution due to charge movement caused by the electronic oscillation in the longitudinal direction.

In one of a coupled oscillation of the dimer, the electronic systems have a phase difference of 0°, in the other 180°. The resultants of the two single moments relevant for absorption and emission thus are the vector sum and difference, the latter of which is zero. This frequency is accordingly missing from the absorption and emission, and the corresponding transition in the quantum mechanical energy level diagram is forbidden.

From this consideration it can also be concluded that the oscillation with a phase difference of 180° is slower, because the electrostatic interaction of the oscillating charges opposes

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[¶¶¶] This is also apparent from the course of the concentration depolarization, taking the quenching perturbation into account.

[****] In such a solution, in which the mean intermolecular distance is around 15 Å, the conditions of the calculation are still fulfilled.

[††††] Translator's note: In the German original, these last two sentences are a single sentence covering 9 lines.

[‡‡‡‡] For a justification, see for example KORTUM.[20]

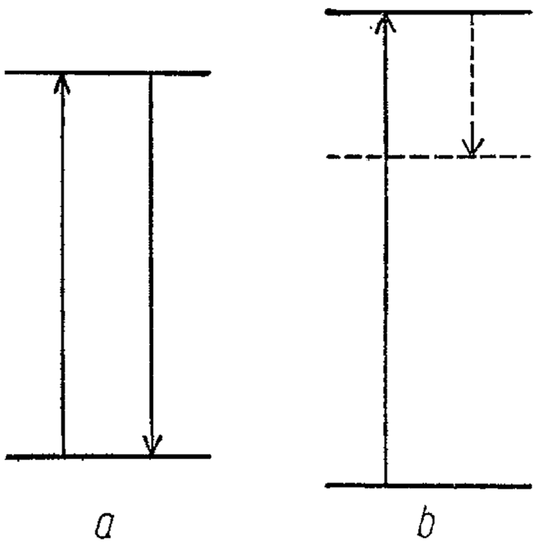


Fig. 5 Energy level diagram. (a) Monomer. (b) Dimer. - - - level of low transition probability. → radiative transition. - - - > non-radiative transition.

the restoring force. The energy level corresponding to the forbidden transition is thus lower.

The quantum mechanical treatment confirms these correspondence-principle considerations.

This energy level diagram, justified here for the first time using a model, was, in 1933, already devised by Jablonski[14] to describe different phenomena of long-lifetime fluorescence and phosphorescence, albeit without assignment to a dimer. Only Kautsky, who also interpreted these phenomena (discovered by himself in the context of this energy level diagram), recognized the link with association.[18b]

The energy level diagram shows that the absorption band of the dimer is at shorter wavelengths than that of the monomer. This corresponds to the observations on fluorescent dyes discussed in Sec. 3, but has also been observed in dyes with low association levels (Scheibe[34a] and Ecker[5]).

The lower of the two energy levels is not perceptible in the absorption spectrum yet determines the fluorescent behavior of the dimer. It nonradiatively transfers from the higher level excited by radiation to the lower one.[§§§§] As this is metastable, the molecule cannot return to its ground-state by fluorescence emission. It ultimately loses its energy by some quenching processes. However, under favorable circumstances, the luminescence phenomena of long duration described by Kautsky can occur, but this will not be discussed here.

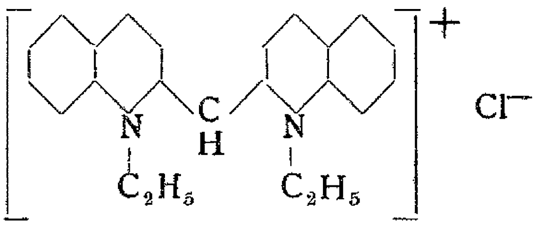
Important for the understanding of the loss of fluorescence by association, as explained here, is the fact that the transition from the *lowest* excited state to the ground-state is forbidden. For dimers this follows from the spatial structure as discussed above. We will encounter a different type of association for Scheibe's polymers below, which has a different energy level diagram and thus different fluorescence properties.

Association of the fluorescent dyes considered here does not occur between neutral molecules but between ionic molecules. It is hindered by repulsive Coulomb forces due to charges of the same sign. Thus it is only significant in water, where the repulsive forces are small due to the very high *dielectric constant*, whereas the association forces (according to our assumption London's dispersion forces) are independent of the static dielectric constant. These attractive forces are large for molecules with large transition moments to low excited states, i.e., especially for the typical dyes and not for the aromatic hydrocarbons which only very weakly absorb in the long wavelength range. As already mentioned in Sec. 3, their quenching is not due to association but due to collisions between excited and ground-state molecules. The excited dimer created by this encounter then annihilates the energy in the manner discussed above.

6 Scheibe's Polymers

When considering dye aggregation, it had to be assumed that the electronic excitation energy transfers from one molecule to another in a time span which is short compared to molecular vibrations. The transfer thus occurs in a manner different from that discussed in Sec. 4. For the dimers, it is, however, limited to a fairly small section of the aggregate.

Now, Scheibe found[34a] that normally non-fluorescent dyes of the pseudoisocyanine type can, above a certain concentration threshold, form *fluorescent polymers* in aqueous solution, i.e. they form aggregates of a high order.



N-N′ diethyl chloride

Polymerization only occurs in one dimension, so that the molecules are arranged like beads on a string. This only takes place in aqueous solution and is reversible. Apart from its sudden occurrence, its circumstances are similar to those of the association of dimers, which point to similar forces.[‖‖‖]

It is most astonishing that nonfluorescent dyes become fluorescent by polymerization, whereas the opposite occurs upon dimerization. Moreover, the optical properties of the polymers found by Scheibe and coworkers are remarkable. They possess a significantly altered absorption spectrum compared to that of the monomer, which owes its special character to an unusually *high* and *narrow band* in its *long wavelength limits*. In mixtures of different dyes of this kind, polymer mixtures are formed which only have such a band. This is located in a region between the two bands of the corresponding pure polymers and is determined by their composition. This particular feature clearly demonstrates that the spectrum is a *collective property* of the individual molecules making up the polymer. The fluorescence of the polymers also shows this property. It emanates solely from the narrow band, both upon short-wavelength excitation and excitation within the band itself, which for absorption and emission is almost at the same wavelength.

[§§§§] Transitions of this kind are the more probable the lower the energy needed for this transition. In general, upon irradiation into higher electronic excited states of a molecule, fluorescence is always observed from the lowest one. For example, chlorophyll always fluoresces red, irrespective of irradiation of its red, blue or ultraviolet absorption band.

[‖‖‖] The role of water as a solvent is clear after the considerations at the end of section 5. There is no need to invoke regular hydrogen bonds between the molecules as has been done occasionally (SHEPPARD[36] MATTOON[24]).

The ability to orient polymers in capillary flows offers the possibility of a range of polarized optical investigations. Scheibe found[34b] that in the region of the narrow band, light with its electrical vector oscillating parallel to the length of the polymer is preferentially absorbed. The fluorescence emitted in this band also has this polarization. However, in the short-wavelength spectrum below this narrow band, the perpendicular polarization is absorbed to a higher degree.

To interpret these properties, it can be assumed that the electrons of all molecules in the polymer, or at least a large proportion thereof, act together for absorption and emission. The general behavior of such systems consisting of many individual elements has been discussed in a range of publications by Peierls[27] and Frenkel.[9] Franck and Teller,[8a] as well as Möglich and Rompe[25] have also included Scheibe's polymers, but not all their properties found an explanation.

For the structure initially assumed by Scheibe[34] where the molecular planes lie perpendicular to the polymer axis *(stack of coins!)*, the observed direction of oscillation of the narrow band along the polymer axis caused problems for a straightforward interpretation. As the electronic oscillations of the monomer occur in the molecular plane,[34c] a coupled oscillation in a plane perpendicular to this is unthinkable.

Recent X-ray studies by Hoppe from Scheibe's institute[35] show, however, that the molecular planes are significantly *tilted* with respect to the polymer axis. In agreement with this we would like to assume that the structure of the polymer roughly corresponds to the one shown schematically in Fig. 6, where neighboring molecules are nearly parallel, but distant ones are randomly oriented because there are no alignment forces between them. Due to the tilt of the molecular plane with respect to the polymer axis, the electronic oscillations of the monomers possess a component in this direction, so that for the whole system oscillations in this direction are also possible.

The energy level diagram for the sum of n molecules is given by a generalization of the considerations for dimers discussed in Sec. 5. It contains n excited states of different energy, which for a sufficiently large n practically form a continuum, the width of which is given by the magnitude of the coupling energy.

The structure depicted in Fig. 6 shows a *symmetry* which expresses itself by the fact that a translation of the molecules along the polymer axis does not change their projection onto the axis. This symmetry implies a *selection rule* for the light oscillations with the electrical vector *parallel* to the polymer axis but not in the direction perpendicular to it. While the latter is allowed for transitions between the ground-state and all excited states of the continuum[¶¶¶¶], for the parallel component only transitions with a fraction of those excited states take place. The calculation shows that those are the states *at the bottom of the continuum*, as depicted in the energy level diagram Fig. 7.

This can be justified by a classical physics consideration, similar to the case of dimers. The amplitudes of the electron oscillations of a monomer are illustrated by the moments in Fig. 6. They are a superposition of n different coupled oscillations with a constant phase difference between 0° and 180° between neighboring molecules. A phase difference of zero yields a large resultant in the direction of the polymer which decreases as the phase difference increases, since the single moments then increasingly compensate each other. Only the transitions corresponding to the oscillations with a small phase difference occur in the spectrum of the parallel components. The corresponding energy levels are of course located near one of the edges of the continuum. For the nearly perpendicular orientation of the molecular planes to the axis of the polymer, this would be the upper edge (corresponding to high frequencies), because then the oscillation with a phase difference of zero is the fastest one, just as in the dimer. With an increasing tilt of the molecules against the polymer axis, the sign of the interaction of neighboring molecules changes. This can be seen from Fig. 4 where the same poles and in Fig. 6 where opposite poles of the moments of adjacent molecules are next to each other. The *tilt* determines the position of the energy levels of the allowed transitions at the *lower* end of the continuum.

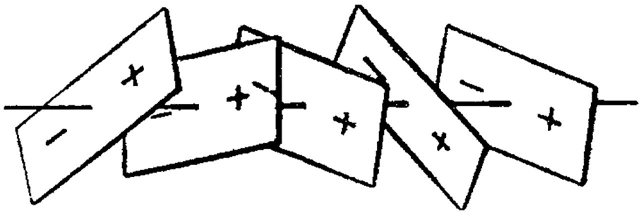


Fig. 6 Structure of the polymers (schematic).

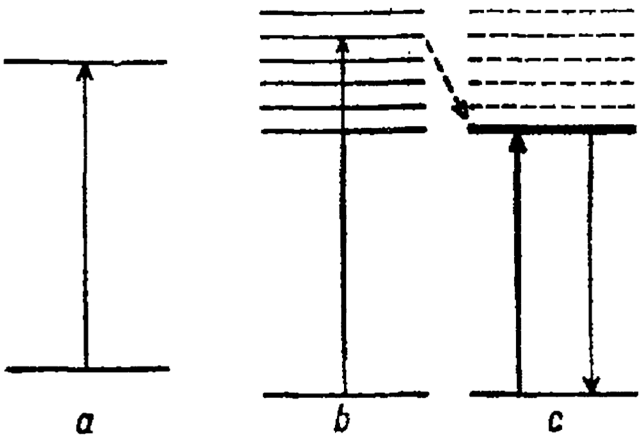


Fig. 7 Energy level diagram. (a) Monomer. (b) Polymers, oscillations perpendicular to the field. (c) Polymers, oscillation parallel to the field. —— level of very high transition probability.

The energy level diagram principally only represents electronic energies. As the excitation is distributed over a large number of monomers, the change in nuclear equilibrium is so small that high-frequency molecular vibrations are not excited. In a monomer, these determine the bandwidth by superposition with the electronic oscillations. The possible excitation of slow oscillations yields no significant energy level broadening, so that Fig. 7 can be considered to be the energy level diagram of the entire system. Despite the nuclear movement, the spectrum's continuum region is narrow and overlaps in absorption and emission.

The energy level diagram describes the absorption and fluorescence of polymers in agreement with experience.[¶¶¶¶] The narrow band which only absorbs light with the electrical vector in the direction of the polymer axis is situated not only on the long wavelength edge of the polymer absorption but is also at longer wavelengths than the band of the monomer (Fig. 7(a)). When exciting higher energy levels by light with perpendicularly oscillating electric fields, the energy is initially transferred nonradiatively into the lower edge of the continuum and is also emitted

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[¶¶¶¶] The intensity in the continuum is of course not constant. To faithfully render the broad bands in the spectra, the model given in Fig. 6 would have to be optimized.

by the narrow band with the polarization associated with it. Light irradiating this narrow band is very rapidly re-emitted as *resonance fluorescence* due to the high transition moment (according to Kirchoff[19] the lifetime is less than 10^{-9} sec).

The opposite fluorescence behavior of dimer and polymer corresponds to the difference of energy level schemes in Figs. 5 and 7. Whereas the *dimer's* lowest excited state is *metastable*, it has a very *high emission probability* for the *polymers*, so that the quenching processes which prevent monomer fluorescence cannot occur there.[*****] The reason for different energy level diagrams is deemed neither a difference in the kind of associating forces nor the number of associating molecules, but a difference in the *type* of association, expressed in the *tilt* of the monomers with respect to the polymer axis. The two types of association are the same, which for dipole association cause as *contra-association* the formation of dimers with a compensation of the moment, and as *co-association* the formation of chains with an increase of the moment.

The energy migration in Scheibe's polymers, which in this context is of great interest to us, is most clearly expressed in the *collective spectral properties* which reveal that it occurs faster than molecular vibrations. This is independently established by the observation of fluorescence quenching by other compounds (Katheder[17]). The fluorescence of the polymers is quenched by phenol-type compounds, just like the fluorescence of other dyes. While usually concentrations of 10^{-3} to 10^{-2} mol/l are required for noticeable quenching, in this case a 10^{-7} mol/l concentration of brenzcatechin is sufficient. At such a low concentration, the quenching cannot be explained in the usual manner by diffusion of quenchers to excited monomers (cf Sveshnikov[39]). It can only be understood by assuming either that the excitation of the molecular chain of polymers wanders along it, toward the quencher, or—which is not very different—that it is distributed over a large number of molecules from the start, and that from each of those it can be released in its entirety.

The speed of energy migration is, however, difficult to gauge from the present quenching experiments. The theory provides some clues. Since the transfer occurs rapidly compared to molecular vibrations, we have exact resonance, and the transfer time is of order h/U according to Eq. (4), where U is again the electrostatic interaction of the oscillating charges of neighboring molecules, which, due to their proximity, can no longer be calculated from that of two point dipoles. As U also determines the bandwidth of the absorption spectrum ($U \sim \hbar\omega$), the transfer time roughly equals its reciprocal value (10^{-14} sec).

The effect of the rapid transfer is limited by the fact that with the number of molecules affected by the excitation, the excited state lifetime decreases because molecules oscillating in phase emit faster than a monomer. An estimation on this basis yields that the spread of the excitation is limited to a maximum of around 1000 molecules. If the number of molecules in polymers is larger, the energy migration in this polymer is limited to such a region.

7 Application to Biological Systems

To return to the initially mentioned biological problems, we will now discuss whether one of the two mechanisms of energy migration outlined here could be considered as an explanation of the phenomena observed there. For the *assimilation problem*,[†††††] the properties of the chlorophyll molecule are very favorable for this kind of energy transfer. The strong absorption band in the red at 6500 Å[38] corresponds to a large transition moment and thus a strong interaction between excited and ground-state molecules. The intense fluorescence in appropriate solvents proves the absence of energy-absorbing processes within the molecule.

The energy transfer mechanism present in the polymers is certainly not applicable. The absorption spectrum of chlorophyll in a leaf differs only very little from that in dilute solution.[8] This mechanism, however, requires a significant change in the spectrum, as present in the case of associates or polymers. Therefore, only the first mechanism discussed in Sec. 4 appears possible, which causes energy migration in dye solutions of modest concentration without spectral change. For fluorescing dyes, however, so far no case of energy migration has been observed over as many molecules as obviously present in the assimilation structure. As already mentioned in Sec. 4, concentrations which allow energy migration over such a scale suffer from quenching where the energy is annihilated either by transfer to existing dimers or by formation of dimers of excited and ground-state monomers.

However, conditions are conceivable which even in high concentrations do not allow associates to be present or to be formed after excitation. It would only be required that each chlorophyll molecule is sufficiently deeply embedded in a corresponding protein, or that it is bound at a defined position of a protein structure. However, a quantitative treatment of such a model on the basis of the theory developed here appears not yet possible for want of sufficient data.

A similar situation arises for the energy migration problem for the *components of the cell nucleus*. The compounds present here which absorb at long wavelengths are nucleic acids and aromatic amino acids which absorb between 2500 and 3000 Å. Nothing appears to be known about their (of course, ultraviolet) fluorescence which would be expected in analogy with related compounds. It is, however, certain that electronic oscillation moments of these compounds are significantly smaller than those of dyes, which severely hinder energy transfer by both mechanisms. Moreover, here also only the first mechanism would be applicable, since the absorption of the substance in the cell nucleus does not differ significantly from that in a normal solution (cf the microspectrograms by Casparsson[3] or the absorption of the tobacco mosaic virus[2]).

It was not the intention of this paper to ascertain the occurrence of the controversial energy migration without a material carrier in biological systems. A final clarification of the mechanisms we are dealing with here will probably only be possible on the basis of a more complete knowledge of the physicochemical structure of the basic matter involved in it. We only wanted to demonstrate that this type of energy migration in nonbiological systems is *more frequent* than generally assumed, and that, by and large, it is a phenomenon which is theoretically understood. We now have an overview of the conditions for its occurrence. Where these are fulfilled in biological systems energy migration has, of course, to be taken into account for the interpretation of the observed effects of light.

[*****] However, the distribution of the excitation energy over a large number of monomers, as well as the viscosity of the polymer solution also reduces the intramolecular quenching.

[†††††] Translator's note: i.e., photosynthesis.

Acknowledgments

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